



# Handbook of herbs and spices

Second edition

Volume 1

Edited by K. V. Peter

# **Handbook of herbs and spices**

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Herbs and spices are among the most versatile and widely-used ingredients in food processing. As well as their traditional roles as flavourants and colourants, they have increasingly been used as natural preservatives and for their potential health-promoting properties, for example as antioxidants. *Handbook of herbs and spices* is an essential reference work for manufacturers wishing to make the most of these important ingredients. The three volumes of the handbook's first edition have been condensed into two indispensable volumes. Comprehensively-updated, they focus on products of commercial significance. Introductory chapters cover fundamental issues such as quality specifications for herbs and spices and their use as antimicrobials in foods. Subsequent chapters each focus on a different herb or spice crop. New chapters on important products such as basil, fennel seeds, mint, kaffir lime leaves and tarragon have been added.

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While products such as bananas, pineapples, kiwifruit and citrus have long been available to consumers in temperate zones, new fruits such as lychee, longan, carambola, and mangosteen are now also entering the market. Confirmation of the health benefits of tropical and subtropical fruit may also promote consumption further. Tropical and subtropical fruits are particularly vulnerable to postharvest losses, and are also transported long distances for sale. Therefore maximising their quality postharvest is essential and there have been many recent advances in this area. Many tropical fruits are processed further into purees, juices and other value-added products, so quality optimization of processed products is also important. These books cover current state-of-the-art and emerging post-harvest and processing technologies. Volume 1 contains chapters on particular production stages and issues, whereas Volumes 2, 3 and 4 contain chapters focused on particular fruit.

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# **Handbook of herbs and spices**

**Second edition**

**Volume 1**

**Edited by  
K. V. Peter**



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# 1

## Introduction to herbs and spices: definitions, trade and applications

**K. V. Peter, World Noni Research Foundation, India and M. R. Shylaja, Kerala Agricultural University, India**

**Abstract:** This chapter discusses the definition and classification of herbs and spices. It also discusses the trade of spices and, in particular, the role of India. The applications of different spices in medicine, the food and beverage industry (including health foods), cosmetics, perfumery and nutraceuticals are summarized. The use of spices as a source of natural colour, flavouring, antioxidants and antimicrobials is commented on. This chapter also highlights the importance of producing high quality, clean spices, by minimizing the use of chemical fertilizers and pesticides. The use of biocontrol agents is also briefly touched upon.

**Key words:** spices, classification, spice trade, uses of spices, medicine, foods, beverages, nutraceutical, health food, cosmetics, perfumery, natural colour, natural flavour, natural antioxidants, natural antimicrobials, quality clean spices.

### 1.1 Definitions

The Geneva-based International Organisation for Standardisation (ISO) defines spices and condiments as:

vegetable products or mixtures thereof, free from extraneous matter, used for flavouring, seasoning and imparting aroma to foods.

Though the term spice can also be used to refer to herbs, the distinction between herbs and spices is usually as follows:

- Herbs may be defined as the dried leaves of aromatic plants used to impart flavour and odour to foods. The leaves are commonly traded separately from the plant stems and leaf stalks.
- Spices may be defined as the dried parts of aromatic plants with the exception of the leaves. This definition is wide-ranging and covers virtually all parts of the plant.

The taxonomic classification of spices is presented in Table 1.1 and a conventional classification in Table 1.2. The various parts of plants used to produce the range of herbs and spices are illustrated in Table 1.3. Herbs and spices have been used in

## 2 Handbook of herbs and spices

**Table 1.1** Taxonomic classification of spices

|              |                  |                  |               |               |                                                                          |
|--------------|------------------|------------------|---------------|---------------|--------------------------------------------------------------------------|
| Angiospermae | Dicotyledoneae   | Sympetales       |               |               |                                                                          |
|              |                  |                  |               | Solanaceae    | chilli, paprika, red pepper                                              |
|              |                  |                  |               | Pedaliaceae   | sesame                                                                   |
|              |                  |                  | Campalunatae  | Compositae    | camomile, chicory, tarragon                                              |
|              |                  | Archichlamydales | Piperales     | Piperaceae    | cubeba, long pepper, pepper                                              |
|              |                  |                  | Ranales       | Myristicaceae | mace, nutmeg                                                             |
|              |                  |                  |               | Lauraceae     | bay leaf, cassia, cinnamon                                               |
|              |                  |                  |               | Magnoliaceae  | star-anise                                                               |
|              |                  |                  | Rhoeadales    | Cruciferae    | mustard, wasabi                                                          |
|              |                  |                  | Myrtiflorae   | Myrtaceae     | allspice, clove                                                          |
|              |                  |                  | Umbelliflorae | Umbelliferae  | anise, caraway, celery, chervil, coriander, cumin, dill, fennel, parsley |
|              | Monocotyledoneae | Liliiflorae      |               | Liliaceae     | garlic, onion                                                            |
|              |                  |                  |               | Iridaceae     | saffron                                                                  |
|              |                  | Scitamineae      |               | Zingiberaceae | cardamom, ginger, turmeric                                               |
|              |                  | Orchidales       |               | Orchidaceae   | vanilla                                                                  |

**Table 1.2** Conventional classification of spices

| Classes             | Spices                                                                                              |
|---------------------|-----------------------------------------------------------------------------------------------------|
| Hot spices          | Capsicum (chillies), cayenne pepper, black and white peppers, ginger                                |
| Mild spices         | Paprika, coriander                                                                                  |
| Aromatic spices     | Allspice (pimento), cardamom, cassia, cinnamon, clove, cumin, dill, fennel, fenugreek, mace, nutmeg |
| Herbs               | Basil, bay leaves, dill leaves, marjoram, tarragon, thyme                                           |
| Aromatic vegetables | Onion, garlic, shallot, celery                                                                      |

**Table 1.3** Plant organs as spices

| Plant organs | Spice crops                                                                               |
|--------------|-------------------------------------------------------------------------------------------|
| Aril         | Mace or nutmeg                                                                            |
| Barks        | Cassia, cinnamon                                                                          |
| Berries      | Allspice, black pepper, chilli                                                            |
| Buds         | Clove                                                                                     |
| Bulbs        | Onion, garlic, leek                                                                       |
| Pistil       | Saffron                                                                                   |
| Kernel       | Nutmeg                                                                                    |
| Leaf         | Basil, bay leaf, mint, marjoram, sage, curry leaf                                         |
| Rhizome      | Ginger, turmeric                                                                          |
| Latex        | Asafoetida                                                                                |
| Roots        | Angelica, horse-radish                                                                    |
| Seeds        | Ajowan, aniseed, caraway, celery, coriander, dill, fennel, fenugreek, mustard, poppy seed |

foods since antiquity. ISO document 676 lists 109 herb and spice plant species useful as ingredients in food (see Appendix 1).

## **1.2 Trade in herbs and spices and trends in their use**

Some of the main spice-producing areas are listed in Appendix 2. The current annual global trade in spices is 0.6–0.7 million tonnes valued at US \$3–3.5 billion. The value of the spice trade is particularly dependent on pepper prices as pepper remains the main spice in international trade. The global trade in spices is expected to increase with growing consumer demand in importing countries for more exotic, ethnic tastes in food. About 85 % of spices are traded internationally in whole form, with importing countries processing and packaging the final product for the food industry and the retail market. The trade in processed and value-added spice ingredients is, however, growing rapidly as importers look for cheaper global sourcing of spice products and exporting businesses develop the appropriate technologies and quality systems. There is limited competition from synthetic products, with the exception of vanilla, particularly given consumer preferences for ‘natural’ ingredients in food products. The USA is the biggest importer of spices and spice products, followed by Germany and Japan. The total value of spice imports in to the USA increased from US \$426 million in 1998 to \$597 million in 2007 ([www.ers.usda.gov](http://www.ers.usda.gov)). Germany is the largest consumer of spices and herbs in the EU. The annual consumption of herbs and spices in Germany amounted to 62 000 tonnes with an annual average growth rate of 9.7 % between 2004 and 2008 ([www.cbi.eu](http://www.cbi.eu)). Other major importing regions are the Middle East and Africa.

### **1.2.1 India: the land of spices**

India is known the world over as the ‘land of spices’. Cultivation of spices started in India in ancient times and it was Indian spices, famous across the globe, that attracted explorers, invaders and traders from various lands to Indian shores. India, with its varied climatic and soil conditions, was the original home of many spices and produces spices of high intrinsic quality. Spices play a vital role in the national economy of India. India is the largest producer, consumer and exporter of spices in the world, contributing 86 % of global spice production followed by China (4 %), Bangladesh (3 %), Pakistan (2 %), Turkey (2 %) and Nepal (1 %) (FAOstat). The domestic market in India absorbs 90 % of the spices produced in the country and the rest is exported. India enjoys a formidable position in world spice trade with a 48 % share in terms of volume and 44 % share in terms of value. India has the monopoly in the supply of spice oils and oleoresins and is a major supplier of curry powders, spice powders, spice mixes and spices in consumer packs. Spices exports have registered substantial growth during the last 5 years, registering an annual average growth rate of 21 % in value and 8 % in volume. During the year 2010–11, spices export from India has registered an all time high both in terms of quantity and value. In 2010–11 the export of spices from India was 525 750 tonnes valued at Rs. 6840.71 crores (US \$1502.85 million) as compared to 502 750 tonnes valued at Rs. 5560.50 crores (US \$1173.75 million).

in 2009–10, registering an increase of 28 % in dollar terms of value and 5 % in volume.

Spices from India are mainly exported to the USA, followed by the EU, Eastern Europe, East and West Asia and Africa. The highest export earning was registered for mint products (Rs. 169 679.00 lakhs) followed by chilli (Rs. 153 554.00 lakhs), turmeric (Rs. 70 285.15 lakhs), cumin (Rs. 39 597.75 lakhs) and black pepper (Rs. 38 318.50 lakhs) ([www.indianspices.com](http://www.indianspices.com)).

### 1.2.2 Uses of herbs and spices

Spices played a prominent role in all the ancient civilizations that prevailed in China–India, Greece–Rome and Babylon–Egypt, and they have long been valued for their medicinal properties. The first authentic record on the uses of spices dates back to the pyramidal age in Egypt. During the period, onion and garlic were fed to workers to preserve their health and cinnamon was used to embalm the dead. Medicinal uses of spices are mentioned in Charaka Samhita and Sushruta Samhita. The first use of spices in food was for meat preservation, due to their antimicrobial properties. With the advent of refrigeration, the demand for spices as a preservative in the western world decreased. However, with the passing of time, spices had become indispensable in the culinary art of cooking to enhance flavour and taste of foods and beverages, so their use did not cease in the West. With the development of procedures for the extraction of spice extracts, spices were extensively used in the perfumery, cosmetics and pharmaceutical industries. In the globalization era, due to consumer resistance to chemical additives, spices have become all the more important as sources of natural colours, flavours, antimicrobials and antioxidants for the food industry. There has also been a tremendous growth in the use of herbal and natural plant products in the cosmetics industry and spices like turmeric, saffron, coriander, basil, fenugreek, etc. have become more important in this sector. In the emerging nutraceutical industry, herbs and spices could play a pivotal role, since for many applications, their therapeutic use has been proven and scientifically validated, and the necessary safety evaluations have been performed.

## 1.3 Herbs and spices in traditional medicine

The medicinal properties of spices have been known to mankind from time immemorial. Spices are used extensively in traditional systems of medicines such as Ayurveda, Sidha and Unani. Long pepper, black pepper and ginger are the widely used spices in the Ayurvedic system of medicine, for example. Spices not only have their own therapeutic role, but also enhance the absorption and utilization of other therapeutic substances administered along with them. They are prepared in a number of ways to extract their active ingredients for internal and external uses. Extracts from herbs and spices are used as infusions, decoctions, macerations, tinctures, fluid extracts, teas, juices, syrups, poultices, oils, ointments and powders and many spices used are thought to have multiple bioactive principles. The most commonly used spices and their medicinal properties are given in Table 1.4.



**Table 1.4** Medicinal properties of spices

| Spices             | Medicinal properties                                                                                          |
|--------------------|---------------------------------------------------------------------------------------------------------------|
| Black pepper       | Carminative, antipyretic, diuretic, antihelminthic, anti-inflammatory, anti-epileptic                         |
| Cardamom           | Antidepressive, carminative, appetizer, diuretic                                                              |
| Ginger             | Carminative, anti-nauseant, diuretic, antifatulence, antihistaminic, aphrodisiac, cholesterol lowering        |
| Turmeric           | Carminative, antibiotic, antifatulence, antiseptic, anti-inflammatory                                         |
| Garlic             | Antimicrobial, diuretic, diaphoretic, antifatulence, cholesterol lowering, anti-inflammatory                  |
| Clove              | Antifatulence, analgesic, stimulant, carminative, antinauseant                                                |
| Nutmeg             | Stimulant, carminative, astringent, aphrodisiac, anti-inflammatory                                            |
| Cinnamon           | Stimulant, carminative, astringent, aphrodisiac, anti-inflammatory                                            |
| Chilli             | Carminative, antirheumatic                                                                                    |
| Saffron            | Stimulant, stomachic, anticarcinogenic                                                                        |
| Allspice           | Stimulant, digestive, carminative                                                                             |
| Basil, sweet       | Stomachic, antihelminthic, diaphoretic, expectorant, antipyretic, carminative, stimulant, diuretic, demulcent |
| Bayleaves (laurel) | Stimulant, narcotic                                                                                           |
| Caraway            | Stomachic, carminative, antihelminthic, lactagogue                                                            |
| Celery             | Stimulant, tonic, diuretic, carminative, emmenagogue, anti-inflammatory                                       |
| Chive              | Stimulant, diuretic, expectorant, aphrodisiac, emmenagogue, anti-inflammatory                                 |
| Coriander          | Carminative, diuretic, tonic, stimulant, stomachic, refrigerent, aphrodisiac, analgesic, anti-inflammatory    |
| Cumin              | Stimulant, carminative, stomachic, astringent, antiseptic                                                     |
| Dill               | Carminative, stomachic, antipyretic                                                                           |
| Fennel             | Stimulant, carminative, stomachic, emmenagogue                                                                |
| Fenugreek          | Carminative, tonic, aphrodisiac                                                                               |
| Leek               | Stimulant, expectorant                                                                                        |
| Marjoram           | Carminative, expectorant, tonic, astringent                                                                   |
| Mint (peppermint)  | Stimulant, stomachic, carminative, antiseptic                                                                 |
| Mint (spearmint)   | Stimulant, carminative, antispasmodic                                                                         |
| Oregano            | Stimulant, carminative, stomachic, diuretic, diaphoretic, emmenagogue                                         |
| Parsley            | Stimulant, diuretic, carminative, emmenagogue, antipyretic, anti-inflammatory                                 |
| Rosemary           | Mild irritant, carminative, stimulant, diaphoretic                                                            |
| Sage               | Mild tonic, astringent, carminative                                                                           |
| Tarragon           | Aperient, stomachic, stimulant, febrifuge                                                                     |
| Thyme              | Antispasmodic, carminative, emmenagogue, antihelminthic, spasmodic, laxative, stomachic, tonic, vermifuge     |

The essential oils of many herbs and spices are used nowadays in aromatherapy to relieve symptoms of various ailments such as aches and pains and emotional problems such as depression, stress and anxiety. For example, the essential oil of coriander and pepper mint is analgesic, dill and anise oils are antipyretic, coriander, celery, parsley, cumin and ginger oils are anti-inflammatory. Recently, anticarcinogenic properties have been reported for essential oils of cumin and basil suggesting their

potential use as protective agents against carcinogenesis. Also, methanol extracts of allspice, marjoram, tarragon and thyme strongly inhibited platelet aggregation induced by collagen in human beings.

## 1.4 Herbs and spices in the food and beverage industries

In the food and beverage industries, spices find application as sources of natural colour and flavour and as antimicrobials and antioxidants.

### 1.4.1 Spices as a source of natural colour

The food sector is now experiencing a return to the use of natural colours due to changes in legislation and consumer preferences. Low tinctorial power, poor stability (to changes in pH, oxygen, heat and light), low solubility, off-flavour issues and high cost limit the use of natural colours. However, these problems can be overcome by using alternatives to traditional extraction and preparation methods, such as methods involving enzymes, supercritical CO<sub>2</sub>, membrane processing and encapsulation techniques. Before synthetic colours came into existence, spices like chilli, saffron, turmeric, etc. were used in Indian cuisines to add colour. The Central Food Technological Research Institute of India (CFTRI) has developed technologies for the manufacture of certain natural food colours such as kokum (red) and chillies (red). Kokum contains 2–3 % anthocyanin and is regarded as a natural colour source for acidic foods. Garcinol is the fat-soluble yellow pigment isolated from rind of kokum fruit. Garcinol is added at a 0.3 % level to impart an acceptable yellow colour to butter. Colour components present in spices and natural shades available with spices are presented in Table 1.5.

**Table 1.5** Colour components in spices

| Colour component  | Tint             | Spice                                 |
|-------------------|------------------|---------------------------------------|
| <b>Carotenoid</b> |                  |                                       |
| β-Carotene        | Reddish orange   | Red pepper, mustard, paprika, saffron |
| Cryptoxanthin     | Red              | Paprika, red pepper                   |
| Lutin             | Dark red         | Paprika, parsley                      |
| Zeaxanthin        | Yellow           | Paprika                               |
| Capsanthin        | Dark red         | Paprika, red pepper                   |
| Capsorubin        | Purple red       | Paprika, red pepper                   |
| Crocetin          | Dark red         | Saffron                               |
| Neoxanthin        | Orange yellow    | Parsley                               |
| Violaxanthin      | Orange           | Parsley, sweet pepper                 |
| Crocin            | Yellowish orange | Saffron                               |
| Flavonoids        | Yellow           | Ginger                                |
| Curcumin          | Orange yellow    | Turmeric                              |
| Chlorophylls      | Green            | Herbs                                 |

Source: Ravindran *et al.* (2006).

### 1.4.2 Herbs and spices as a source of natural flavours

The increasing demand in developed countries for natural flavours means there is tremendous potential to increase the trade in spices. Spices are added to foods in several forms, such as whole spices, ground spices and spice extracts. As spice extracts are highly concentrated, they are either encapsulated or emulsified in edible bases like salt or dextrose to obtain a uniform dispersion of the extracts in food materials. Extraction of oils and oleoresins is accomplished using a range of methods, including steam distillation, hydrocarbon extraction, chlorinated solvent extraction, enzymatic treatment and fermentation, supercritical CO<sub>2</sub> extraction. Carbon dioxide extraction from solid botanicals is now adopted on a commercial scale. The resulting extracts have no solvent residues and fewer terpenes. Enzymatic treatment and fermentation of raw botanicals also result in higher yields and improved quality of essential oil. More recently, the use of genetic engineering and recombinant DNA technology has resulted in *in vitro* production of natural esters, ketones and other flavouring materials. Cloning and single cell culture techniques are also of benefit to the flavourist. The recovery of essential oils and oleoresins from spices is presented in Table 1.6 and the main flavour compounds present in herbs and spices are presented in Table 1.7.

### 1.4.3 Herbs and spices as a source of natural antioxidants

Antioxidants are added to foods to preserve the lipid components from quality deterioration. Synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) are the ones commonly used. Due to the suspected action of these compounds as promoters of carcinogenesis, there is growing demand for natural antioxidants. Antioxidants also play a role in defence mechanisms of the body against cardiovascular diseases, cancer, arthritis, asthma and diabetes. Many herbs and spices are known as excellent sources of natural antioxidants, and consumption of fresh herbs in the diet may therefore contribute to the daily antioxidant intake. Phenolic compounds are the primary antioxidants present in spices, and a linear relationship exists between the total phenolic content and the antioxidant properties of spices. Essential oils, oleoresins and even aqueous extracts of spices possess

**Table 1.6** Recovery of essential oil and oleoresin from spices

| Spice            | Essential oil (%)                   | Oleoresin (%) |
|------------------|-------------------------------------|---------------|
| Black pepper     | 1–4.0                               | 10–13         |
| Cardamom (small) | 6–10                                | 10–12         |
| Cardamom (large) | 1–3                                 | –             |
| Ginger           | 1–3                                 | 5–10          |
| Turmeric         | 2–6                                 | 8–10          |
| Nutmeg           | 7–16                                | 10–12         |
| Clove            | 16–18                               | 20–30         |
| Cinnamon         | 1–3                                 | 10–12         |
| Allspice         | 1–3 (leaf oil)<br>3–4.5 (berry oil) | –             |

**Table 1.7** Important flavour compounds in spices

| Spice            | Important flavour compounds                                   |
|------------------|---------------------------------------------------------------|
| Allspice         | Eugenol, $\beta$ -caryophyllene                               |
| Anise            | (E)-anethole, methyl chavicol                                 |
| Black pepper     | Piperine, S-3 Carene, $\beta$ -caryophyllene                  |
| Caraway          | <i>d</i> -Carvone, crone derivatives                          |
| Cardamom         | $\alpha$ -Terpinyl acetate, 1-80-cineole, linalool            |
| Cinnamon, cassia | Cinnamaldehyde, eugenol                                       |
| Chilli           | Capsaicin, dihydro capsaicin                                  |
| Clove            | Eugenol, eugenyl acetate                                      |
| Coriander        | <i>d</i> -Linalool, C10-C14-2-alkenals                        |
| Cumin            | Cuminaldehyde, <i>p</i> -1,3-mentha-dienal                    |
| Dill             | <i>d</i> -Carvone                                             |
| Fennel           | (E)-anethole, fenchone                                        |
| Ginger           | Gingerol, shogaol, neral, geranial                            |
| Mace             | $\alpha$ -Pinene, sabinene, 1-terpenin-4-ol.                  |
| Mustard          | Allyl isothiocynate                                           |
| Nutmeg           | Sabinene, $\alpha$ -pinene, myristicin                        |
| Parsley          | Apiol                                                         |
| Saffron          | Safranol                                                      |
| Turmeric         | Turnerone, zingiberene, 1,8-cineole                           |
| Vanilla          | Vanillin, <i>p</i> -OH-benzyl-methyl ether                    |
| Basil, sweet     | Methylchavicol, linalool, methyl eugenol                      |
| Bay laurel       | 1,8-Cineole                                                   |
| Marjoram         | <i>e</i> - and <i>t</i> -Sabinene hydrates, terpinen-4-ol     |
| Oregano          | Carvacrol, thymol                                             |
| Origanum         | Thymol, carvacrol                                             |
| Rosemary         | Verbenone, 1,8-cineole, camphor, linalool                     |
| Sage, clary      | Salvial-4 (14)-en-1-one, linalool                             |
| Sage, Dalmatian  | Thujone, 1,8-cineole, camphor                                 |
| Sage, Spanish    | <i>e</i> - and <i>t</i> -Sabinylacetate, 1,8-cineole, camphor |
| Savory           | Carvacrol                                                     |
| Tarragon         | Methyl chavicol, anethole                                     |
| Thyme            | Thymol, carvacrol                                             |
| Peppermint       | 1-Menthol, menthone, menthufuran                              |
| Spear mint       | 1-Carvone, carvone derivatives                                |

antioxidative properties. The plants of the lamiaceae family are universally considered as important sources of natural antioxidants. Rosemary is widely used as antioxidant in Europe and the USA. Oregano, thyme, marjoram, sage, basil, fenugreek, fennel, coriander and pimento also possess antioxidant properties better than those of the synthetic antioxidant BHT. Important natural antioxidants and components responsible for the property are presented in Table 1.8.

#### 1.4.4 Herbs and spices as a source of natural antimicrobials

Herbs and spices are important sources of antimicrobials, and the use of spices, their essential oils or active ingredients for controlling microbial growth in food materials constitutes an alternate approach to chemical additives. Some of the spice essential oils (either used individually or in combinations) are highly inhibitory to selected pathogenic and spoilage microorganisms. The fractionation of essential oils helps to

**Table 1.8** Antioxidants isolated from herbs and spices

| Spice         | Antioxidants                                                       |
|---------------|--------------------------------------------------------------------|
| Black pepper  | Phenolic amides, flavonoids                                        |
| Ginger        | Gingerol                                                           |
| Tumeric       | Curcumin                                                           |
| Red pepper    | Capsaicin                                                          |
| Chilli pepper | Capsaicin, capsaicinol                                             |
| Clove         | Eugenol                                                            |
| Rosemary      | Carnosic acid, carnosol, rosmarinic acid, rosmanol                 |
| Sage          | Carnosol, carnosic acid, rosmanol, rosmarinic acid                 |
| Oregano       | Derivatives of phenolic acid, flavonoids, tocopherols              |
| Thyme         | Carvacrol thymol, <i>p</i> -cymene, caryophyllene, carvone borneol |
| Summer savory | Rosmarinic acid, carnosol, carvacrol, thymol                       |
| Marjoram      | Flavanoides                                                        |
| Allspice      | Pimentol                                                           |

improve the level of activity in some cases. The optical isomers of carvone from *Mentha spicata* and *Anethum sowa* (Indian dill) were found to be highly active against a wide spectrum of human pathogenic fungi and bacteria than the essential oils, for example. Mixing compounds like carvacrol and thymol at different proportions may totally inhibit the growth of *Psuedomonas aeruginosa* and *Staphylococcus aureus*. The inhibition is due to loss of membrane integrity which further affects pH homeostasis and equilibrium of inorganic ions. Knowledge of the mode of action of natural antimicrobials helps to inform the application of spice extracts/ingredients in foods. Also, application of active ingredients instead of essential oil will not cause much flavour change to the foodstuff. Of the various herbal spices, oregano and thyme show the highest antimicrobial activity. Carvacrol present in essential oils of oregano and thyme has been proven to be the most important fungitoxic compound. The activity of herbs and spices against fungi and bacteria and the mode of application is given in Table 1.9.

## 1.5 Herbs and spices in the cosmetics and perfumery industries

The importance of spices in cosmetics, perfumery and personal care is well known from ancient times. The cosmetics and perfumery industries employ the oils of many spices for blending with other volatile and fixed oils to make high-quality perfumes. The toiletries and allied industries also make use of spices and their fragrant oils for manufacture of soaps, toothpastes, talcum powder, aftershave lotions, freshness sachets, toilet waters, powders and hair oils. The uses of spices in the cosmetic industry are outlined in Table 1.10.

## 1.6 Modern research into the medicinal and nutraceutical properties of herbs and spices

In the nutraceutical and health food industry, the antidiabetic, antihypercholesterolemic, anticarcinogenic and anti-inflammatory effects of spices are of paramount

**Table 1.9** Antimicrobial properties of herbs and spices

| Spice                                 | Mode of application        | Activity against bacteria                                                                                                                                                                                                                                      | Activity against fungus                                                                                          |
|---------------------------------------|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| Basil                                 | Essential oil              |                                                                                                                                                                                                                                                                | <i>Ascophaera apis</i>                                                                                           |
| Basil                                 | Methyl chavicol            | <i>Aeromonas hydrophylla</i> ,<br><i>Pseudomonas fluorescens</i>                                                                                                                                                                                               |                                                                                                                  |
| Coriander                             | Essential oil              |                                                                                                                                                                                                                                                                | <i>A. apis</i>                                                                                                   |
| Fenugreek                             | Seed saponins              |                                                                                                                                                                                                                                                                | <i>Fusarium oxysporum</i><br>f. sp. <i>lycopersici</i>                                                           |
| Fenugreek                             | Essential oil              | <i>Bordetella bronchiseptica</i> ,<br><i>Bacillus cereus</i> ,<br><i>B. pumilus</i> , <i>B. subtilis</i> ,<br><i>Micrococcus flavus</i> ,<br><i>Staphylococcus aureus</i> ,<br><i>Sarcinia lutea</i> ,<br><i>Escherichia coli</i> ,<br><i>Proteus vulgaris</i> |                                                                                                                  |
| Cumin                                 | Essential oil              |                                                                                                                                                                                                                                                                | <i>Penicillium notatum</i> ,<br><i>Aspergillus niger</i> ,<br><i>A. fumigatus</i> ,<br><i>Microsporium canis</i> |
| Fennel                                | Essential oil              | <i>S. aureus</i> , <i>B. subtilis</i>                                                                                                                                                                                                                          |                                                                                                                  |
| Ajowan                                | Seed extracts              |                                                                                                                                                                                                                                                                | <i>Pythium aphanidematum</i> ,<br><i>Macrophomina phaseolina</i> ,<br><i>Rhizactonia solani</i>                  |
| Allspice                              | Plant extract              |                                                                                                                                                                                                                                                                | <i>Fusarium</i> spp.,<br><i>Alternania</i> spp.,<br><i>Cladosporium</i> spp.                                     |
| Oregano, coriander, basil             | Essential oil              | <i>Listeria monocytogenes</i> ,<br><i>S. aureus</i> , <i>E. coli</i> ,<br><i>Yersinia enterocolitica</i> ,<br><i>Psuedomonas aeruginosa</i> ,<br><i>Lactobacillus plantarum</i>                                                                                | <i>A. niger</i>                                                                                                  |
| <i>Anethum graveolens</i> , coriander | Seed diffusates            |                                                                                                                                                                                                                                                                | <i>Alternaria alternata</i> ,<br><i>F. solani</i> ,<br><i>Macrophomina phaseolina</i>                            |
| Peppermint, thyme, caraway            | Essential oil              | <i>Agrobacterium tumefaciens</i> ,<br><i>Rhizactonia solanacearum</i> , <i>Erwinia carotovora</i>                                                                                                                                                              |                                                                                                                  |
| Spearmint, basil, parsley             | Essential oil              | <i>S. aureus</i> , <i>E. coli</i>                                                                                                                                                                                                                              | <i>Candida albicans</i> ,<br><i>A. niger</i>                                                                     |
| Oregano, mint                         | Essential oil              |                                                                                                                                                                                                                                                                | <i>A. ochraceus</i>                                                                                              |
| Oregano                               | Essential oil or carvacrol |                                                                                                                                                                                                                                                                | <i>C. albicans</i>                                                                                               |
| Oregano, thyme                        | Essential oil or carvacrol | <i>Streptococcus pneumoniae</i> R36 A, <i>B. cereus</i>                                                                                                                                                                                                        |                                                                                                                  |

**Table 1.10** Spices in cosmetics

| Spice     | Beauty care                         |
|-----------|-------------------------------------|
| Turmeric  | Improves skin glow and complexion   |
| Basil     | Improves skin complexion            |
| Fenugreek | Removes wrinkles on skin            |
| Coriander | Skin tonic                          |
| Cinnamon  | Removes skin blemishes              |
| Saffron   | Improves skin colour and complexion |

importance, as diabetes, cardiovascular diseases, arthritis and cancer are key health problems currently facing mankind. Extensive investigations undertaken at the CFTRI among many other research centres, have revealed the multiple health beneficial effects of spices. Safety evaluation studies conducted in animal models also indicate that spices could be consumed at higher dietary levels without any adverse effects on growth, organ weight, food efficiency ratio and blood constituents. Spices or their active principles could thus be used as possible ameliorative or preventive agents for various health disorders. Spices do not contribute significantly to the nutritional makeup of our food *per se* because of the small quantities added to food stuffs. However, due to promising health beneficial physiological effects, spices have immense potential in the nutraceutical industry. Spices like turmeric, ginger, fenugreek, garlic and red pepper are important in the nutraceutical industry due to their promising biological effects.

Turmeric is reported to have antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic and hypocholesterolemic properties. The anti-inflammatory, anticarcinogenic and antioxidant activities are clinically exploited to control rheumatism, cancer and oxidative stress related pathogenesis. Curcumin, derivatives of curcumin, aqueous and organic solvent extracts of turmeric, turmeric powder, essential oil and Ar-turmerone were found to be biologically active. Of the various forms/compounds, the colouring pigment curcumin is responsible for most of the medicinal properties. Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at very high doses without any toxic effects.

Ginger played an important role in primary healthcare in ancient India, China and Japan. In traditional medicine, ginger finds a wide range of applications. Because of its carminative, stimulant and digestive properties, ginger is commonly used in fever, cough, vomiting, cardiac complaints, constipation, flatulence, colic, swelling, diarrhoea, cholera, diabetes and neurological disorders. Ginger powder, aqueous and ethanol extracts of ginger, oleoresin and active principles of ginger like gingerol, shogaol, paradol, zingiberine, zingerone and zingerol have been found to be biologically active. The efficacy of ginger extracts or active principles of ginger like gingerol and shogaol in lowering serum cholesterol level in relation to atherosclerosis and coronary heart diseases have been investigated by several workers. Dietary intake of ginger reduced the risk of atherosclerosis by virtue of its hypolipidemic and anti-atherogenic effects. Ginger is used as an anti-inflammatory drug in the treatment of arthritis. Patients receiving 3–7 g of powdered ginger daily for 56 days had significant reduction in pain and swelling associated with either rheumatoid or osteoarthritis.

Fenugreek seeds are traditionally considered to a carminative and a galactagogue and are used to treat dysentery, diarrhoea, dyspepsia, cough and enlargement of liver and spleen, ricket and gout. Fenugreek seed, sprouted seed, seed powder, sprouted seed powder, decoction of seed, methanol extract of seed, diosgenin, fibre and 4-hydroxy isoleucine, an amino acid extracted from seeds, have been found to be biologically active. The hypoglycaemic activity of fenugreek seed has been well documented by several workers. Fenugreek reduces fasting and post-prandial blood glucose levels in diabetic patients. Supplementation of the diet with fenugreek seeds has been found to reduce total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides. Oxidative stress plays a key role in diabetes and fenugreek seed extract also exhibits antioxidant properties.

Garlic, the bulbous spice, is known for its spicy flavour and medicinal properties. It acts as a stimulant, carminative, emmenagogue, antirheumatic, antihelminthic and vermifuge. Garlic lowers cholesterol in the blood and is recommended for heart diseases and arteriosclerosis. Daily use of garlic in the Mediteranean diet is thought to lower incidence of heart disease in these areas. The active therapeutic compounds present in garlic are S-containing compounds like allicin, iso-allicin, diallyl/disulphide, S-allylcysteine and ajoene. Garlic, garlic powder, garlic oil, aqueous, garlic extract, alliin, allicin, diallyldisulphide, S-allyl cysteine, isoallicin and ajoene have been found to be biologically active.

Aged garlic extract (AGE) has widespread use against cardiovascular diseases. Extracts of fresh garlic that are aged over a prolonged period will have unique water-soluble organosulphur compounds, lipid-soluble organosulphur components and flavanoids. AGE exerts antioxidant action by scavenging reactive oxygen species (ROS) enhancing the activity of cellular antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase and increasing glutathione in the cells. AGE has cholesterol lowering and blood pressure reducing effects. AGE also inhibits platelet aggregation adhesion to collagen but only at higher intake levels. The reputation of garlic as an effective remedy for tumors extend back to the Egyptian Codex Ebers of 1550 BC. Several garlic compounds, including allicin and its corresponding sulphide, inhibit proliferation and induce apoptosis in several human non-leukaemia malignant cells including breast, bladder, colorectal, hepatic, prostate cancer, lymphoma and skin-tumour cell lines.

Capsaicin, the pungent principle of chillies, is a potent anti-inflammatory and analgesic agent. Chillies are used for the treatment of headaches, toothaches and muscular sprains. Capsaicin has cholesterol lowering effects and is used as an anti-obesity agent. It is effective against gaseous irritant induced pulmonary damages. It is digestive and has potent antimicrobial properties. Capsaicin treatment significantly reduces tissue damage, induces certain cells to undergo apoptosis and has putative role in cancer chemoprevention. Capsaicin can induce body heat and enhance blood flow and increase energy expenditure and prevent oxidative stress. Capsaicin is a potent antioxidant and can lower LDL even when consumed for a short period.

The phytochemicals present in spice crops hold promise for preventing or ameliorating various health disorders. India, the land of spices, could exploit the fast-growing nutraceutical sector with her high intrinsic quality spices. Proven therapeutic uses of spices in traditional systems of medicine and safety of spices for consumption without side-effects are the basic strengths in this field. Numerous *in*



*vitro* evaluation studies, *in vivo* studies in animal models and clinical validation studies conducted on the health beneficial effects of spices are the stepping stones on the way to exploiting spices in the nutraceutical and health food industry.

On the other hand, most of the evaluation studies conducted on the health beneficial effects of spices are short-term and clinical studies are lacking in the majority of spices, with the exception of fenugreek. The mode of action nutraceuticals, bioavailability of nutraceuticals and interaction of nutraceuticals with drugs need thorough investigations. The quality of raw materials for the nutraceutical industry should be ensured and quality and quantity of bioactive compounds in the raw material should be ascertained. The importance of good agricultural practices (GAP) and good manufacturing practices (GMP) could be emphasized in this context. 'Clean spices production, not cleaned spices production' is the slogan. Quality clean spices could thus make a major breakthrough in the nutraceutical and health food industry.

### **1.6.1 Bioprospection**

Bioprospection of herbs and spices could isolate new and novel therapeutic molecules. This area of research has high impetus around the world. A classical example of such a study is the Piperine alkaloid isolated from black pepper and marketed as Bioperine (98 % pure piperine). This alkaloid could increase bioavailability of certain drugs and nutrients like  $\beta$ -carotene. Bioinformatics plays an essential role in the *in silico* analysis of active compounds from herbs and spices, screening of new drugs and studies on their biological activities. The bioinformatic approaches consequently provide a new insight for treatment of various diseases using traditional drugs from spices and herbs. Further, creation of a database on the topic through bioinformatic tools will help to strengthen the research and development activities in this field.

## **1.7 Production of quality herbs and spices**

Production of quality clean spices without any pesticide/chemical residues is important in this era of free international trade resulting from globalization. Organic spices, which fetch 20–50 % higher prices than spices from conventional farms, are devoid of pesticides and chemical residues and are superior in quality. The adoption of good agricultural practices helps to reduce the above contaminants. Quality assurance systems such as hazard analysis critical control point (HACCP) are highly relevant in the production of quality spices. Decontamination techniques and proper packaging and storage techniques also play a major role in maintaining the quality of spices.

### **1.7.1 The use of pesticides and chemicals in herb and spice production**

The world over, people are becoming more and more concerned about the health problems that may arise due to consumption of foods contaminated with pesticide residues. Promotion of a farming technique adopting ecologically sound plant protection measures, organic recycling and biowaste management would go a long way

in restoring soil health and reducing pesticide residues in farm produces. The role played by various beneficial microorganisms, including mycorrhizae, biocontrol agents and plant growth promoting rhizobacteria, is enormous in enhancing crop growth and disease control without leaving any chemical residues on plants. Effective bioagents for the control of major diseases of spice crops are listed in Table 1.11. Guidelines for production of organic spices are being developed for various producing countries. The Spices Board of India (2001) published guidelines for production of organic spices in India. The nutrient composition of selected organic cakes and recommended quantity of organic manure for various spice crops are presented in Tables 1.12 and 1.13.

### **1.7.2 Irradiation of herbs and spices**

Radiation processing offers good scope for increasing shelf-life and enhancing quality and microbial safety without changing the natural flavour attributes of spices. This technique is widely practised in North America and Europe to decontaminate imported spices. The various producing countries also started installing facilities for radiation processing of spices. Irradiation, along with good agricultural and manufacturing practices, helps to produce clean, high-quality spices free from pesticide and chemical residues. Being a non-thermal process, it does not affect the delicate aroma and flavour compounds in spices. The risk of post-treatment contamination can be eliminated by subjecting prepacked spices to irradiation. Tables 1.14 and 1.15 give the list of countries which have approved irradiation processing of food products and spices items permitted for irradiation under Indian Prevention of Food Adulteration Act (PFA) rules.

A low doses of irradiation (<1 K.Gy) helps to inhibit sprouting in onion, garlic, ginger, etc., medium-dose application (1–10 K.Gy) to eliminate spoilage microbes and food pathogens and high-dose application (>10 K.Gy) to sterilize food for special requirements and for shelf-stable foods without refrigeration.

### **1.7.3 Packaging of herbs and spices for quality maintenance**

Spice products are hygroscopic in nature and, since they are highly sensitive to moisture, absorption of moisture may result in caking, discolouration, hydrolytic rancidity, mould growth and insect infestation. As spices contain volatile aromatic principles, loss of these principles and the absorption of foreign odours as a result of inefficient packaging may pose serious problems. In addition, heat and light accelerate deterioration of aroma and flavour components.

Spices containing natural colouring pigments need protection from light (capsicum, cardamom, turmeric and saffron). Spice powders like onion and garlic contain highly volatile sulphur compounds and need rigorous protection from loss/absorption of flavour. The essential oil components naturally present in most of the spices are subject to oxidation by atmospheric oxygen, particularly at high storage temperature, resulting in the development of off-flavours. Packing of spice oils and oleoresins is done in epoxylined steel drums and high-density polythene containers. For certain oils and oleoresins, aluminium and stainless steel containers are used. Polyethylene terephthalate (PET) bottles which possess very good odour barrier

**Table 1.11** Effective bio-agents for the control of major diseases in spice crops

| Crops                 | Major diseases                                                                   | Causal organisms                                                                                     | Biocontrol agents                                                                                                            |
|-----------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| Cardamom (small)      | Azhukal                                                                          | <i>Phytophthora meadii</i> and <i>P. nicotianae</i> var. <i>nicotianae</i>                           | A consortium of <i>Trichoderma viride</i> , <i>T. harzianum</i> , <i>Laetisaria arvalis</i> and <i>Gliocladium virens</i>    |
|                       | Rhizome rot                                                                      | <i>Rhizoctonia solani</i> , <i>Pythium vexans</i> and <i>Fusarium oxysporum</i>                      | A consortium of arbuscular mycorrhizal fungi (AMF) and <i>Trichoderma</i> sp.                                                |
|                       | Seed rot (seedling rot, root rot)                                                | <i>R. solani</i> , <i>P. vexans</i> and <i>F. oxysporum</i>                                          | A consortium of <i>Pseudomonas fluorescens</i> and <i>Bacillus subtilis</i>                                                  |
| Black pepper          | Foot rot (quick wilt)                                                            | <i>Phytophthora capsici</i>                                                                          | A consortium of AMF, <i>T. viride</i> , <i>T. harzianum</i> , <i>Gliocladium virens</i> and <i>Paecilomyces lilacinus</i>    |
|                       | Slow decline (slow wilt)                                                         | <i>Rodopholus similes</i> and <i>Meloidogyne incognita</i>                                           | A consortium of <i>T. harzianum</i> , AMF, <i>Verticillium</i> sp, <i>Chlamydosporium</i> sp. and <i>Pasteuria penetrans</i> |
| Vanilla               | Root rot                                                                         | <i>F. oxysporum</i> and <i>Sclerotium rolfsii</i>                                                    | A consortium of <i>T. viride</i> and <i>T. harzianum</i>                                                                     |
|                       | Stem rot (stem blight, beans rot, beans yellowing and rotting and shoot tip rot) | <i>P. meadii</i> , <i>F. oxysporum</i> , <i>S. rolfsii</i> and <i>Colletotrichum gloeosporioides</i> | A consortium of <i>B. subtilis</i> , <i>P. fluorescens</i> , <i>T. viride</i> and <i>T. harzianum</i>                        |
| Ginger                | Soft rot (rhizome rot)                                                           | <i>Pythium aphanidermatum</i>                                                                        | A consortium of <i>T. viride</i> and <i>T. harzianum</i>                                                                     |
|                       | Ginger yellows                                                                   | <i>Pythium myriotylum</i> and <i>Fusarium</i> sp.                                                    | <i>Trichoderma</i> sp.                                                                                                       |
| Turmeric              | Rhizome rot                                                                      | <i>Rhizoctonia solani</i>                                                                            | <i>Trichoderma</i> sp.                                                                                                       |
|                       | Storage rot                                                                      | <i>S. rolfsii</i>                                                                                    | <i>Trichoderma</i> sp                                                                                                        |
| Chillies and paprikas | Damping off in seedlings                                                         | <i>Pythium</i> sp. and <i>Phytophthora</i> sp.                                                       | A consortium of <i>T. viride</i> and <i>T. harzianum</i>                                                                     |
|                       | Anthraxnose (fruit rot)                                                          | <i>Colletotrichum lindemuthianum</i>                                                                 | A consortium of <i>P. fluorescens</i> , <i>B. subtilis</i> and <i>Trichoderma</i> sp.                                        |
| Thyme                 | Wilt disease                                                                     | <i>F. oxysporum</i>                                                                                  | <i>T. viride</i>                                                                                                             |
|                       | Leaf rot                                                                         | <i>F. oxysporum</i>                                                                                  | <i>T. harzianum</i>                                                                                                          |
| Rosemary              | Thread blight                                                                    | <i>R. solani</i>                                                                                     | <i>T. harzianum</i>                                                                                                          |
| Sage                  | Wilt                                                                             | <i>R. solani</i>                                                                                     | <i>T. harzianum</i>                                                                                                          |
| Mint                  | Wilt                                                                             | <i>F. oxysporum</i>                                                                                  | <i>T. harzianum</i>                                                                                                          |
| Horse radish          | Leaf blight                                                                      | <i>Colletotrichum</i> sp.                                                                            | <i>T. harzianum</i>                                                                                                          |
|                       | Root rot and wilt                                                                | <i>Verticillium</i> sp.                                                                              | <i>T. harzianum</i>                                                                                                          |
| Burmese-coriander     | Wilt                                                                             | <i>Fusarium</i> sp.                                                                                  | <i>T. harzianum</i>                                                                                                          |
| Marjoram              | Leaf blight and leaf spot                                                        | <i>Colletotrichum</i> sp.                                                                            | <i>T. harzianum</i>                                                                                                          |
| Oregano               | Leaf spot                                                                        | <i>Phoma</i> sp. and <i>Curvularia lunata</i>                                                        | <i>T. harzianum</i>                                                                                                          |

**Table 1.12** Nutrient composition of selected organic cakes

| Oil cakes                      | Nutrient contents (%) |            |        |
|--------------------------------|-----------------------|------------|--------|
|                                | Nitrogen              | Phosphorus | Potash |
| <b>Edible cakes</b>            |                       |            |        |
| Coconut cake                   | 3.0                   | 1.9        | 1.8    |
| Niger cake                     | 4.7                   | 1.8        | 1.2    |
| Sesamum cake                   | 6.2                   | 2.0        | 1.2    |
| Sunflower cake                 | 7.9                   | 2.2        | 1.9    |
| Groundnut cake                 | 7.3                   | 1.5        | 1.3    |
| <b>Non-edible cake</b>         |                       |            |        |
| Cotton seed cake (with shells) | 6.4                   | 2.9        | 2.2    |
| Mahua cake                     | 2.51                  | 0.80       | 1.85   |
| Neem cake                      | 5.22                  | 1.08       | 1.48   |

**Table 1.13** Recommended quantity of organic manure for various spice crops

| Spice crops    | Organic manure                         | Quantity                         |
|----------------|----------------------------------------|----------------------------------|
| Black pepper   | Farmyard manure                        | 4–10 kg/plant                    |
| Small cardamom | Neem cake/vermicompost/poultry manure  | 4–5 kg/plant                     |
| Large cardamom | Cattle manures/organic cakes           | 2 kg/plant                       |
| Vanilla        | Farmyard manure/vermicompost           | 4–5 kg/plant                     |
| Chilli         | Farmyard manure/sheep manure/neem cake | 4–5 t/ha<br>3–5 q/ha<br>3–4 q/ha |
| Ginger         | Farmyard manure/neem cake              | 5–6 t/ha<br>2 t/ha               |
| Turmeric       | Farmyard manure/neem cake              | 5–6 t/ha<br>2 t/ha               |
| Fennel         | Farmyard manure                        | 10–12 t/ha                       |
| Coriander      | Farmyard manure                        | 4 t/ha                           |
| Cumin          | Farmyard manure                        | 4–5 t/ha                         |
| Fenugreek      | Farmyard manure                        | 4–5 t/ha                         |
| Celery         | Farmyard manure                        | 10–12 t/ha                       |
| Clove          | Farmyard manure                        | 15–40 kg/plant                   |
| Nutmeg         | Farmyard manure                        | 15–40 kg/plant                   |

Source: Spices Board of India (2001).

properties and food grade high molecular weight high-density polyethylene (HMHDPE) containers are also used for storing essential oils and oleoresins. Most of the whole spices are protected by pericarp, and the natural antioxidants present therein need less rigorous protection than ground spices. The packaging materials suitable for different spice products are listed in Table 1.16.

## 1.8 The structure of this book

This book contains introductory chapters on quality specifications for herbs and spices and quality indices for spice essential oils. The following individual spices and

**Table 1.14** Countries which have approved radiation processing of food products

| Sl. No. | Country        | Sl. No. | Country           | Sl. No. | Country            |
|---------|----------------|---------|-------------------|---------|--------------------|
| 1       | Argentina      | 19      | Ghana             | 37      | Philippines        |
| 2       | Australia      | 20      | Greece            | 38      | Poland             |
| 3       | Austria        | 21      | Hungary           | 39      | Portugal           |
| 4       | Bangladesh     | 22      | India             | 40      | Russian Federation |
| 5       | Belgium        | 23      | Indonesia         | 41      | South Africa       |
| 6       | Brazil         | 24      | Iran              | 42      | Spain              |
| 7       | Canada         | 25      | Ireland           | 43      | Sweden             |
| 8       | Chile          | 26      | Israel            | 44      | Syria              |
| 9       | China          | 27      | Italy             | 45      | Thailand           |
| 10      | Costa Rica     | 28      | Japan             | 46      | Turkey             |
| 11      | Croatia        | 29      | Republic of Korea | 47      | Ukraine            |
| 12      | Cuba           | 30      | Libya             | 48      | UK                 |
| 13      | Czech Republic | 31      | Luxemburg         | 49      | Uruguay            |
| 14      | Denmark        | 32      | Mexico            | 50      | USA                |
| 15      | Egypt          | 33      | Netherlands       | 51      | Vietnam            |
| 16      | Finland        | 34      | New Zealand       | 52      | Yugoslavia         |
| 17      | France         | 35      | Norway            |         |                    |
| 18      | Germany        | 36      | Pakistan          |         |                    |

**Table 1.15** Spice items permitted for irradiation under Indian Prevention of Food Adulteration Act (PFA) rules

| Name of spice          | Dose of irradiation |         | Purpose                   |
|------------------------|---------------------|---------|---------------------------|
|                        | Minimum             | Maximum |                           |
| Onion                  | 0.03                | 0.09    | Sprout inhibition         |
| Shallots (small onion) | 0.03                | 0.15    | Sprout inhibition         |
| Garlic                 | 0.03                | 0.15    | Sprout inhibition         |
| Ginger                 | 0.03                | 0.15    | Sprout inhibition         |
| Spices                 | 6.0                 | 14.0    | Microbial decontamination |

Source: Sharma *et al* (2003).

herbs are then covered: basil, bay leaves, black pepper, capsicum, cardamom, chives, cinnamon, cloves, coriander, cumin, curry leaf, dill, fenugreek seed, garlic, ginger, marjoram, mint, mustard, nutmeg and mace, onion, parsley, rosemary, saffron, thyme, turmeric and vanilla. Contributors were asked to cover issues relating to:

- **Definition and classification:** such issues can be very significant in establishing appropriate standards of quality and authenticity.
- **Chemical structure:** essential in assessing such issues as quality, potential applications and processing functionality.
- **Production:** a description of the principal methods of cultivation and post-harvest processing and how they impact on quality and functionality.
- **Uses in food processing:** a review of current and potential applications.
- **Functional properties:** As has already been noted, there is increasing interest in herbs and spices as functional ingredients, for example as natural antioxidants. Where appropriate, contributors summarize the current state of research on the

**Table 1.16** Packaging in spices

| Spice              | Product         | Type of packaging | Packing material                                                                                                                 |
|--------------------|-----------------|-------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Black pepper       | Whole pepper    | Bulk              | Gunny bags (Burlap bags).<br>Polyethylene lined double burlap bags.                                                              |
|                    | Whole pepper    | Retail            | HDPE pouches 200 gauge.                                                                                                          |
|                    | Ground pepper   | Retail            | Laminated heat-stable aluminium foil (polyethylene coated).<br>Moisture-proof cellulose film.<br>Double lined polyethylene bags. |
| Cardamom           | Green cardamom  | Bulk              | Wooden boxes or tins lined with heavy gauge black polyethylene, metal foil or waterproof paper.                                  |
|                    | Cardamom seed   | Retail            | Air-tight tin.<br>Wooden chests lined with aluminium foil laminate.                                                              |
|                    | Cardamom powder | Retail            | Lacquered cans.<br>PVDC & HDPE pouches.                                                                                          |
| Ginger<br>Turmeric | Dry ginger      | Bulk              | Single/double gunny bags.                                                                                                        |
|                    | Dry turmeric    | Bulk              | Double gunny bags.                                                                                                               |
|                    | Turmeric powder | Retail            | Aluminium foil laminate.                                                                                                         |
|                    | Turmeric powder | Bulk              | Fibre board drums, multiwall bags and tin containers.                                                                            |
| Chilli             | Dry chilli      | Bulk              | Wooden crate dunnage with a layer of matting.                                                                                    |
|                    | Chilli powder   | Retail            | Plastic laminate and aluminium combination pouches with nitrogen gas. 3000 gauge low-density polyethylene pouches.               |

Source: Pruthi (1993).

nutritional and functional benefits of individual spices and herbs. Issues of toxicity and allergy are also addressed where necessary.

- **Quality and regulatory issues:** a summary of the key quality standards and indices relating to the herbs and spices.

Individual chapters vary in structure and emphasis, depending on the nature of spice in question and the particular issues and body of research surrounding it. It is hoped that the revised and updated book will help manufacturers and others to make even fuller use of the valuable resource that herbs and spices provide.

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**Appendix 1** ISO list of plant species

| No. | Botanical name of the plant                  | Family                  | Common name                                                             | Name of plant part used as spice |
|-----|----------------------------------------------|-------------------------|-------------------------------------------------------------------------|----------------------------------|
| 1.  | <i>Acorus calamus</i>                        | Araceae                 | Sweet flag, myrtle flag, calamus, flag root                             | Rhizome                          |
| 2.  | <i>Aframomum angustifolium</i>               | Zingiberaceae           | Madagascar cardamom                                                     | Fruit, seed                      |
| 3.  | <i>Aframomum hanburyi</i>                    | Zingiberaceae           | Cameroon cardamom                                                       | Fruit, seed                      |
| 4.  | <i>Aframomum koranima</i>                    | Zingiberaceae           | Korarima cardamom                                                       | Fruit, seed                      |
| 5.  | <i>Aframomum melegueta</i>                   | Zingiberaceae           | Grain of paradise, Guinea grains                                        | Fruit, seed                      |
| 6.  | <i>Allium ascalonicum</i>                    | Liliaceae               | Shallot                                                                 | Bulb                             |
| 7.  | <i>Allium cepa</i>                           | Liliaceae               | Onion                                                                   | Bulb                             |
| 8.  | <i>Allium cepa</i> var. <i>aggregatum</i>    | Liliaceae               | Potato onion                                                            | Bulb                             |
| 9.  | <i>Allium tuberosum</i>                      | Liliaceae               | Indian leek, Chinese chive                                              | Bulb, leaf                       |
| 10. | <i>Allium fistulosum</i>                     | Liliaceae               | Stony leek, Welsh onion, Japanese bunching onion                        | Leaf and bulb                    |
| 11. | <i>Allium porrum</i>                         | Liliaceae               | Leek, winter leek                                                       | Leaf and bulb                    |
| 12. | <i>Allium sativum</i>                        | Liliaceae               | Garlic                                                                  | Bulb                             |
| 13. | <i>Allium schoenoprasum</i>                  | Liliaceae               | Chive                                                                   | Leaf                             |
| 14. | <i>Alpinia galanga</i>                       | Zingiberaceae           | Greater galangal, Longwas, Siamese ginger                               | Rhizome                          |
| 15. | <i>Alpinia officinarum</i>                   | Zingiberaceae           | Lesser galangal                                                         | Rhizome                          |
| 16. | <i>Amomum aromaticum</i>                     | Zingiberaceae           | Bengal cardamom                                                         | Fruit, seed                      |
| 17. | <i>Amomum kepulaga</i>                       | Zingiberaceae           | Round cardamom, Chester cardamom, Siamese cardamom, Indonesian cardamom | Fruit, seed                      |
| 18. | <i>Amomum krervanh</i>                       | Zingiberaceae           | Cambodian cardamom                                                      | Fruit, seed                      |
| 19. | <i>Amomum subulatum</i>                      | Zingiberaceae           | Greater Indian cardamom, large cardamom, Nepalese cardamom              | Fruit, seed                      |
| 20. | <i>Amomum tsao-ko</i>                        | Zingiberaceae           | Tsao-ko cardamom                                                        | Fruit, seed                      |
| 21. | <i>Anethum graveolens</i>                    | Apiaceae (Umbelliferae) | Dill                                                                    | Fruit, leaf, top                 |
| 22. | <i>Anethum sowa</i>                          | Apiaceae (Umbelliferae) | Indian dill                                                             | Fruit                            |
| 23. | <i>Angelica archangelica</i>                 | Apiaceae (Umbelliferae) | Garden angelica                                                         | Fruit, petiole                   |
| 24. | <i>Anthriscus cereifolium</i>                | Apiaceae (Umbelliferae) | Chervil                                                                 | Leaf                             |
| 25. | <i>Apium graveolens</i>                      | Apiaceae (Umbelliferae) | Celery, garden celery                                                   | Fruit, root, leaf                |
| 26. | <i>Apium graveolens</i> var. <i>rapaceum</i> | Apiaceae (Umbelliferae) | Celeriac                                                                | Fruit, root, leaf                |



**Appendix 1** *Continued*

| No.  | Botanical name of the plant  | Family                       | Common name                             | Name of plant part used as spice |
|------|------------------------------|------------------------------|-----------------------------------------|----------------------------------|
| 27.  | <i>Armoracia rusticana</i>   | Brassicaceae<br>(Cruciferae) | Horse radish                            | Root                             |
| 28.  | <i>Artemisia dracunculus</i> | Asteraceae<br>(Compositae)   | Tarragon, estragon                      | Leaf                             |
| 29.  | <i>Averrhoa bilimbi</i>      | Averrhoaceae                 | Belimbing, bilimbi<br>cucumber tree     | Fruit                            |
| 30.  | <i>Averrhoa carambola</i>    | Averrhoaceae                 | Carambola, caramba                      | Fruit                            |
| 31.  | <i>Brassica juncea</i>       | Brassicaceae                 | Indian mustard                          | Seed                             |
| 32.  | <i>Brassica nigra</i>        | Brassicaceae                 | Black mustard                           | Seed                             |
| 33.  | <i>Bunium persicum</i>       | Apiaceae<br>(Umbelliferae)   | Black caraway                           | Seed, tuber                      |
| 34.  | <i>Capparis spinosa</i>      | Capparidaceae                | Caper, common caper,<br>caper bush      | Floral bud                       |
| 35.  | <i>Capsicum annuum</i>       | Solanaceae                   | Capsicum, chillies,<br>paprika          | Fruit                            |
| 36.  | <i>Capsicum frutescens</i>   | Solanaceae                   | Chillies, bird's eye<br>chilli          | Fruit                            |
| 37.  | <i>Carum bulbocastanum</i>   | Apiaceae<br>(Umbelliferae)   | Black caraway                           | Fruit, bulb                      |
| 38.  | <i>Carum carvi</i>           | Apiaceae<br>(Umbelliferae)   | Caraway, blond<br>caraway               | Fruit                            |
| 39.  | <i>Cinnamomum aromaticum</i> | Lauraceae                    | Cassia, Chinese cassia                  | Bark, leaves                     |
| 40.  | <i>Cinnamomum burmanii</i>   | Lauraceae                    | Indonesian cassia                       | Bark                             |
| 41.  | <i>Cinnamomum loureirii</i>  | Lauraceae                    | Vietnamese cassia                       | Bark                             |
| 42.  | <i>Cinnamomum tamala</i>     | Lauraceae                    | Tejpat, Indian cassia                   | Leaf, bark                       |
| 43.  | <i>Cinnamomum zeylanicum</i> | Lauraceae                    | Sri Lankan cinnamon,<br>Indian cinnamon | Bark, leaf                       |
| 44.  | <i>Coriandrum sativum</i>    | Apiaceae<br>(Umbelliferae)   | Coriander                               | Leaf, fruit                      |
| 45.  | <i>Crocus sativus</i>        | Iridaceae                    | Saffron                                 | Stigma                           |
| 46.  | <i>Cuminum cyminum</i>       | Apiaceae<br>(Umbelliferae)   | Cumin                                   | Fruit                            |
| 47.  | <i>Curcuma longa</i>         | Zingiberaceae                | Turmeric                                | Rhizome, leaf                    |
| 48.  | <i>Cymbopogon citratus</i>   | Poaceae                      | West Indian<br>lemongrass               | Leaf                             |
| 49.  | <i>Cymbopogon nardus</i>     | Poaceae                      | Sri Lankan citronella                   | Leaf                             |
| 50.  | <i>Elettaria cardamomum</i>  | Zingiberaceae                | Small cardamom                          | Fruit, seed                      |
| 51.  | <i>Elettaria cardamomum</i>  | Zingiberaceae                | Sri Lankan cardamom                     | Fruit, seed                      |
| 52.1 | <i>Ferula assa-foetida</i>   | Apiaceae<br>(Umbelliferae)   | Asafoetida                              | Rhizome                          |
| 52.2 | <i>Ferula foetida</i>        |                              |                                         |                                  |
| 52.3 | <i>Ferula narthex</i>        |                              |                                         |                                  |
| 53.  | <i>Foeniculum vulgare</i>    | Apiaceae                     | Bitter fennel                           | Leaf, twig, fruit                |
| 54.  | <i>Foeniculum vulgare</i>    | Apiaceae                     | Sweet fennel                            | Leaf, twig, fruit                |

*Continued*

**Appendix 1** *Continued*

| No.  | Botanical name of the plant    | Family        | Common name                                                  | Name of plant part used as spice |
|------|--------------------------------|---------------|--------------------------------------------------------------|----------------------------------|
| 55.  | <i>Garcinia cambogia</i>       | Clusiaceae    | Garcinia, Camboge                                            | Pericarp of the fruit            |
| 56.  | <i>Garcinia indica</i>         | Clusiaceae    | Garcinia, Kokum                                              | Pericarp of the fruit            |
| 57.  | <i>Hyssopus officinalis</i>    | Lamiaceae     | Hyssop                                                       | Leaf                             |
| 58.  | <i>Illicium verum</i>          | Illiciaceae   | Star anise, Chinese anise                                    | Fruit                            |
| 59.  | <i>Juniperus communis</i>      | Cupressaceae  | Common juniper                                               | Fruit                            |
| 60.  | <i>Kaempferia galanga</i>      | Zingiberaceae | Galangal                                                     | Rhizome                          |
| 61.  | <i>Laurus nobilis</i>          | Lauraceae     | Laurel, true laurel, bay leaf, sweet flag                    | Leaf                             |
| 62.  | <i>Levisticum officinale</i>   | Apiaceae      | Garden lovage, lovage                                        | Fruit, leaf                      |
| 63.1 | <i>Lippia graveolens</i>       | Verbenaceae   | Mexican oregano                                              | Leaf, terminal shoot             |
| 63.2 | <i>Lippia berlandieri</i>      |               |                                                              |                                  |
| 64.  | <i>Mangifera indica</i>        | Anacardiaceae | Mango                                                        | Immature fruit (rind)            |
| 65.  | <i>Melissa officinalis</i>     | Lamiaceae     | Balm, lemon balm, melissa                                    | Leaf, terminal shoot             |
| 66.  | <i>Mentha arvensis</i>         | Lamiaceae     | Japanese mint, field mint, corn mint                         | Leaf, terminal shoot             |
| 67.  | <i>Mentha citrata</i>          | Lamiaceae     | Bergamot                                                     | Leaf, terminal shoot             |
| 68.  | <i>Mentha x piperita</i>       | Lamiaceae     | Peppermint                                                   | Leaf, terminal shoot             |
| 69.  | <i>Mentha spicata</i>          | Lamiaceae     | Spearmint, garden mint                                       | Leaf, terminal shoot             |
| 70.  | <i>Murraya koenigii</i>        | Rutaceae      | Curry leaf                                                   | Leaf                             |
| 71.  | <i>Myristica argentea</i>      | Myristicaceae | Papuan nutmeg                                                | Kernel                           |
|      |                                |               | Papuan mace                                                  | Aril                             |
| 72.  | <i>Myristica fragrans</i>      | Myristicaceae | Indonesian type nutmeg, Indonesian type mace, Siau type mace | Kernel<br>Aril                   |
| 73.  | <i>Nigella damascena</i>       | Ranunculaceae | Damas black cumin, love in a mist                            | Seed                             |
| 74.  | <i>Nigella sativa</i>          | Ranunculaceae | Black cumin                                                  | Seed                             |
| 75.  | <i>Ocimum basilicum</i>        | Lamiaceae     | Sweet basil                                                  | Leaf, terminal shoot             |
| 76.  | <i>Origanum majorana</i>       | Lamiaceae     | Sweet marjoram                                               | Leaf, floral bud                 |
| 77.  | <i>Origanum vulgare</i>        | Lamiaceae     | Oregano, organ                                               | Leaf, flower                     |
| 78.  | <i>Pandanus amaryllifolius</i> | Pandanaceae   | Pandan wangi                                                 | Leaf                             |
| 79.  | <i>Papaver somniferum</i>      | Papaveraceae  | Poppy, blue maw, mawseed                                     | Seed                             |
| 80.  | <i>Petroselinum crispum</i>    | Apiceae       | Parsley                                                      | Leaf, root                       |
| 81.  | <i>Pimenta dioica</i>          | Myrtaceae     | Pimento, allspice, Jamaica pepper                            | Immature fruit, leaf             |
| 82.  | <i>Pimenta racemosa</i>        | Myrtaceae     | West Indian bay                                              | Fruit, leaf                      |
| 83.  | <i>Pimpinella anisum</i>       | Apiaceae      | Aniseed                                                      | Fruit                            |

**Appendix 1** *Continued*

| No.  | Botanical name of the plant                                                        | Family         | Common name                                 | Name of plant part used as spice |
|------|------------------------------------------------------------------------------------|----------------|---------------------------------------------|----------------------------------|
| 84.  | <i>Piper guineense</i>                                                             | Piperaceae     | West African or Benin pepper                | Fruit                            |
| 85.  | <i>Piper longum</i>                                                                | Piperaceae     | Long pepper, Indian long pepper             | Fruit                            |
| 86.  | <i>Piper nigrum</i>                                                                | Piperaceae     | Black pepper, white pepper, green pepper    | Fruit                            |
| 87.  | <i>Punica granatum</i>                                                             | Punicaceae     | Pomegranate                                 | Seed (dried with flesh)          |
| 88.  | <i>Rosmarinus officinalis</i>                                                      | Lamiaceae      | Rosemary                                    | Terminal shoot, leaf             |
| 89.  | <i>Salvia officinalis</i>                                                          | Lamiaceae      | Garden sage                                 | Terminal shoot, leaf             |
| 90.  | <i>Satureja hortensis</i>                                                          | Lamiaceae      | Summer savory                               | Terminal shoot, leaf             |
| 91.  | <i>Satureja montana</i>                                                            | Lamiaceae      | Winter savory                               | Leaf, twig                       |
| 92.  | <i>Schinus molle</i>                                                               | Anacardiaceae  | American pepper, Californian pepper tree    | Fruit, wall (rind)               |
| 93.  | <i>Schinus terebenthifolius</i>                                                    | Anacardiaceae  | 'Brazilian pepper'                          | Fruit                            |
| 94.  | <i>Sesamum indicum</i>                                                             | Pedaliaceae    | Sesame, gingelly                            | Seed                             |
| 95.  | <i>Sinapis alba</i>                                                                | Brassicaceae   | White mustard, yellow mustard               | Seed                             |
| 96.  | <i>Syzygium aromaticum</i>                                                         | Myrtaceae      | Clove                                       | Flower bud                       |
| 97.  | <i>Tamarindus indica</i>                                                           | Cesalpiniaceae | Tamarind                                    | Fruit                            |
| 98.  | <i>Thymus serpyllum</i>                                                            | Lamiaceae      | Mother of thyme, wild thyme, creeping thyme | Terminal shoot, leaf             |
| 99.  | <i>Thymus vulgaris</i>                                                             | Lamiaceae      | Thyme, common thyme                         | Terminal shoot, leaf             |
| 100. | <i>Trachyspermum ammi</i>                                                          | Apiaceae       | Ajowan                                      | Fruit                            |
| 101. | <i>Trigonella</i>                                                                  | Fabaceae       | Fenugreek                                   | Seed, leaf                       |
| 102. | <i>Foenumgracecum</i><br><i>Vanilla planifolia</i><br>syn. <i>Vanilla fragrans</i> | Orchidaceae    | Vanilla                                     | Fruit (pod)                      |
| 103. | <i>Vanilla tahitensis</i>                                                          | Orchidaceae    | Vanilla                                     | Fruit (pod)                      |
| 104. | <i>Vanilla pompona</i>                                                             | Orchidaceae    | Pompona vanilla                             | Fruit (pod)                      |
| 105. | <i>Xylopi aethiopica</i>                                                           | Annonaceae     | Negro pepper, Guinean pepper                | Fruit                            |
| 106. | <i>Zanthoxylum bungei</i>                                                          | Rutaceae       | Chinese prickly ash pepper, Sechuang pepper | Fruit                            |
| 107. | <i>Zanthoxylum acanthopodium</i>                                                   | Rutaceae       | Chinese pepper                              | Fruit                            |
| 108. | <i>Zanthoxylum piperitum</i>                                                       | Rutaceae       | Japanese pepper                             | Fruit                            |
| 109. | <i>Zingiber officinale</i>                                                         | Zingiberaceae  | Ginger                                      | Rhizome                          |

**Appendix 2** Major spice-producing areas

| Spices           | Edible part(s)       | Major source/origin                                                     |
|------------------|----------------------|-------------------------------------------------------------------------|
| Allspice         | Berry, leaf          | Jamaica, Mexico                                                         |
| Aniseed          | Fruit                | Mexico, The Netherlands, Spain                                          |
| Basil, Sweet     | Leaf                 | France, Hungary, USA, Yugoslavia                                        |
| Caraway          | Fruit                | Denmark, Lebanon, The Netherlands, Poland                               |
| Cardamom         | Fruit                | India, Guatemala,                                                       |
| Celery           | Fruit                | France, India                                                           |
| Chervil          | Leaf                 | USA                                                                     |
| Chilli           | Fruit                | Ethiopia, India, Japan, Kenya, Mexico, Nigeria, Pakistan, Tanzania, USA |
| Cinnamon         | Stem bark            | Sri Lanka                                                               |
| Cassia           | Stem bark            | China, Indonesia, South Vietnam                                         |
| Clove            | Buds                 | Indonesia, Malaysia, Tanzania                                           |
| Coriander        | Fruit                | Argentina, India, Morocco, Romania, Spain, Yugoslavia                   |
| Cumin            | Fruit                | India, Iran, Lebanon                                                    |
| Dill             | Fruit                | India                                                                   |
| Fennel           | Fruit                | Argentina, Bulgaria, Germany, Greece, India, Lebanon                    |
| Fenugreek        | Fruit                | India                                                                   |
| Garlic           | Bulb/clove           | Argentina                                                               |
| Ginger           | Rhizome              | India, Jamaica, Nigeria, Sierra Leone                                   |
| Laurel           | Leaf                 | Portugal, Turkey                                                        |
| Marjoram (sweet) | Leaf                 | Chile, France, Lebanon, Mexico, Peru                                    |
| Mint             | Leaf, terminal shoot | Bulgaria, Egypt, France, Germany, Greece, Morocco, Romania, Russia, UK  |
| Mustard          | Seed                 | Canada, Denmark, Ethiopia, UK                                           |
| Nutmeg           | Aril, seed kernel    | Grenada, Indonesia                                                      |
| Onion            | Bulb                 | Argentina, Romania                                                      |
| Oregano          | Leaf                 | Greece, Mexico                                                          |
| Paprika          | Fruit                | Bulgaria, Hungary, Morocco, Portugal, Spain, Yugoslavia                 |
| Parsley          | Leaf                 | Belgium, Canada, France, Germany, Hungary                               |
| Black pepper     | Fruit                | Brazil, India, Indonesia, Malaysia, Sri Lanka                           |
| Poppy            | Seed                 | The Netherlands, Poland, Romania, Turkey, Russia                        |
| Rosemary         | Leaf, terminal shoot | France, Spain, USA, Yugoslavia                                          |
| Saffron          | Pistil of flower     | Spain                                                                   |
| Sage             | Leaf                 | Albania, Yugoslavia                                                     |
| Sesame           | Seed                 | China, El-Salvador, Ethiopia, Guatemala, India, Mexico, Nicaragua       |
| Star anise       | Fruit                | China, North Vietnam                                                    |
| Tarragon         | Leaf                 | France, USA                                                             |
| Thyme            | Leaf                 | France, Spain                                                           |
| Turmeric         | Rhizome              | China, Honduras, India, Indonesia, Jamaica                              |
| Vanilla          | Fruit/beans          | Indonesia, Malagasy Republic, Mexico                                    |

Source: Mahindru, S.N. (1994). *S.N. Mahindru's Manual of Indian Spices*. Academic Foundation, New Delhi, p. 380.

## Quality specifications for herbs and spices

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**Abstract:** This chapter deals with the definition of the main quality parameters relevant to herbs and spices and the associated applicable limits. The terms authenticity and quality are sometimes at odds. Authenticity can be defined as freedom from adulteration, most obviously in the sense of absence of foreign bodies or extraneous matter, but it also suggests freedom from impurities in the product itself. A more appropriate term is quality, which can be defined in the case of herbs and spices as ‘fit (and customary) for the purpose intended’. The main trade bodies defining the required quality parameters and details of recommended quality management schemes are included.

**Key words:** quality specifications, adulteration, authenticity, foreign bodies, extraneous matter.

### 2.1 Introduction: defining quality

Within the herb and spice industry, the terms authenticity and quality are sometimes at odds. Authenticity can be defined as freedom from adulteration, most obviously in the sense of absence of foreign bodies or extraneous matter, but it also suggests freedom from impurities in the product itself. However, in practice, the term authenticity is not always helpful in the case of herbs and spices. As an example, sage in virtually all textbooks is defined as *Salvia officinalis*. But there are some 300 species of sage, and some of the major ones, which are traded throughout the world at present, are not the ‘classic’ *Salvia officinalis*. *Salvia trilobula* is widely traded and this is accepted universally as sage. Oregano is typically a blend of *Origanum vulgare* and *Origanum onites*. Similarly with thyme, references are usually to *Thymus vulgaris*, but most thyme traded is a mixture of *Thymus capitatus*, *Thymus serpyllum* and *Thymus vulgaris*. This blend is universally accepted as thyme. Turning to examples of spices, turmeric is defined as *Curcuma longa*, but there are sub-species such as Alleppy turmeric, which is dark red orange in colour with a rough outer appearance to the root, whereas Cuddapah turmeric is lighter lemon yellow in colour with a smoother root. Each type has its own market niche. The reason for these variations is that most herbs and spices were originally wild rather than cultivated crops, gathered from their natural habitat where mixing of the species and sub-species occurred.

A more appropriate term is quality which can be defined in the case of herbs and spices as 'fit (and customary) for the purpose intended'.

Herbs and spices have traditionally been traded as dried products for reasons of preservation. The industry goes back before the time of Christ (fragmentary written records exist from 2600 BC) when drying was one of the main forms of food preservation. Drying was then by means of the sun, and this method is still widely used. With the advent of modern transport methods and methods of preservation, frozen herbs and fresh herbs and spices have made an appearance as items of trade, but the industry remains dominated by the trade in dried products. The major quality specifications are based mainly on dried herbs and spices.

## 2.2 Major international quality specifications

Herbs and particularly spices have always been highly-priced commodities and vulnerable to adulteration. In consequence, simple standards evolved early. As an example, in 1180 in England in the reign of Henry II, a 'peppers' guild was established in London to set and enforce standards for spices. In 1429, it was incorporated into the Grocers Company which is still in existence. This guild was granted a charter by Henry VI to manage the trade in spices. This organisation was given exclusive power to garble (e.g. cleanse and separate) spices. The term is still in use today, for example in classifying types of pepper such as Tellicherry Garbled Extra Bold Black Pepper (TGEB). Today the two major international standards are those set by the USA and those set by the European Union (EU). Standards relying on the same general parameters also exist in those countries responsible for growing herbs and spices, for example the Indian Spices Board and the Pepper Marketing Board. These standards are influenced by those set by the major importing countries.

There are various types of test which make up the range of international standards:

- **Cleanliness:** This is a measure of the amount of foreign and extraneous matter, for example insect contamination, excreta or foreign bodies. Measurement is by physical determination (using microscopic analysis (x30)) of contamination within aliquots (samples) of the product).
- **Ash level:** This is a measure of the level of impurities in a product, obtained by burning off the organic matter and measuring the residue of ash. This measurement is carried out by incinerating the herb or spice at 550°C to constant weight. Characteristic maximum figures exist for most herbs and spices.
- **Acid insoluble ash (AIA) (or sand content):** This is a classic determination of the cleanliness of the herb or spice. The measure is usually made in conjunction with the ash content by boiling the ash in 2N HCl and incinerating the residue (again at 550°C) to a constant weight. Again, maximum figures exist for most herbs and spices. Prosecutions have in the past been based on high AIA levels within Europe, which are seen as indicating an unacceptably high level of gross contamination.
- **Volatile oil (V/O) determination:** This measure helps to identify whether the herb or spice has been adulterated, perhaps by addition of foreign materials, low-quality materials or spent herb or spices (by product of essential oil or

oleoresin production). The herb or spice is boiled with water under reflux conditions. The oil separates on top of the water and is reported as a proportion of the mass of the product under test. Minimum percentage levels of oil exist for most major herbs and spices.

- **Moisture content:** This measure is important since moisture content determines weight, and weight is used in pricing. With highly priced commodities traded on weight, a 1 % moisture increase in the product as shipped can result in increased weight and increased profits for the original exporter. Maximum moisture contents are set for all herbs and spices, based on the maximum allowable amount of moisture for the product to remain stable. Moisture content is generally determined within the herb and spice industry using the Dean & Stark methodology. This involves refluxing a known weight of the herb or spice in petroleum spirit and measuring the water that condenses at the bottom of the reflux chamber from the known weight of herb or spice. Generally the level is 12 % max.
- **Water activity:** In recent years, moisture content has been related to the water activity ( $A_w$ ) of the herb or spice. A level of 0.6  $A_w$  is generally accepted as a figure below which microbial growth cannot occur. However, this figure does not take into account other natural antimicrobial factors, and several herbs and spices may be stored at significantly higher water activity levels without problem due to the preservative effect of other components, especially of the oils they contain. Examples are cinnamon, oregano and cloves where the oils have very strong antimicrobial properties.
- **Microbiological measures:** There are a range of techniques available for enumerating microorganisms (both spoilage species and pathogens) in herbs and spices. Whilst new technologies for microbiological analysis are continually being developed and improved, the standard methods are slow to change (usually described in ISO standards), and it is these standard methods that tend to be used in cases of dispute and which are quoted on specifications.
- **Pesticide levels:** Pesticide levels are not seen as a major health problem given the (low) average daily intakes of herbs and spices by consumers. EC Regulation 396/2005 which came into force on 21 September 2008 details the maximum residue levels (MRLs) permitted. A list of dehydration factors for fresh herbs and capsicums can be accessed from the ESA website ([www.esa-spices.org](http://www.esa-spices.org)). At the time of writing legislation is in a state of flux in the USA and limits may be introduced. In the meantime, Codex limits for the nearest equivalent commodity may be a useful guide. Pesticide levels are usually assessed either by gas chromatography (GC) or high-performance liquid chromatography (HPLC), depending on the pesticide in question.
- **Mycotoxin levels:** Mycotoxins, particularly aflatoxin and ochratoxin A, have been of increasing concern within the industry. EC Regulation 1881/2006 governing the maximum levels of aflatoxins and EU Regulation 105/2010 governing the maximum levels of ochratoxin A in capsicum species, piper species, nutmeg, mace, ginger and turmeric have been implemented in recent years. Within the USA, the maximum limit for aflatoxins is currently 20 ppb and there are no levels for ochratoxin A. HPLC linked to immunoaffinity columns is the reference methodology employed in these determinations.

- **Bulk density/bulk index:** This is an important measure, particularly in filling retail containers of herbs and spices. The herb or spices must be sifted or ground to give a certain density so that retail units not only appear satisfactorily full but also comply with the declared weight. Densities may be measured packed down, e.g. after tapping the product so that it assumes a minimum density, or untapped (as it falls into the container without compression). This measure is usually reported as grams/litre or ml/100 g.
- **Mesh/particle size:** Many spices and herbs are ground to give easier dispersion in the final food product. This process also aids the dispersion of flavour. Particle size is generally specified and is carried out using standardised sieves. Products are ground to pass through a specified sieve, whilst coarse matter is recycled through the mill until it finally passes through the sieve. Sieves are often characterised in 'micron sizes' and typical specification requirements will be for a 95 % pass on a specified size of sieve. The older method of measuring sieve mesh (hole) sizes was related to the number of holes per inch. However, confusing differences exist between US and UK mesh sizes. The mesh size depends on the diameter of the wire making up the sieves and this differs between nations. Thus a 25 mesh US sieve is equivalent to a 30 mesh BS (UK) sieve and both are equivalent to a 500 micron aperture size. Tables are available giving the relationships between national sieve sizes and micron sizes. There are a number of internationally-approved standards for testing procedures, established by the International Standards Organisation (ISO) as referenced in Appendix I.

### 2.2.1 Adulteration

Adulteration is the commercially motivated deliberate and intentional inclusion in herbs and spices of constituents whose presence is not declared. The blending together of different qualities of the same material in order to reduce the variation in the flavour profile is not considered adulteration. In addition, naturally occurring contaminants such as heavy metals, mycotoxins, pathogens, etc. are not considered adulterants.

Product may be adulterated with:

- ingredients, additives or constituents not approved for use in food;
- ingredients, additives or constituents approved for use in food but not declared;
- material that has had any valuable constituent omitted or removed.

### 2.2.2 Prevention

Responsible companies are well aware of the risk of adulteration and closely monitor the products they handle. They will use risk assessment to determine the most appropriate tools to combat this problem, including:

- evaluation of the supply chain
  - history of supply
  - supplier capability of meeting legal and other requirements
  - adherence to good agricultural practices (GAP) and good manufacturing practices (GMP)



- adherence to hazard analysis critical control point (HACCP) principles
- traceability;
- evaluation of the raw material
  - form (whole or ground)
  - price
  - species;
- evaluation of raw material control through appropriate product testing.

Responsible companies are constantly working with agencies and suppliers around the world to increase the awareness of this issue and eliminate the practice of adulteration of herbs and spices.

## 2.3 Product-specific quality parameters

There are a number of other tests used in the industry, some of which are for specific herbs or spices. Some of the best-known and widely used are:

- **Piperine levels:** The test is specifically for peppers of the *Piper* genus. This involves extraction and measurement of the characterising heat portion of the pepper (the piperine content). After refluxing in alcohol to extract the piperine, absorbency is compared to a standard using a spectrophotometer at 342–345 nm.
- **(ASTA) Colour values:** This is a measurement of the extractable colour of products of the *Capsicum* genus and is used as a quality indicator for paprika. Extraction is in acetone (16 hour ambient extraction), followed by spectrophotometric analysis (against a standard at 460 nm). The methodology was developed by the American Spice Trade Association (ASTA) and results are still often expressed as the ASTA colour value.
- **Capsaicin content:** Capsaicin is the pungent principle that gives heat to species of *Capsicum*. Extraction of capsaicin by refluxing with alcohol is followed by determination by HPLC using acetonitrile/water as the carrier. Results can be related to the Scoville test (see below).
- **Scoville heat units:** The Scoville heat unit is a measure of the capsaicin content of *Capsicum* species. The test involves extraction of the capsaicin in alcohol and tasting of successively stronger dilutions in sugar syrup until the chilli heat (burning sensation) is detected. It gives a compatible result to capsaicin content but obviates a need for sophisticated laboratory equipment. A trained tasting panel is required. (Scoville units divided by 150 000 = percent capsaicin.)
- **Curcumin content:** This is a test specific to the measurement of the extractive colour of turmeric. This is carried out by reflux extraction in acetone followed by measurement using a spectrophotometer at 415–425 nm.
- **Non-permitted colours:** These colours are sometimes added illegally to spices such as chillies, cassia, fennel, paprika, saffron and star anise to enhance their physical appearance and therefore their value. Non-permitted colours are assessed by HPLC with a diode array detector (DAD) or by liquid chromatography, tandem mass spectrometry (LC/MS/MS) dependent on the limit of detection (LOD) required.

## 2.4 World spice organisations

### 2.4.1 The American Spice Trade Association (ASTA)

ASTA was established at the beginning of the twentieth century. Given its long involvement in regulating the quality of herbs and spices entering the USA, ASTA standards are recognised and endorsed by the US Food & Drug Administration (US FDA).

Cleanliness specifications exist for all major herbs and spices, in terms of permitted amounts of extraneous matter or filth, mould (visible), excreta, insects, and insect damaged material. The amount of contamination is measured by microscopic analysis ( $\times 30$ ) of aliquots of the material. The ASTA cleanliness specifications are shown in Table 2.1. For the purposes of these specifications, extraneous matter is defined as everything foreign to the product itself, including, but not restricted to: stones, dirt, wire, string, stems, sticks, excreta, and other animal contamination, non-toxic foreign seeds and other plant material (in some cases) such as foreign leaves.

The level of contaminants must fall below those shown in Table 2.1, except for the column 'Whole insects, dead' which must not exceed the limit shown. Herbs and spices not meeting this standard must be re-cleaned/re-conditioned before distribution and sale within the USA. ASTA also sets a range of other standards and recommended analytical methods (ASTA, 1997, 2004, 2008). These are broadly comparable to those set by the European Spice Association (ESA), which are discussed in the next section. Microbiological standards in particular now play an increasingly important role in determining the quality of herbs and spices. They are becoming a crucial quality parameter due to the increasingly varied uses of herbs and spices in the developed world.

Increased travel has led to a society demanding multicultural foods. This, coupled with ready meals, cook–chill products, etc., has meant that herbs and spices are not 'always cooked' as was assumed in the past.

**Table 2.1** American Spice Trade Association Cleanliness Specifications (effective 28 April, 1999; data courtesy of the ASTA) (SF = see footnote)

| Name of spice, seed or herb | ▲ Whole insects, dead by count | Excreta, mammalian by mg./lb. | Excreta, other by mg./lb. | Mould % by wgt. | Insect defiled/infested % by wgt. | Extraneous/foreign matter % by wgt. |
|-----------------------------|--------------------------------|-------------------------------|---------------------------|-----------------|-----------------------------------|-------------------------------------|
| Allspice                    | 2                              | 5                             | 5.0                       | 2.00            | 1.00                              | 0.50                                |
| Anise                       | 4                              | 3                             | 5.0                       | 1.00            | 1.00                              | 1.00                                |
| Sweet basil                 | 2                              | 1                             | 2.0                       | 1.00            | 1.00                              | 0.50□                               |
| Caraway                     | 4                              | 3                             | 10.0                      | 1.00            | 1.00                              | 0.50                                |
| Cardamom                    | 4                              | 3                             | 1.0                       | 1.00            | 1.00                              | 0.50                                |
| Cassia                      | 2                              | 1                             | 1.0                       | 5.00            | 2.50                              | 0.50                                |
| Cinnamon                    | 2                              | 1                             | 2.0                       | 1.00            | 1.00                              | 0.50                                |
| Celery seed                 | 4                              | 3                             | 3.0                       | 1.00            | 1.00                              | 0.50                                |
| Chillies                    | 4                              | 1                             | 8.0                       | 3.00            | 2.50                              | 0.50                                |
| Cloves*                     | 4                              | 5                             | 8.0                       | 1.00            | 1.00                              | 1.00*                               |
| Coriander                   | 4                              | 3                             | 10.0                      | 1.00            | 1.00                              | 0.50                                |
| Cumin seed                  | 4                              | 3                             | 5.0                       | 1.00            | 1.00                              | 0.50                                |

**Table 2.1** *Continued*

| Name of spice, seed or herb | ▲ Whole insects, dead by count | Excreta, mammalian by mg./lb. | Excreta, other by mg./lb. | Mould % by wgt. | Insect defiled/infested % by wgt. | Extraneous/foreign matter % by wgt. |
|-----------------------------|--------------------------------|-------------------------------|---------------------------|-----------------|-----------------------------------|-------------------------------------|
| Dill seed                   | 4                              | 3                             | 2.0                       | 1.00            | 1.00                              | 0.50                                |
| Fennel seed                 | SF(2)                          | SF(2)                         | SF(2)                     | 1.00            | 1.00                              | 0.50                                |
| Ginger                      | 4                              | 3                             | 3.0                       | SF(3)           | SF(3)                             | 1.00                                |
| Laurel leaves <sup>†</sup>  | 2                              | 1                             | 10.0                      | 2.00            | 2.50                              | 0.50                                |
| Mace                        | 4                              | 3                             | 1.0                       | 2.00            | 1.00                              | 0.50                                |
| Marjoram                    | 3                              | 1                             | 10.0                      | 1.00            | 1.00                              | 1.00□                               |
| Nutmeg (broken)             | 4                              | 5                             | 1.0                       | SF(4)           | SF(4)                             | 0.50                                |
| Nutmeg (whole)              | 4                              | 0                             | 0.0                       | SF(5)           | SF(5)                             | 0.00                                |
| Oregano <sup>‡</sup>        | 3                              | 1                             | 10.0                      | 1.00            | 1.00                              | 1.00□                               |
| Black pepper                | 2                              | 1                             | 5.0                       | SF(6)           | SF(6)                             | 1.00                                |
| White pepper <sup>¶</sup>   | 2                              | 1                             | 1.0                       | SF(7)           | SF(7)                             | 0.50                                |
| Poppy seed                  | 2                              | 3                             | 3.0                       | 1.00            | 1.00                              | 0.50                                |
| Rosemary leaves             | 2                              | 1                             | 4.0                       | 1.00            | 1.00                              | 0.50□                               |
| Sage <sup>†</sup>           | 2                              | 1                             | 4.0                       | 1.00            | 1.00                              | 0.50                                |
| Savory                      | 2                              | 1                             | 10.0                      | 1.00            | 1.00                              | 0.50□                               |
| Sesame seed                 | 4                              | 5                             | 10.0                      | 1.00            | 1.00                              | 0.50                                |
| Sesame seed, hulled         | 4                              | 5                             | 1.0                       | 1.00            | 1.00                              | 0.50                                |
| Tarragon                    | 2                              | 1                             | 1.0                       | 1.00            | 1.00                              | 0.50□                               |
| Thyme                       | 4                              | 1                             | 5.0                       | 1.00            | 1.00                              | 0.50□                               |
| Turmeric                    | 3                              | 5                             | 5.0                       | 3.00            | 2.50                              | 0.50                                |

Ground processed spice (cannot exceed limit shown)

| Spices         | Whole equivalent insects | Insect fragments                       | Mites | Other insects | Rats/mouse hairs                          | Animal hairs |
|----------------|--------------------------|----------------------------------------|-------|---------------|-------------------------------------------|--------------|
| Ground paprika |                          | Average of more than 75 fragments/25 g |       |               | Average of more than 11 rodent hairs/25 g |              |

\* Clove Stems: Less than 5 % allowance by weight for unattached clove stems over and above the tolerance for other extraneous matter is permitted.

<sup>†</sup> Laurel leaves/sage: 'Stems' will be reported separately for economic purposes and will not represent a pass/fail criteria.

<sup>‡</sup> Oregano: Analysis for presence of Sumac shall not be mandatory if samples are marked 'Product of Mexico.'

<sup>¶</sup> White pepper: 'Percent Black Pepper' will be reported separately for economic purposes and will not represent a pass/fail criteria.

(2) Fennel seed: In the case of Fennel Seed, if 20 % or more of the sub-samples contain any rodent, other excreta or whole insects, or an average of 3 mg/lb or more of mammalian excreta, the lot must be reconditioned.

(3) Ginger: More than 3 % mouldy pieces and/or insect infested pieces by weight.

(4) Broken nutmeg: More than 5 % mould/insect defiled combined by weight.

(5) Whole nutmeg: More than 10 % insect infested and/or mouldy pieces, with a maximum of 5 % insect defiled pieces by count.

(6) Black pepper: 1 % mouldy and/or infested pieces by weight.

(7) White pepper: 1 % mouldy and/or infested pieces by weight.

▲ *Whole insects, dead*: Cannot exceed the limits shown.

□ Extraneous matter: Includes other plant material, e.g. foreign leaves.

A certain level of microbiological contamination is unavoidable on plant materials grown outdoor. This, coupled with low levels of technology employed in harvesting, handling and storage in the countries of origin of many herbs and spices plus the concentration that occurs due to drying, means these products can have high microbiological counts. Total counts in excess of  $10^6$  colony forming units (cfu) per gram are common and food poisoning bacteria such as *Salmonella* are estimated to be present in approximately 10 % of consignments.

There are currently three major methods of microbiological control within the USA, the principal method being fumigation with ethylene oxide (a biocidal gas). Sometimes multiple fumigations are used to achieve a satisfactory microbiological reduction. In recent years, concern about residues left by ethylene oxide (ETO) and ethylene chlorhydrin (ECH) has led to bans on its use (within the EU for example,) and the USA has recently set maximum residue levels for ETO and ECH. This has led to the use of heat treatment for decontamination, generally using high-pressure steam in highly specialised equipment. Irradiation is permitted for microbiological control of herbs and spices in many countries of the world. However, the use of irradiation must be declared on the consumer packaging, and public concern about the safety of irradiated foods has prevented the widespread application of this undeniably efficient process in many areas where its use is permitted by law.

#### **2.4.2 The European Spice Association (ESA)**

ESA represents national seasoning and spice trade associations within Europe. ESA members are involved in the processing, marketing, distribution and trading of herbs, spices and seasoning mixes. Its objectives are:

- to promote and protect the interests of its membership;
- to be the leading organisation of the European Seasoning & Spice Industry;
- to ensure members are fully aware of all current and future legislation;
- to act as central medium of communication with the EU Commission, European Parliament and Confederation of the Food and Drink Industries of the EU (CIAA);
- to strengthen links with worldwide spice trade associations and non-governmental organisations (NGOs), including ASTA, the International Organisation of Spice Trade Associations (IOSTA), the Indian Spices Board (ISB), Codex Alimentarius and the ISO.

Quality standards in Europe are typified by the standards set by ESA (2008, 2010). These draw upon standards set by AFNOR (the French standards authority) and the British Standards Institute (BSI) (4540 and 7087 series), together with the international standards issued by the ISO.

The ESA minimum general quality standards for all herbs and spices are summarised in Table 2.2, whilst values for specific herbs and spices are shown in Table 2.3. (For analytical methods, see Appendix 1.)

The ESA general standards are more relaxed and represent *minimum* standards allowable for trade. They do not preclude buyer and seller setting additional, stricter standards fit for the final purpose for which the herb and spice is to be used.

**Table 2.2** European Spice Association (ESA) specifications of quality minima for herbs and spices

|                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Subject</b>                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Sampling                          | ISO 948<br>For mycotoxins: See the relevant Commission Regulation at: <a href="http://www.esa-spices.org">www.esa-spices.org</a>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Chemical/physical analysis</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Ash                               | For values see Table 2.3; for analysis see Appendix 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Acid Insoluble Ash                | For values see Table 2.3; for analysis see Appendix 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Moisture                          | For values see Table 2.3; for analysis see Appendix 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Volatile oil                      | For values see Table 2.3; for analysis see Appendix 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Water activity                    | Water activity is a key parameter that affects microbiological growth. Therefore ESA recommends a target value of max. 0.65.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Bulk density                      | Due to methodology variability, both method and value should be agreed between buyer and seller.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Microbiology                      | The product shall be free from microorganisms at such levels which may represent a hazard to health. If the product is treated to reduce microbial loads before being imported into destination country the treatment will be such as to render/ensure the microbiological safety of consumers. Specific requirements to be agreed between buyer and seller.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Contaminants/residues</b>      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Pesticides                        | Shall be utilised in accordance with good agricultural practice. Application and residue limits must comply with existing EU and/or national legislation.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Heavy metals                      | Must comply with national/EU legislation (e.g. cadmium, lead)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Mycotoxins                        | Herbs and spices must be grown, harvested, handled and stored in such a manner as to prevent the occurrence of mycotoxins. If found, levels must comply with existing national and / or EU legislation.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Treatments                        | Only legally permitted processing procedures may be applied in any treatment used for product quality or safety.<br>EC approved fumigants may be used in accordance with manufacturers' instructions but this must be indicated on the accompanying documents. Ethylene oxide (ETO) treatment has been banned under European legislation. This ban covers both material treated within and outside the EU (i.e. the use of material that has been ETO treated before importation is also illegal).<br>Irradiation, at present, does not have full consumer acceptability, so the treatment has to be agreed between buyer and seller. If it is agreed irradiation is only permitted in EU approved irradiation plants. However, EU legislation requires that the irradiated product is declared at all levels within the food chain.<br>Members of ESA support the use on environmentally friendly fumigants (Montreal protocol) and non-toxic processes (e.g. microbial reduction under pressure, steam treatment).<br>All products subject to processing (for example, grinding, microbial reduction) are not in the scope of this document, unless otherwise stated. |

*Continued*

**Table 2.2** *Continued*

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|                           |                                                                                                                                                                                               |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Purity</b>             |                                                                                                                                                                                               |
| Species                   | To be agreed between buyer and seller.                                                                                                                                                        |
| Adulteration              | Must be free from.                                                                                                                                                                            |
| Infestation               | Should be free in practical terms from live and/or dead insects, insect fragments and rodent contamination visible to the naked eye (corrected if necessary for abnormal vision).             |
| Extraneous matter         | Herbs max. 2 %, spices max. 1 %.                                                                                                                                                              |
| Foreign matter            | The European food operator has to evaluate whether products fully comply with safety requirements before selling them to the final consumer. If not, additional processing will be necessary. |
| <b>Sensory properties</b> | Must be free from off odour or off-flavour.                                                                                                                                                   |
| <b>Packaging</b>          | The packaging must not be a source of contamination, should be food grade and must protect the product quality during transportation and storage.                                             |

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**2.4.3 The International Organisation of Spice Trade Associations (IOSTA)**

IOSTA is made up of spice associations from around the world whose members meet regularly to discuss issues relevant to the spice industry. Its Mission Statement reads:

IOSTA brings together spice associations from around the world to address common issues and seek sensible solutions to ensure the sustainability of the spice industry.

IOSTA has developed a Good Agricultural Practices Guide to be used as a resource in the growing and harvesting of spices (General Guidelines for Good Agricultural Practices Spices, April 2008: Issue 1. Produced by IOSTA with assistance from the International Trade Centre, Geneva).

The current IOSTA membership comprises: the All Nippon Spice Association (ANSA), ASTA, the Canadian Spice Association, ESA, the International General Produce Association (IGPA), ISB, All India Spices Exporters Forum, the Spice Council of Sri Lanka and the Vietnam Pepper Association.

**2.4.4 The All Nippon Spice Trade Association (ANSA)**

ANSA membership comprises leading Japanese spice, flavour and fragrance and seasoning companies. Its Mission Statement is:

to increase the consumption of spices in Japan through stable supply of good quality spices and to contribute to the better, healthier and safer food life of consumers.

ANSA's activities include providing information on food safety issues to consumers and communicating with the relevant governmental bureaux to establish realistic and adequate food safety regulations for spices. The most recent activities were to request the Ministry of Health, Labour and Welfare to undertake a risk assessment on the irradiation of spices, which is currently banned in Japan, and to provide information on approved chemical residue levels in spices.

**Table 2.3** Chemical / physical parameters; dry base for ASH, AIA, V/O

| Product <sup>1</sup>             | Ash<br>% W/W<br>max <sup>2</sup> | AIA<br>% W/W<br>max <sup>2</sup> | H <sub>2</sub> O<br>% W/W<br>max <sup>2</sup> | V/O<br>ml/100 g<br>min <sup>2</sup> | NOTES                                                                                                                                                                                                                                               |
|----------------------------------|----------------------------------|----------------------------------|-----------------------------------------------|-------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Anis                             | 9.0                              | 2.5                              | 12                                            | 1.0                                 |                                                                                                                                                                                                                                                     |
| Basil                            | 16                               | 2.0                              | 12                                            | 0.5                                 |                                                                                                                                                                                                                                                     |
| Caraway                          | 8.0                              | 1.5                              | 13                                            | 2.5                                 |                                                                                                                                                                                                                                                     |
| Cardamom                         | 9.0                              | 2.5                              | 12                                            | 4.0                                 |                                                                                                                                                                                                                                                     |
| Celery seed                      | 12                               | 3.0                              | 11                                            | 1.5                                 |                                                                                                                                                                                                                                                     |
| Celery leaves                    | 20                               | 1.0                              | 8.0                                           | Traces <sup>3</sup>                 |                                                                                                                                                                                                                                                     |
| Chervil                          | 17                               | 2.0                              | 8.0                                           | Traces                              |                                                                                                                                                                                                                                                     |
| Chilli                           | 10                               | 1.6                              | 11                                            | –                                   |                                                                                                                                                                                                                                                     |
| Chives                           | 13                               | 2.0                              | 8.0                                           | Traces                              |                                                                                                                                                                                                                                                     |
| Cinnamon<br>(Ceylon)<br>(cassia) | 7.0                              | 2.0                              | 14                                            | 0.7–1.0<br>(ISO 6539<br>& 6538)     | <i>The use of SO<sub>2</sub> is only<br/>permitted for<br/>Ceylon cinnamon<br/>Annex III part B<br/>Directive 95/2/EC</i><br>Styrene off notes can<br>be prevented<br>through the control<br>of moisture content<br>throughout the<br>supply chain. |
| Cloves                           | 7.0                              | 0.5                              | 12                                            | 14                                  |                                                                                                                                                                                                                                                     |
| Coriander seed                   | 7.0                              | 1.5                              | 12                                            | 0.6                                 |                                                                                                                                                                                                                                                     |
| Microcarpum                      |                                  |                                  |                                               | Traces                              |                                                                                                                                                                                                                                                     |
| Macrocarpum                      |                                  |                                  |                                               |                                     |                                                                                                                                                                                                                                                     |
| Coriander leaves                 | 15                               | 1.0                              | 8.0                                           | Traces                              |                                                                                                                                                                                                                                                     |
| Cumin                            | 14                               | 3.0                              | 13                                            | 1.5                                 |                                                                                                                                                                                                                                                     |
| Dill seed                        | 10                               | 2.5                              | 12                                            | 1.0                                 |                                                                                                                                                                                                                                                     |
| Dill tops                        | 15                               | 2.0                              | 8.0                                           | Traces                              |                                                                                                                                                                                                                                                     |
| Fennel                           | 10                               | 2.0                              | 12                                            | 1.5                                 |                                                                                                                                                                                                                                                     |
| Fenugreek                        | 7.0                              | 1.5                              | 11                                            | Traces                              |                                                                                                                                                                                                                                                     |
| Galangal<br>(ground)             | 9.0                              | 4.0                              | 10                                            | Traces                              |                                                                                                                                                                                                                                                     |
| Garlic products                  | 6.0                              | 0.5                              | 6.5                                           | –                                   | Due to the hygro-<br>scopic nature of<br>these products<br>lower moisture<br>content may be<br>required for pow-<br>dered items.                                                                                                                    |
| Ginger                           | 8.0                              | 2.0                              | 12                                            | 1.5                                 |                                                                                                                                                                                                                                                     |
| Juniper berries                  | 5.0                              | 1.0                              | 16                                            | 1.4                                 |                                                                                                                                                                                                                                                     |
| Laurel leaves                    | 7.0                              | 2.0                              | 8.0                                           | 1.0                                 |                                                                                                                                                                                                                                                     |
| Lemon grass                      | 8.0                              | 2.5                              | 10                                            | Traces                              |                                                                                                                                                                                                                                                     |
| Mace                             | 4.0                              | 0.5                              | 10                                            | 5.0                                 |                                                                                                                                                                                                                                                     |
| Marjoram                         | 10                               | 2.0                              | 12                                            | 0.7                                 |                                                                                                                                                                                                                                                     |
| Mustard                          | 6.5                              | 1.0                              | 10                                            | –                                   |                                                                                                                                                                                                                                                     |

*Continued*

**Table 2.3** *Continued*

| Product <sup>1</sup>     | Ash<br>% W/W<br>max <sup>2</sup> | AIA<br>% W/W<br>max <sup>2</sup> | H <sub>2</sub> O<br>% W/W<br>max <sup>2</sup> | V/O<br>ml/100 g<br>min <sup>2</sup> | NOTES                                                                                                      |
|--------------------------|----------------------------------|----------------------------------|-----------------------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------|
| Nutmeg                   | 3.0                              | 0.5                              | 10                                            | 5.0–6.5<br>Depending<br>on grade    |                                                                                                            |
| Onion products           | 5.0                              | 0.5                              | 6.0–8.0<br>(depending<br>on origin)           | –                                   | Due to the hygroscopic nature of these products lower moisture content may be required for powdered items. |
| Oregano                  | 10                               | 2.0                              | 12                                            | 1.5                                 |                                                                                                            |
| Paprika powder           | 10                               | 2.0                              | 11                                            | –                                   |                                                                                                            |
| Parsley                  | 14                               | 1.5                              | 7.5                                           | Traces                              | English origin is not covered.                                                                             |
| Pepper black             | 7.0                              | 1.5                              | 12                                            | 2.0                                 |                                                                                                            |
| Pepper white             | 3.5                              | 0.3                              | 12                                            | 1.5                                 |                                                                                                            |
| Pepper green<br>(dried)  | 3.0                              | 0.3                              | 13 <sup>2</sup>                               | 1.0                                 | <sup>2</sup> If freeze dried: 8 %.                                                                         |
| Pimento                  | 4.5                              | 0.4                              | 12                                            | 3.0                                 |                                                                                                            |
| Jamaica                  | 5.0                              | 1.0                              | 12                                            | 2.0                                 |                                                                                                            |
| Other origins            |                                  |                                  |                                               |                                     |                                                                                                            |
| Pink pepper<br>(Schinus) | 7.0                              | 1.8                              | 14 <sup>2</sup>                               | 2.0                                 | <sup>2</sup> If freeze dried: 8 %.                                                                         |
| Poppy seeds              | 8                                | 1                                | 8                                             | –                                   |                                                                                                            |
| Rosemary                 | 8.0                              | 1.0                              | 10                                            | 1.0                                 |                                                                                                            |
| Saffron whole            | 8.0                              | 1.0                              | 12                                            | –                                   |                                                                                                            |
| Saffron ground           | 8.0                              | 1.5                              | 10                                            | –                                   |                                                                                                            |
| Sage                     | 12                               | 2.0                              | 12                                            | 1.5                                 |                                                                                                            |
| Savoury                  | 12                               | 1.0                              | 12                                            | 0.5                                 |                                                                                                            |
| Spearmint                | 12                               | 2.5                              | 13                                            | 0.5                                 |                                                                                                            |
| Star anise               | 3.0                              | 0.5                              | 8.0                                           | 7.0                                 |                                                                                                            |
| Tarragon                 | 12                               | 1.5                              | 8.0                                           | 0.5                                 |                                                                                                            |
| Thyme                    | 12                               | 3.5                              | 12                                            | 1.0                                 |                                                                                                            |
| Turmeric                 | 8.0                              | 2.0                              | 12                                            | 2.5                                 |                                                                                                            |
| Whole                    | 9.0                              | 2.5                              | 10                                            | 1.5                                 |                                                                                                            |
| Ground                   |                                  |                                  |                                               |                                     |                                                                                                            |

<sup>1</sup> The parameters listed shall apply to the whole product unless otherwise specified.

<sup>2</sup> see Appendix 1.

<sup>3</sup> Traces – low levels of volatiles (in general <0.5 %) for which analytical quantification by using EN ISO 6571 is not accurate and reliable. The sensorial flavouring properties should be agreed between buyer and seller.

## 2.5 Quality management system (QMS)

A food business must have a full understanding of the products produced, manufactured and distributed and have systems in place to identify and control hazards significant to food safety. There are many global standards that can be implemented



and, in general, they are all based on two key components: senior management commitment and the hazard analysis critical control point (HACCP) system, which is a step-by-step approach to managing food safety risks. One of the most widely used quality management standards is *ISO 9001:2008 Quality management systems – Requirements*. ISO 9001 is founded on the following eight principles:

- customer focus
- leadership
- involvement of people
- process approach
- system approach to management
- continual improvement
- factual approach to decision making
- mutually beneficial supplier relationships.

ISO9001 can be certified and verified by independent audit.

Related documents to ISO 9001 are:

- *ISO 9000:2005 Quality Management Systems – Fundamentals and vocabulary*
- *ISO 9004:2000 Quality Management Systems Guidance for Performance Improvement*
- *ISO 19011:2002 Guidelines on Quality and Environmental Auditing*

### 2.5.1 Senior management commitment

Within a food business, food safety must be seen as a cross-functional responsibility that draws upon the skills and management expertise in many disciplines within the organisation. Effective food safety management extends beyond technical departments and must involve commitment from senior management and those with delegated responsibility for production operations, engineering and distribution management, procurement of raw materials, customer feedback and human resource activities such as training. The starting point for an effective food safety plan is the commitment of senior management to the development of an all-encompassing policy as a means to guide the quality management activities that collectively assure food safety.

### 2.5.2 Hazard analysis critical control point (HACCP)

Quality assurance (QA) systems for herbs and spices should be planned and documented using HACCP principles. HACCP systems rely on a team of experts who between them have the necessary knowledge and expertise to identify and develop control procedures for all consumer safety hazards. All HACCP analyses will be different and tailored to the operation of the team involved; however, many prerequisite programmes (good hygiene practices) are common. Good hygiene practices are set out in Regulation 852/2004 and include:

- **Raw material (including water) and packaging specifications and supplier management:** Any QA system should start with a comprehensive raw material specification agreed with the supplier, where this is possible. Specifications need to be

supported by an audit programme to verify that suppliers have the expertise, technology and QA systems required to meet the agreed specifications. In many cases, allowance will need to be made for the lack of infrastructure and resources within many supplier countries, and the company purchasing the material must ensure that is able to make up for this through the thorough and consistent application of its own rigorous and effective procedures and systems:

- Transport hygiene
- **Personnel/personal hygiene and training:** It should be emphasised that effective QA systems rely on trained and experienced personnel.
- Pest management
- Scheduled cleaning
- Planned maintenance
- Waste management
- Spillage and emergency procedures
- **Traceability and bar coding:** It is a fundamental requirement of QMS that product can be identified and traced at all stages of processing and distribution of the finished product to the customer. The traceability system should be able to locate and track product in a timely manner. The system should be challenged at least annually to ensure effective operation and should include a mass balance.

Bar coding of products is an extremely effective and useful tool and is widely used in storage and distribution operations. Most stock management systems commonly available use bar coding as one of the key elements.

HACCP-based procedures for controlling process hazards will not be effective unless good hygiene practices are being followed.

The HACCP analysis will usually result in the identification of critical control points (CCPs) in the process. Control measures are focused on these steps (CCPs) in order to eliminate or reduce consumer safety risks to an acceptable level. The 7 HACCP principles set out in the Codex Alimentarius document CAC/RCP 1-1969, rev. 4-2003 ([www.codexalimentarius.org](http://www.codexalimentarius.org)) are:

1. Identify any hazards that must be prevented, eliminated, or reduced to acceptable levels (typically these hazards will include those posed by chemicals, allergens, microorganisms and foreign bodies).
2. Identify the critical control points at the step or steps at which control is essential to prevent or eliminate a hazard or to reduce it to acceptable levels.
3. Establish critical limits at critical control points which separate acceptability from unacceptability for the prevention, elimination or reduction of identified hazards.
4. Establish and implement effective monitoring procedures at critical control points.
5. Establish corrective actions when monitoring indicates that a critical control point is not under control.
6. Establish procedures, which shall be carried out regularly, to verify that the above measures are working effectively.
7. Establish documents and records commensurate with the nature and size of the food business to demonstrate the effective application of the above measures.

A HACCP approach may also be applied to quality parameters. Furthermore, HACCP may be incorporated within a wider management system such as ISO9001 as required.

### *Quality checks*

A typical testing plan would begin with testing/inspection of raw materials, on delivery for gross contamination or infestation. The first inspection is usually an overall sensory inspection (appearance and odour) of the product as the doors of the container are opened or the load is made accessible. This inspection should identify large-scale infestation with insects or rodents, mould growth, unacceptable or damaged packaging, or an unsuitable container, e.g. one previously used for chemicals, which may have contaminated/tainted the spice or herb.

Further, more detailed sampling and examination of the raw material usually follows.

- It is important that samples are representative of the bulk under test and sampling is usually on a 'square root + 1 basis' (i.e. the number of samples taken is equivalent to the square root of the number of sacks or containers + one). Samples should be taken from throughout the load, usually to a maximum of 10 samples. The assessment should include tests for the amount of dust (with the aid of sieves as appropriate), stalks, stem, extraneous matter, etc. and, most importantly, the colour, odour, taste (if possible, taste tests should not be carried out until after the results of microbiological analysis are available) and general appearance of the product.
- Sensory assessment should be backed up with analytical tests relevant to the product, e.g. volatile oil, moisture, ash, AIA.
- Microbiological testing may also be carried out for the presence of indicator organisms (e.g. total viable count, aerobic spore formers count) and/or for pathogenic organisms such as *Salmonella* and *E. coli* before it is further processed or released (positive release) on the attainment of the specification parameters (typical market requirements include: *Salmonella* not detected (ND) in 25 g, *E. coli* < 100 cfu/g).

Unacceptable material is usually rejected or, in certain cases, may be re-conditioned, e.g. re-cleaned, dried, etc., to bring it within the required parameters. This may be carried out in-house or by a specialist processor. Microbiologically unsatisfactory material may be gas treated (where this is permitted) or heat treated. Positive microbiological release may be employed on a pallet basis subsequent to heat treatment.

Also incorporated within the QMS should be procedures for detecting and removing metal of all types. Characteristically, this consists of the use of metal detection/magnets/sieves at appropriate stages during processing and with obligatory use immediately prior to or (in the case of metal detection) after packing.

## **2.6 Environmental safety: ISO 14001**

ISO 14001 was first published in 1996 and specifies the requirements for an environmental management system. It applies to those environmental aspects over

which an organisation has control and which it can be expected to be able to influence. The standard itself does not state specific environmental performance criteria, but it outlines key areas that an organisation must address. Examples of the key areas are:

- implement, maintain and improve an environmental management system;
- assure itself of its conformance with its own stated environmental policy;
- demonstrate conformance;
- ensure compliance with environmental laws and regulations.

Particular areas of environmental concern linked to the processing of herbs and spices are energy usage, food miles, factory emissions (dust and odour) and packaging waste.

## 2.7 Sources of further information

- Regulation EC 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC  
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:070:0001:0016:en:PDF>
- Regulation EC 1881/2006 setting maximum levels for certain contaminants in foodstuffs  
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF>
- Regulation EC 105/2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A  
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:035:0007:0008:EN:PDF>
- Regulation EC 852/2004 on the hygiene of foodstuffs  
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:139:0001:0054:EN:PDF>
- IOSTA (2008) *General Guidelines for Good Agricultural Practices Spices*  
[http://www.astaspice.org/files/public/IOSTA\\_GAP\\_Final.pdf](http://www.astaspice.org/files/public/IOSTA_GAP_Final.pdf)
- ISO TC 34 SC/7 Spices, culinary herbs and condiments  
[http://www.iso.org/iso/iso\\_catalogue/catalogue\\_tc/catalogue\\_tc\\_browse.htm?commid=47912](http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=47912)

## 2.8 References

- ASTA (1997) *Official Analytical Methods of the American Spice Trade Association*. American Spice Trade Association, Washington DC.
- ASTA (1999) *ASTA Cleanliness Specification for Spices, Seeds and Herbs*. American Spice Trade Association, Washington DC.
- ASTA (2004) *Official Microbiological Methods of the American Spice Trade Association*. American Spice Trade Association, Washington DC.
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- BS7087 series: *Specification for Herbs and Spices*. British Standards Institute, London.
- BS4540 series: *Methodology for Analysis of Herbs and Spice*. British Standards Institute, London.
- CAC (2003) *Hazard analysis and critical control point (HACCP) system and guidelines for its application*, ANNEX to Recommended International Code of Practice/General Principles of Food Hygiene, CAC/RCP 1-1969, Rev 4. FAO/WHO Codex Alimentarius Commission.
- ESA (2008) *Dehydration Factors for Products of the Spice Industry*. European Spice Association.
- ESA (2011) *European Spice Association: Quality Minima for Herbs and Spices*. European Spice Association.

## Appendix 1: Recommended analytical methods

Unless otherwise agreed between buyer and seller, ESA recommends the following methods:

1. Spices and condiments – Sampling EN ISO 948 – 2009
2. Spices and condiments – Preparation of a ground sample for analysis ISO 2825 – 1981
3. Spices and condiments – Determination of extraneous matter and foreign matter content ISO 927 – 2009
4. Spices and condiments – Determination of total ash ISO 928 – 1997
5. Spices and condiments – Determination of acid insoluble ash ISO 930 – 1997
6. Spices and condiments – Determination of moisture content – Entrainment method ISO 939 – 1980
7. Spices, condiments and herbs – Determination of volatile oil content (hydrodistillation method) EN ISO 6571 2009
8. Determination of loss in mass of capsicum and allium species and of vegetables when dried in a vacuum oven – DIN 10236 – 2001–12 (German standard)

## Quality indices for spice essential oils

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**Abstract:** Quality assurance of essential oils is imperative for therapeutic purposes, as well as to ensure authenticity and product quality. Natural variation in the quality of spices also affects the quality of their essential oils. There have been numerous approaches to the setting of quality standards for essential oils. This chapter reviews the major constituents of spice essential oils and common methods for their adulteration such as the addition of synthetic flavourants or edible and mineral oils, dilution with ethanol or blending with isolates with other natural essential oils. Methods for the detection of adulteration are also covered. Then the chapter gives an overview of the authentication of the botanical and geographical origin of essential oil by GC/MS of natural constituents, isotope ratio mass spectrometry (IRMS), site-specific natural isotope fractionation (such as  $^{13}\text{C}$ ,  $^{14}\text{C}$  and  $^2\text{H}$ ) by NMR combined with IRMS, and DNA markers.

**Key words:** essential oils, authenticity, blending/adulteration, botanical/geographical origin, benchmarks of quality.

### 3.1 Introduction

Essential oil fractions and oleoresins derived from spices are widely used in the food industry to provide flavour. Besides applications in the food sector, they are vital components of many cosmetics, perfumery products and phytopharmaceuticals (Schulz *et al.*, 2003). Essential oils are hydrophobic concentrates that contain the essence of the plant material from which they are extracted by steam or hydrodistillation. They are largely composed of saturated or partly unsaturated cyclic and linear molecules of relatively low molecular mass such as hydrocarbons and oxygenated compounds (hydroxy and carbonyl derivatives) (Marriott *et al.* 2001). Some of the major essential oils and their origins are shown in Table 3.1. The essential oil constituents provide fragrance, have healing properties, serve as antioxidants and also enhance the stability of the product. The terpenoids of the spice essential oils are responsible for their medicinal properties. For example, cinnamon's medicinal properties are attributed to eugenol and cinnamaldehyde, two of its very important terpenoids, which act as potent antifungal agents, and its anti-allergenic properties are due to its diterpenes (Faix *et al.*, 2009). Hence, when essential oils are used for therapeutic purposes, it becomes imperative to assess their complete composition to rule out any possible form of adulteration or contamination.

**Table 3.1** Major essential oils – their production and adulterants

| Essential oil     | Origin                                                           | Major countries                                                               | Adulterants employed                                                                     |
|-------------------|------------------------------------------------------------------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Bergamot oil      | <i>Citrus auranticum</i><br>(Rutaceae)                           | Italy, Ivory Coast, Brazil<br>Argentina, Spain,<br>Russia                     | Synthetic linalool and<br>linalyl acetate; orange<br>and lime terpenes                   |
| Cassia oil        | <i>Cinnamomum cassia</i><br>(Lauraceae)                          | China, Indonesia,<br>Vietnam, Taiwan                                          | Cinnamaldehyde                                                                           |
| Cinnamon oil      | <i>Cinnamomum zeylanicum</i><br>(Lauraceae)                      | Sri Lanka, India                                                              | Leaf oil to bark oil and<br>cinnamaldehyde                                               |
| Clove leaf oil    | <i>Eugenia caryophyllata</i><br>(Myritaceae)                     | Madagascar, Indonesia,<br>Tanzania, Brazil, Sri<br>Lanka                      | Clove stem oil                                                                           |
| Clove bud oil     | <i>Eugenia caryophyllata</i><br>(Myritaceae)                     | Indonesia, Madagascar                                                         | Clove stem oil, leaf oil,<br>eugenol, and stem oil<br>terpenes                           |
| Coriander oil     | <i>Coriandrum sativum</i><br>(Umbelliferae)                      | Russia                                                                        | Synthetic linalool                                                                       |
| Cornmint oil      | <i>Mentha arvensis</i><br>(Labiatae)                             | China, Brazil, India,<br>Paraguay, Taiwan,<br>Thailand, North<br>Korea, Japan | Not a commercially<br>attractive proposition                                             |
| Dill oil          | <i>Anethum graveolens</i><br>(Umbelliferae)                      | US, Hungary, Bulgaria,<br>Russia Egypt                                        | Distilled orange<br>terpenes                                                             |
| Eucalyptus oil    | <i>Eucalyptus globus</i><br>(Myrtaceae)                          | Portugal, S. Africa,<br>Spain, China, India,<br>Austria, Paraguay             | –                                                                                        |
| Garlic oil        | <i>Allium sativum</i><br>(Liliaceae)                             | Mexico, Italy, Egypt                                                          | Nature identical raw<br>materials                                                        |
| Ginger oil        | <i>Zingiber officinale</i><br>(Zingiberaceae)                    | China, India                                                                  | Not often adulterated                                                                    |
| Grapefruit        | <i>Citrus paradisi</i><br>(Rutaceae)                             | Brazil, US, Israel,<br>Argentina, New<br>Zealand                              | Orange terpenes                                                                          |
| Lemon oil         | <i>Citrus limon</i><br>(Rutaceae)                                | Argentina, US, Italy,<br>Brazil, Greece, Spain,<br>Australia, Peru            | Distilled oil and<br>terpenes                                                            |
| Lemongrass        | <i>Citrus flexuosus</i> and<br><i>C. citratus</i><br>(Gramineae) | India, China, Guatemala,<br>Brazil, Russia, Sri<br>Lanka, Haiti, Russia       | Synthetic citral                                                                         |
| Lime oil          | <i>Citrus aurantifolia</i><br>(Rutaceae)                         | Mexico, Peru, Haiti,<br>Brazil Ivory Coast,<br>Cuba, Ghana, Jamaica,<br>China | Synthetic terpineol<br>terpinolene, and<br>other components of<br>lime terpenes          |
| Litsea cubeba oil | <i>Litsea cubeba</i><br>(Lauraceae)                              | China                                                                         | Synthetic citral                                                                         |
| Nutmeg            | <i>Myristica fragrens</i><br>(Myristicaceae)                     | Indonesia, Sri Lanka,                                                         | Terpenes and nature<br>identical raw<br>materials                                        |
| Sweet orange oil  | <i>Citrus sinensis</i><br>(Rutaceae)                             | Brazil, US, Israel, Italy,<br>Australia                                       | Adulteration infrequent<br>but higher priced oils<br>diluted with cheaper<br>substitutes |
| Peppermint oil    | <i>Mentha piperita</i><br>(Labiatae)                             | US, Russia, Yugoslavia,<br>Hungary, France                                    | Cornmint oil, terpenes                                                                   |

**Table 3.1** *Continued*

| Essential oil  | Origin                                          | Major countries                                           | Adulterants employed                                                  |
|----------------|-------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------------------|
| Rose oil       | <i>Rosa damascena</i><br>(Rosaceae)             | Turkey, Russia, Bulgaria,<br>Morocco                      | Nature identical<br>components such as<br>citronellol and<br>geraniol |
| Rosemary oil   | <i>Rosmarinus<br/>officinalis</i><br>(Labiatae) | Spain, Morocco, Tunisia,<br>Russia, Yugoslavia,<br>Turkey | Camphor and<br>eucalyptus fraction                                    |
| Spearmint oil  | <i>Mentha spicata</i><br>(Labiatae)             | US, China, Italy, Brazil,<br>Japan, France                | Laevo carvone                                                         |
| Star anise oil | <i>Illicium verum</i><br>(Magnoliaceae)         | China, Vietnam, North<br>Korea, Russia                    | Anethole                                                              |
| Tangerine oil  | <i>Citrus reticulata</i><br>(Rutaceae)          | Brazil, US, Russia, Spain,<br>South Africa                | Synthetic methyl-n-<br>methyl anthranilate                            |

Source: Singhal *et al.* (1997).

Determining the quality and purity of essential oils faces similar difficulties to establishing the quality of raw spices, given the accepted variations in and mixing of varieties within a particular spice. There are, however, a number of properties that can be used to set quality standards. These include physical characteristics such as:

- specific gravity
- molecular refractive index
- optical rotation
- solubility.

It is also possible to use chemical properties to benchmark quality. These include determination of acids, esters, alcohols, aldehydes and ketones, phenols and iodine number. Some specific flavour tests for halogens can also be used to set standards (Guenther, 1972). An overview of these quality indices is provided in the appendix at the end of this chapter.

### 3.2 Major chemical constituents of spice essential oils

- **Clove oil:** The essential oil extracted from the dried flower buds of clove, *Eugenia caryophyllata* (Myrtaceae), is used topically to relieve pain and to promote healing. The main constituents of the essential oil are phenylpropanoids such as carvacrol, thymol, eugenol, and cinnamaldehyde (Chaieb *et al.*, 2007).
- ***Coriandrum sativum*:** Coriander, an annual herb of the parsley family (Apiaceae) and native to the Mediterranean region, is grown widely as a spice or for essential oil production. While aliphatic aldehydes (mainly C<sub>10</sub>–C<sub>16</sub> aldehydes) are predominant in the fresh herb oil, the major components in the oil isolated from coriander fruit include linalool and some other oxygenated monoterpenes and monoterpene hydrocarbons (Coleman and Lawrence, 1992; Leung and



Foster, 1996). The coriander seed oil contains linalool (60–70 %) and 20 % hydrocarbons (Guenther, 1950). The essential oils from leaves and fruits of *Coriandrum sativum* were analysed by gas chromatography–mass spectrometry (GC–MS) by Bhuiyan *et al.* (2009). The leaf oil contained 44 compounds, mostly of aromatic acids such as 2-decenoic acid (30.8 %), E-11-tetradecenoic acid (13.4 %), capric acid (12.7 %), undecyl alcohol (6.4 %), tridecanoic acid (5.5 %), and undecanoic acid (7.1 %), as the major constituents. The seed oil contained 53 compounds where the major compounds detected were linalool (37.7 %), geranyl acetate (17.6 %), and  $\gamma$ -terpinene (14.4 %).

- ***Cinnamomum zeylanicum*:** In the Lauraceae family, the *Cinnamomum* genus (cinnamon) is a very popular spice all over the world. The sensorial qualities of *Cinnamomum zeylanicum* are slightly sweet, pleasant, warm, and bitter, besides being strongly aromatic. Trajano *et al.* (2010) found the essential oil from *C. zeylanicum* leaves to contain eugenol (76.10 %), *trans*- $\beta$ -caryophyllene (6.7 %), linalool (3.7 %), eugenol acetate (2.8 %), and benzyl benzoate (1.9 %). Lima *et al.* (2005) noted a qualitatively and quantitatively different profile of constituents between the analysed oils from the leaves and branches of *C. zeylanicum*. Eugenol (60 %) was the major compound found in the essential oil from leaves, whereas the oil from branches showed a higher range of compounds with linalool (10.6 %),  $\alpha$ -pinene (9.9 %), and  $\alpha$ -phellandrene (9.2 %) as prevalent compounds.
- **Indian bayleaf (*Cinnamomum tejpata*):** The essential oil from the leaves contains mostly monoterpenoids. Linalool (50 %) is the major compound;  $\alpha$ -pinene, *p*-cymene,  $\beta$ -pinene, and limonene constitute from 5–10 % ([http://www.uni-graz.at/~katzer/engl/Cinn\\_tam.html](http://www.uni-graz.at/~katzer/engl/Cinn_tam.html)).
- ***Juniperus phoenicea*** (family Cupressaceae): The essential oil of *Juniperus phoenicea* has been used to increase the flow of digestive fluids, improve digestion, and eliminate gas and stomach cramping. Juniper is effective as a steam inhalant for bronchitis and to control arthritis. In a study, the major compound of the essential oils of the aerial parts of *J. phoenicea* (family Cupressaceae) determined by GC–MS and gas chromatography–flame ionization detection (GC–FID) was found to be  $\alpha$ -pinene (49.15 %) followed by  $\alpha$ -phylloandrene (7.39 %), mycene (5.24 %),  $\beta$ -pinene (3.58 %), linalool (2.54 %), piperitone (1.56 %),  $\gamma$ -terpinene (1.28 %), *trans*-pinocarveole (1.23 %), *p*-cymene (1.10 %),  $\alpha$ -terpineol (1.02 %), and  $\gamma$ -cardinene (1.01 %) (Derwich *et al.*, 2011).
- ***Lippia origanoides*:** *Lippia origanoides* (Verbenaceae) leaves are used as a spice in cooking and in traditional medicine. The chemical composition of the essential oil obtained from its leaves, analysed by GC and GC–MS, showed a high content of oxygenated monoterpenes (66.0 %), carvacrol (38.6 %), and thymol (18.5 %) (Oliveira *et al.*, 2007).
- **Nutmeg (*Myristica fragrans*):** Nutmeg contributes two spice products namely, nutmeg, which is the kernel of the seed, and mace, the dried aril that surrounds the seed within the fruit. Gopalakrishnan (1992) showed that  $\alpha$ -pinene,  $\beta$ -pinene, and sabinene together constituted 77.38 % and 60.76 % in nutmeg and mace oil, respectively. The concentration of myristicin (5-methoxy safrole) and elemicin were found to be high in Indian nutmeg oils. Mullavarapu and Ramesh (1998) reported that nutmeg oil contained 76.8 % monoterpenes, 12.1 % oxygenated

monoterpenes, and 9.8 % phenyl propanoid ether. The mace oil was found to consist of 51.2 % monoterpenes and 18.8 % phenyl propanoid ether.

- **Oregano (*Origanum vulgare* L.):** The oil of *Origanum vulgare* has many medicinal attributes such as antifungal, antibacterial, antithrombin, anti-inflammatory, antiaggregant, antioxidant, and antihyperglycaemic activity. The leaf essential oil may contain variable amounts of the two phenols, carvacrol and thymol. A variety of monoterpene hydrocarbons such as limonene, terpinene, ocimene, caryophyllene,  $\beta$ -bisabolene, and *p*-cymene and monoterpene alcohols (linalool, 4-terpineol) are reported ([http://www.uni-graz.at/~katzner/engl/Orig\\_vul.html](http://www.uni-graz.at/~katzner/engl/Orig_vul.html)). In a study by Derwich *et al.* (2010), the most abundant Moroccan leaf oil component was found to be carvacrol (18.06 %). The other major components were found to be thymol (7.36 %),  $\gamma$ -terpinene (5.25 %), *p*-cymene (5.02 %), limonene (4.68 %), caryophyllene (4.12 %), cymene (3.56 %), ledene (3.41 %), linalool (2.47 %),  $\alpha$ -pinene (2.15 %),  $\gamma$ -terpineol (2.10 %), and germacrene (2.08 %).
- ***Pimenta dioica*:** The tropical, evergreen dried fruits of the *Pimenta dioica* tree are used worldwide as a valuable spice. The essential oil is obtained from the fruits or leaves with yields of 1.5–4.5 %, and the major compound of the *P. dioica* oils is eugenol (70–80 %). In the pimento leaf oils, 1,8-cineole,  $\alpha$ -humulene,  $\beta$ -caryophyllene, and cadinene-derivatives are found as further important constituents at levels of up to 10 %. The essential oils of *P. dioica* leaves and fruits are utilized in the meat and canning industries as well as in perfumery compositions and cosmetic products (Tucker *et al.*, 1991; Maringiu *et al.*, 2005).
- ***Rosmarinus officinalis* (Lamiaceae):** In traditional medicine, rosemary is used to treat different diseases including depression, insomnia, and arthritic pain. Moreover, the volatile compounds have shown antimicrobial, antifungal, and insecticidal activities. In the analysis of the chemical composition of the essential oil of the leaves of *Rosmarinus officinalis* by GC–MS and GC–FID, the major component was found to be  $\alpha$ -pinene (18.25 %), followed by camphor (6.02 %), 1,8-cineole (5.25 %), camphene (5.02 %),  $\beta$ -pinene (4.58 %), bornyl acetate (4.35 %), limonene (3.56 %), borneol (3.10 %),  $\alpha$ -terpineol (2.89 %), and cymene (2.02 %) (Derwich *et al.*, 2011).
- ***Salvia pratensis* and *S. bertolonii* (Lamiaceae):** *Salvia bertolonii* has been considered a synonym, a problematic sub-species, and a form of *S. pratensis* (Anackov *et al.*, 2008). Both the species are used in traditional medicine instead of sage (*S. officinalis*) (Lamiaceae) or as an adulterant. In order to confirm the status of *S. bertolonii*, together with the potential identification characteristics for differentiation from sage, both taxa were analysed through the analysis of their essential oils. There were clear differences in the quantity of essential oil (0.073 % for *S. pratensis* and 0.0016 % for *S. bertolonii*). The major compound in the essential oil of *S. pratensis* was found to be E-caryophyllene (26.4 %) while in the *S. bertolonii* essential oil caryophyllene oxide was the major component (35.1 %). Oniga *et al.* (2010) found the essential oils of *S. officinalis* analysed by GC–MS to contain  $\alpha$ -thujone as the major compound (31.23–52.86 %) followed by camphor and viridiflorol. The presence of monoterpene ketones ( $\alpha$ -thujone,  $\beta$ -thujone, camphor) in high amounts in the essential oils of Romanian sage are indicated to induce toxicity, thereby limiting their use for medical purposes.

### 3.3 The problem of adulteration

Essential oils, in general, should be 100 % pure and wholly derived from the stated botanical source. However, adulteration has been a serious problem for many years in the area of essential oils. The economic incentive to blend synthetic flavourants with natural oil is for some too great to resist. Some essential oils naturally contain a single compound at high concentration, and often the synthetic counterpart of this major component is available at a low cost. Addition of this single compound to natural essential oils without declaration on the label amounts to adulteration. The possibility of detecting the blending of natural essential oils with synthetic substances or with isolates from other essential oils thus becomes an important tool for authenticity testing.

#### 3.3.1 Addition of synthetic flavourants

Synthetic flavour compounds contain impurities characteristic of the synthetic route used to prepare them. These impurities can be quantified by selected ion monitoring (SIM), GC–MS of essential oils, and their absence is an indication of the essential oil being natural (Frey, 1988). For instance, the presence of impurities such as phenyl pentadienal, benzyl alcohol, and eugenol in synthetic cinnamaldehyde forms the basis of its detection in natural cassia oil. These impurities could be quantified by GC–MS, and levels as low as 0.55 parts of synthetic cinnamaldehyde in natural cassia oil can be detected (Zhu *et al.*, 1996). The common adulterants for spice essential oils are listed in Table 3.1. A mass spectrometer usually scans over a range of trace compounds in order to obtain data on every component in a mixture. The decrease in the number of masses detected using SIM results in a 10–100-fold increase in detection sensitivity for a single compound.

#### 3.3.2 Addition of edible and mineral oils

Both edible and mineral oils are often used for adulteration (Nour-el-Din *et al.*, 1977). The mixing of expensive oils with cheaper oils can often be detected by running a GC profile of the oil. One approach is to look for components in the expensive oil which are not commercially available, and are unique to the oil. An example is  $\beta$ -selinene in the oil of celery. A good quality oil should contain 7.0–7.5 %  $\beta$ -selinene (Straus and Wolstromer, 1974). Oils containing less than 7.0 %  $\beta$ -selinene should be suspected of being adulterated.

#### 3.3.3 Dilution with ethyl alcohol

Ethyl alcohol represents the main alcohol usually used in moderate quantities to dilute essential oils (Mostafa *et al.*, 1990a). Dilution of essential oils with ethanol was checked using refractometric methods which were found to be unreliable (Kaminski and Dytkowska, 1960). These and many other adulterations can be identified by infra-red (IR), GC, and thin-layer chromatography (TLC) (Giacomo and Calvarano, 1973). TLC has been found to be a simple method of checking adulteration in essential oils of caraway, coriander, parsley, and anethum (Hoerhammer *et al.*,

1964). Detection of nature-identical flavouring substances in high-value genuine onion oil is based on the GC–MS or IR spectroscopy of the onion furanone, 2-n-hexyl-5-methyl-3(2H) furanone (Losing, 1999). This technique is both simple and rapid.

### 3.3.4 Blending with isolates of other natural essential oils

Blending in cheap essential oils is customary in the oil trade. For instance, adulteration of commercial Mediterranean oregano has been reported with the species of the Lamiaceae family (*Satureja montana* and *O. majorana*) due to a similar superimposing essential oil profile (Marieschi *et al.*, 2011).

### 3.3.5 Iodine number for detection of adulteration

Iodine number has been suggested as a means of detecting adulteration in essential oils (Karthi and Mishra, 1963), but the iodine number has not attained significance in assessing the quality of essential oils, probably due to unpredictable behaviour of these oils in the presence of solutions commonly employed for iodination. The observation that the iodine monobromide–mercuric acetate reagent brings about quantitative fission of the cyclopropane and cyclobutane rings in essential oils prompted Kumar and Madaan (1979) to make use of such iodine absorption values for this purpose. Table 3.2 gives the recommended iodine values for pure specimens of various essential oils and isolates. The method could detect adulteration successfully in samples considered to be unadulterated on the basis of conventional analytical procedures.

**Table 3.2** Recommended iodine values for pure specimens of various oils and isolates

| Essential oil/isolate                                         | Recommended iodine value |
|---------------------------------------------------------------|--------------------------|
| Oil of ajowan, lab. distilled <sup>a</sup>                    | 232–265                  |
| Oil of fennel, lab. distilled <sup>a</sup>                    | 160–185                  |
| Oil of dill, lab. distilled <sup>a</sup>                      | 265–307                  |
| Oil of clove, lab. distilled <sup>a</sup>                     | 232–243                  |
| Oil of cinnamon leaf, lab. distilled                          | 46–52                    |
| Oil of black pepper, lab. distilled                           | 300–324                  |
| Oil of cumin seed, lab. distilled                             | 193–195                  |
| Oil of lavidin (abrialis) <sup>b</sup>                        | 167                      |
| Oil of parsley seeds, lab. distilled <sup>a</sup>             | 248                      |
| Oil of spike lavender <sup>b</sup>                            | 135                      |
| Oil of black jeera, lab. distilled <sup>a</sup>               | 230                      |
| Oil of <i>Curcuma amada</i> , lab. distilled <sup>a</sup>     | 266                      |
| Oil of <i>Piper longum</i> lab. distilled <sup>a</sup>        | 265                      |
| Oil of dry ginger, lab. distilled <sup>a</sup>                | 185                      |
| Oil of <i>Pimpinella anisum</i> , lab. distilled <sup>a</sup> | 296                      |
| Vanillin, pure, BDH <sup>b</sup>                              | 58                       |
| Menthol, pure, lab. distilled                                 | 0                        |
| Eugenol, pure, lab. distilled                                 | 275                      |
| Oil of peppermint, dementholized (Japan) <sup>b</sup>         | 68                       |

<sup>a</sup> Samples collected from different places.

<sup>b</sup> Samples procured from different companies.

Source: Kumar and Madaan (1979) (reproduced with permission).

**Table 3.3** Critical region (border line) for detection of adulterated oils by different adulterants<sup>a</sup>

| Properties<br>Adulterants added (%) | Marjoram | Petit grain<br>bigrade | Fennel  |
|-------------------------------------|----------|------------------------|---------|
| Specific gravity at 25 °C           | 0.94139  | 0.90820                | 0.97336 |
| Ethanol                             | >10      | >0.5                   | >15     |
| Paraffin oil                        | >10      | >0.5                   | >1      |
| Cottonseed oil                      | >20      | >5                     | >15     |
| Refractive index at 25 °C           | 1.44520  | 1.4919                 | 1.5198  |
| Ethanol                             | >20      | >0.5                   | >10     |
| Paraffin oil                        | >0.5     | –                      | >10     |
| Cottonseed oil                      | >0.5     | –                      | >10     |
| Specific optical rotation:          | 13.45    | 3.08                   | 6.39    |
| Ethanol                             | >5       | –                      | >10     |
| Paraffin oil                        | >5       | >40                    | >10     |
| Cottonseed oil                      | >5       | 40 only                | >105    |
| Ester number                        | 45.16    | 193.4                  | 17.22   |
| Ethanol                             | >15      | >5                     | >0.5    |
| Paraffin oil                        | >20      | >10                    | >0.5    |
| Cottonseed oil                      | >0.5     | –                      | >2      |

<sup>a</sup> Significant at 5 % level.

– Not detected.

### 3.3.6 Physical methods for detection of adulteration

Physical methods such as specific gravity at 25 °C, refractive index at 25 °C, specific optical rotation, freezing point, and chemical parameters such as ester number have been useful in detecting adulteration. Table 3.3 gives the critical region (borderline) for detection of a sample of essential oils by these different methods. Such physical properties including ester number should be considered as presumptive tests and should be confirmed by other, more specific, analysis. A freezing point lower than 10.5 °C is indicative of turpentine in peppermint oil (Lu, 1994). Colourimetric analysis of glycerol can indicate adulteration with edible oils. TLC of the hydrocarbon fraction, GLC, and IR are effective in detecting adulterant ethanol, edible oils, and liquid paraffins (Mostafa *et al.*, 1990b). The presence of cottonseed oil in different essential oils gave absorption bands characteristic of esters and unsaturated esters (at 1705–1720 cm<sup>-1</sup>), acetates (at 1245 cm<sup>-1</sup>), and the carbonyl group (at 1250–1170 cm<sup>-1</sup>), while the presence of paraffin oil gave a broadened absorption band at 3000 cm<sup>-1</sup> which characterizes the saturated and unsaturated hydrocarbons. Mineral oil in peppermint oil can be detected as turbidity, when peppermint oil is added to 60–80 % ethanolic solution (Lu, 1994).

### 3.3.7 Authentication of the botanical and geographical origin of essential oils

Authentication methods that can trace the botanical and geographical origin of essential oil constituents are of increasing importance to the perfume, pharmaceutical,

food, and fragrance industries. Aroma constituents of essential oils such as linalool and linalyl acetate have been traced to various botanical sources such as coriander, lavender, etc. Analysis of major volatile constituents has demonstrated the ratio of carvaerol/thymol to differentiate essential oils from four oregano species (Pino *et al.*, 1993). In recent times, essential oil authenticity, quality, extraction technique, geographic origin, and biogenesis have been demonstrated with high-resolution GC of the volatile fraction or enantioselective GC employing chiral stationary phases. Chirality can be used as a criterion for differentiation between components of natural and nature-identical types as the natural chiral signature can rarely be reproduced using synthetic compounds (Werkhoff *et al.*, 1991; Marriott *et al.*, 2001). GC has been used for the detection of adulteration of cinnamon bark oil with cinnamon leaf oil that has a higher content of eugenol compared to the bark oil. Addition of reconstituted oils to genuine lemon oils that causes an increase in the ratio of (–) to (+)-limonene has been detected by GC with non-chiral and chiral capillary columns (Dugo *et al.*, 1993). The enantiometric purity of carvone from essential oils of caraway, dill, and spearmint has been determined using appropriate enantioselective columns. While *S*(+)-carvone is detected in herb oils of caraway and dill, spearmint oils from various countries contain only *R*(–)-carvone (Ravid *et al.* 1992). GC on modified cyclodextrin phases has been demonstrated to detect the adulteration of caraway oil with added *R*(–)-carvone that normally appears only in small amounts in the genuine essential oil (Braun *et al.*, 2000).

GC–MS has been employed for the authentication and quality control issues associated with sandalwood oils (Howes *et al.*, 2004). The majority of trade oils, reportedly from *S. album*, contained approximately 50–70 % santalols (*Z*- $\alpha$  and *Z*- $\beta$ ) and did not comply with the internationally recognized standard of a 90 % santalol content. Rosemary essential oil of different geographical origins has been differentiated on the basis of GC–MS determination of natural constituents. While Spanish oils are rich in  $\alpha$ -pinene (19.4–24.7 %), 1,8-cineole (19.0– 21.8 %), and camphor (16.3–18.9 %), the French oils contain  $\alpha$ -pinene (19.9–35.1 %), 1,8-cineole (5.3–24.8 %), and bornyl acetate (1.2–14.3 %). Moroccan oils are typically rich in 1,8-cineole (43.5–57.7 %) (Chalchat *et al.*, 1993).

Isotope ratio mass spectrometry (IRMS) is another technique that shows promise in the discrimination of the natural or synthetic origin of essential oils. Some major compounds of essential oils such as carvacrol, thymol, and anethole are not chiral and hence are inaccessible to enantioselective analysis (Greule *et al.*, 2008). This is when the multi-element IRMS provides an accurate authenticity assessment of the essential oils. IRMS measurement of the major essential oil components is a proven tool for the authenticity assessment of savory and thyme oil.  $^2\text{H}/^1\text{H}$ -,  $^{13}\text{C}/^{12}\text{C}$ - and  $^{18}\text{O}/^{16}\text{O}$  IRMS analysis of carvacrol from origanum and savory oil, thymol from thyme oil, and *trans*-anethole from fennel oil, together with the IRMS analysis of the minor constituents, has been demonstrated for authenticity assessment of these essential oils (Greule *et al.*, 2008). The authenticity of several mandarin essential oils has been assessed with GC hyphenated to IRMS, conventional GC–FID, enantioselective GC, and HPLC (Schipilliti *et al.*, 2010). The combination of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values is useful in the authenticity control of mandarin oils (Faulhaber *et al.*, 1997). Authentication of saffron oil on the basis of  $\delta^{13}\text{C}/^{12}\text{C}$  of safranols, as measured by IRMS, has also been reported (Bigois *et al.* 1994).



Site-specific natural isotope fractionation studied by nuclear magnetic resonance (SNIF–NMR) combined with IRMS has been shown to characterize linalool and linalyl acetate from chemical synthesis and that extracted from essential oils of well-defined botanical and geographical origins (Hanneguëlle *et al.*, 1992). Site-specific hydrogen isotope ratios and the overall carbon isotopic parameter enabled the natural and synthetic species to be clearly distinguished.

The GC–IRMS method, however, has serious limitations, since the  $\delta^{13}\text{C}$  values of most C3 plants (including spices) partially overlap with those of synthetic substances of fossil origin. This can be overcome by using internal isotopic standards, which can then be used to obtain an ‘isotopic fingerprint’, typical of a plant. A genuine natural essential oil would then have  $\delta^{13}\text{C}$  values that are identical with the ‘isotopic fingerprint’. This approach has been successful with coriander essential oils (Frank *et al.*, 1995). The presence of  $^{14}\text{C}$  in cinnamaldehyde, as the main constituent in cinnamon essential oil, and its absence in the synthetic counterpart formed the basis of their distinction. Unfortunately, this technique was overcome by addition of  $^{14}\text{C}$  enriched cinnamaldehyde.

A strategy wherein the cinnamaldehyde is transformed into benzaldehyde via a controlled retroaldolization reaction followed by measuring the deuterium content in the  $^2\text{H}$ -NMR at a very high magnetic field can distinguish as little as 10–15 % synthetic cinnamaldehyde in cinnamon oil. This technique is superior to the IRMS technique, which determines the total deuterium content (Remaud *et al.*, 1997). Further, model studies with linalool and linalyl acetate have shown  $\delta^{13}\text{C}$  values to be influenced by the method and conditions used in their extraction (Weinrich and Nitz, 1992). Non-random distribution of deuterium exhibits large variations as a function of the origin of the sample. Discriminant analysis performed over the natural and synthetic families shows all synthetic samples to belong to the same group. Natural linalool is characterized by a strong depletion in the heavy isotope in site 1 and by a relative enrichment at site 6. Semi-synthetic linalool obtained from pinene can also be distinguished from natural linalool by virtue of its deuterium at site 3 of the sample. The discrimination between linalools of various botanical origins is however reported to be only 82 % effective (Hanneguëlle *et al.*, 1992). Very recently, an on-line gas chromatography pyrolysis isotope ratio mass spectrometry (GC–P–IRMS) has been developed that can easily bring out clear-cut origin-dependent differences in  $^2\text{H}/^1\text{H}$  ratios in case of E-2-hexenal and E-2-hexenol, demonstrating the importance and potential of this technique in authenticity studies of flavour constituents in complex natural matrices (Hor *et al.*, 2001). The combination of gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS) and GC–P–IRMS has been applied to the authenticity assessment of cinnamaldehyde from various sources (Sewenig *et al.*, 2003).  $\delta^2\text{HV}$ –SMOW and  $\delta^{13}\text{CV}$ –PDB values of cinnamaldehyde and characteristic and authenticity ranges allowed differentiation between natural and synthetic samples.

### 3.4 Future trends

Recently, DNA markers have become an increasingly popular means for the identification and authentication of a large range of food products and aromatic plants.

Sequence characterized amplified regions markers (SCAR) primers specific for adulterants have been developed from random amplified polymorphic DNA (RAPD) markers to give rise to amplification of specific bands that allow detection of adulterating plants (Marieschi *et al.*, 2011). Juliani *et al.* (2006) has demonstrated the use of near-infra-red (NIR) technology as a quality tool for the rapid identification of individual essential oils and detection of adulteration. The method studied could discriminate between *Ravintsara* and *Ravensara* essential oils wrongly labelled as *R. aromatica* in the marketplace. LC–MS equipped with an APCI (atmospheric pressure chemical ionization) source has been successfully used for the rapid identification and characterization of oxygen heterocyclic compounds of citrus oils such as coumarins, psoralens, and polymethoxylated flavones and for the detection of authenticity and adulteration of the oils (Dugo *et al.*, 1999). Tandem mass spectrometry (ESI–MS/MS) has been able to help authenticate and detect adulteration of spice essential oils (Nhu-Trang *et al.*, 2006). Polar components of the essential oils of the spices *O. dictamnus*, *O. vulgare*, *O. majorana*, and *R. officinalis* belonging to the Labiatae family, investigated by ESI–MS, both in the negative and positive ion modes have been shown to serve for a fast identification of these species (Moller *et al.*, 2007).

The differentiation between compounds that are grown naturally, produced by fermentation, or synthesized chemically is projected to reflect in legal regulations in the coming years. Hence, intensive and comprehensive basic investigations on the analytical origin assessment of flavours will gain ground.

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## 4

# Basil

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**Abstract:** Basil (*Ocimum basilicum*) is an annual spicy herb indigenous to India which has been cultivated for several millennia for its aromatic and medical uses. This chapter gives a definition of basil before examining the chemical composition of the different varieties of the plant. Production of basil is discussed in relation to soil and climate requirements, propagation, planting and fertilizing; organic production is also covered. The chapter then looks at postharvest handling and storage of the leaves and essential oil and extraction of the oil. The culinary and medicinal uses of basil and its functional properties are described before the chapter finishes with a discussion of quality issues and toxicity.

**Key words:** basil, *Ocimum basilicum*, essential oil, functional properties, quality, toxicity.

### 4.1 Introduction: the origin of basil

Basil (*Ocimum basilicum*, Lamiaceae) (also known as sweet basil) is an annual spicy herb, indigenous to India. Several species of *Ocimum* are cultivated in India, where their medicinal and aromatic uses have been known for several millennia. The ancient Ayurvedic surgeon Sushruta classified *Ocimum* as a green leafy vegetable, while Bhavamisra, a famous Ayurvedic Acharya, referred to *Ocimum basilicum* as ‘barbari’ (Pushpangadan *et al.*, 1993). It is also mentioned in classical Ayurvedic texts such as *Sushruta Samhita*, *Charaka Samhita*, *Bhavaprakasham*, and *Ashtangahridayam*, among others. Sweet basil is native to India and tropical Asia, and now grows wild in tropical and sub-tropical regions (Ayurnepal, 2012) including Central Africa and South East Asia (Simon, 1998). It is cultivated commercially in many warm and temperate countries worldwide, including France, Hungary, Greece and other southern European countries, Egypt, Morocco and Indonesia. It is also cultivated in several US states (Christman, 2010), including Arizona, New Mexico and North Carolina, as well as in California, where a superior quality of leaf is grown (Prakash, 1990).

**Basil can be classified as follows** (Kartesz, 2009):

- kingdom: *Plantae* – plants
- sub-kingdom: *Tracheobionta* – vascular plants

- superdivision: *Spermatophyta* – seed plants
- division: *Magnoliophyta* – flowering plants
- class: *Magnoliopsida* – dicotyledons
- sub-class: *Asteridae*
- order: *Lamiales*
- family: *Lamiaceae* – mint family
- genus: *Ocimum* L. – basil

#### 4.1.1 Definition of basil

*Ocimum basilicum* is an erect, almost glabrous herb, which grows to between 30 and 90 cm high. The leaves are ovate, lanceolate, cucuminate, toothed or entire, glabrous on both surfaces and glandular. When mature, they reach approximately 5 cm in length, excluding the petiole, which is approximately 2 cm long. The upper surface is smooth and lustrous; on the lower surface along the midrib and on the petiole short, stiff hairs occur sparingly (Prakash, 1990).

The flowers are white or pale purple and are borne in long terminal racemose inflorescences, in simple or many branched racemes. The greenish corolla is small and inconspicuous. The calyx is partly grown together with the branches, and enlarges itself after flowering, remaining dry on the plant with the branches. The capitate hairs have commonly a two-celled head with a stalk so short as to appear sessile.

Polymorphism and cross-pollination under cultivation have given rise to a number of sub-species and varieties differing in height, habitat and growth, degree of hairiness and colour of stems and in their leaves and flowers.

## 4.2 Chemical composition of the basil plant

The flowers yield an average of 0.4 % oil while the whole plant contains 0.1–0.25 % oil (figures refer to Indian basil). By taking the initial three to four harvests of flowers (including main and sub inflorescences) and final harvest of the whole herb, approximately 3–4 t of flowers and 13 t of whole herb per hectare can be obtained, corresponding to about 13 kg of the flower oil and about 27 kg of whole herb oil, a total of 40 kg of oil per hectare. The oil of sweet basil produced both from the herb and flowers has commercial value: it has a clove-like scent with an aromatic and somewhat saline taste, and is used as a flavouring agent and as a perfume (Pruthi, 1976; Farrell, 1985).

Pushpangadan and Bradu (1995) reported that the essential oil composition of *O. basilicum* differed between varieties, finding different varieties of the herb to be rich in methyl chavicol, linalool, methyl cinnamate and geraniol. Based on thin-layer chromatography (TLC) and gas chromatographic studies they established that *O. basilicum* L. var. *minima* Benth. contained geraniol (45 %) and eugenol (25 %) as the major compounds; *O. basilicum* L. var. *glabratum* Benth., chemotype No. 1 contained methyl chavicol (38 %) and linalool (35 %); *O. basilicum* L. var. *glabratum* Benth., chemotype No. 2 contained linalool (47 %) and eugenol (20 %) as the major components; *O. basilicum* L. var. *glabratum* Benth., chemotype No. 3 contained

linalool (40 %), eugenol (20 %) and camphor (20 %); *O. basilicum* L. var *purpurascence* Benth. contained methyl cinnamate (20 %) and linalool (60 %); *O. basilicum* L. var *tryrsiflora* Benth. contained methyl cinnamate (35 %) and linalool (60 %); *O. basilicum* L. var *crispum* Benth. contained methyl chavicol (50 %) and linalool (28 %); and *O. basilicum* L. var *darkapal* contained geraniol (35 %), linalool (35 %) and eugenol (25 %).

Ji-Wen *et al.* (2009) studied the composition of essential oil obtained from the aerial parts of *O. basilicum* Linn. var. *pilosum* (Willd.) Benth., an endemic medicinal plant growing in China. They identified linalool (29.68 %), (*Z*)-cinnamic acid methyl ester (21.49 %), cyclohexene (4.41 %),  $\alpha$ -cadinol (3.99 %), 2,4-diisopropenyl-1-methyl-1-vinylcyclohexane (2.27 %), 3,5-pyridine-dicarboxylic acid, 2,6-dimethyl-diethyl ester (2.01 %),  $\beta$ -cubebene (1.97 %), guaia-1(10),11-diene (1.58 %), cadinene (1.41 %), (*E*)-cinnamic acid methyl ester (1.36 %) and  $\beta$ -guaiene (1.30 %) all present in this oil.

Koba *et al.* (2009) described five chemotypes of *O. basilicum* (Lamiaceae) from Togo. They are the estragole type; the linalool/estragole type; the methyleugenol type; the methyleugenol/t-anethole type and the t-anethole type.

Thus the essential oil composition of *O. basilicum* varies according to the variety, geographic origin, harvesting season, etc. This considerably affects the aroma and flavour characteristics of the oil. A literature search conducted by Lee *et al.* (2005) revealed that the oil constituents belonged to different classes of compounds, including mono and sesquiterpene hydrocarbons, oxygenated mono and sesquiterpenes, aliphatic alcohols, aldehydes, esters, ketones, acids, aromatic compounds, and so on. A list of the compounds identified in basil oil is presented in Table 4.1.

## 4.3 Production of basil

### 4.3.1 Soil and climate requirements

*Ocimum* species thrive well in a variety of soils and climatic conditions. Soils suitable for cultivation are rich loam to poor laterite, and saline and alkaline to moderately acidic. Well-drained soil helps to encourage improved vegetative growth. Basil flourishes well under fairly to high rainfall and humid conditions, and long days and high temperature have been found to be favourable for plant growth and higher oil production. *O. sanctum* can be grown in partially shaded conditions, but this leads to a low oil yield. The above factors make tropical and sub-tropical climates ideal for basil cultivation (Pushpangadan and Bradu, 1995).

### 4.3.2 Seed and propagules

Since the *Ocimum* species are generally highly cross-pollinated, a certain amount of heterozygosity is essential for vigorous growth, high oil yield and high-quality oil. These characteristics are mostly controlled by polygenes whose effect is mostly additive. The seeds are therefore likely to deteriorate in future generations unless the selected lines from which the polycross seed is produced are maintained. It is also crucial that fresh seed is collected from the polycross lines each time. For fresh

**Table 4.1** Different compounds identified in basil oil

|                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Monoterpene hydrocarbons</b>   | $\alpha$ -Pinene, sabinene, myrcene, <i>p</i> -cymene, limonene, $\alpha$ -terpinene, (Z)- $\beta$ -ocimene, (E)- <i>cis</i> -ocimene, $\gamma$ -terpinene, terpinolene                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Oxygenated monoterpenes</b>    | 1,8-Cineole, linalool <i>cis</i> -furanoid, linalool oxide <i>cis</i> -furanoid, <i>trans</i> -sabinene hydrate, linalool <i>trans</i> -furanoid, linalool oxide <i>trans</i> -furanoid, ocimene oxide, camphor, 3,7-dimethyl-1,6-octadien-3-ol, linalool, linalyl acetate, <i>trans</i> - <i>p</i> -menth-2-en-1-ol, bornyl acetate, carvacryl methyl ether, exo-methylcamphenilol, 4-terpineol, <i>cis</i> -dihydrocarvone, hotrienol, terpinen-1-ol, l-menthol, <i>trans</i> -pinocarveol, d-terpineol, lavandulol, <i>trans</i> -verbenol, <i>p</i> -menth-1,8-dien-4-ol, terpinyl formate, $\alpha$ -terpineol, borneol, verbenone, exo-2-hydroxycineole acetate, dihydrocarveol, $\alpha$ -citral, exo-2-hydroxycineole, l-carvone, linalool oxide <i>cis</i> -pyranoid, <i>trans</i> -piperitol, linalool oxide <i>trans</i> -pyranoid, citronellol, yrtanol, nerol, <i>trans</i> -carveol, <i>p</i> -cymen-8-ol, geraniol, geranyl acetate, guaiacol, exo-2-hydroxycineole, piperitenone, l-perillyl alcohol, cuminyl alcohol, fenchone, estragole, <i>t</i> -anethole, carvacrol, thymol, bornyl acetate, methyleugenol, geranyl formate |
| <b>Sesquiterpene hydrocarbons</b> | $\beta$ -Cubebenec, $\delta$ -cadinene, valencene, $\alpha$ -amorphene, $\delta$ -selinene, dehydroaromadendrene, $\beta$ -elemene, $\alpha$ -copaene, $\beta$ -caryophyllene, $\alpha$ -humulene, (–) calamenene (E)- $\alpha$ -bergamotene, $\alpha$ -caryophyllene, germacrene D, $\beta$ -selinene, $\alpha$ -zingiberene, bicyclgermacrene, $\alpha$ -muurolene, germacrene A, germacrene D, $\gamma$ -cadinene                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Oxygenated sesquiterpenes</b>  | $\Gamma$ -Cadinol, spathulenol, caryophyllene oxide, $\alpha$ -humulene oxide, elemol, viridiflorol, spathulenol, $\alpha$ -cadinol, $\Gamma$ -murolol, $\beta$ -bisabolol, $\beta$ -bisabolol isomer, $\alpha$ -eudesmol, isospathulenol, $\beta$ -eudesmol, caryophylla-4(12),8(13)-dien-5 $\beta$ -ol, dihydroactinidiolide, caryophylla-3,8(13)-dien-5 $\alpha$ (or $\beta$ )-ol                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Aliphatic alcohols</b>         | 1-Penten-3-ol, 3-methyl-3-buten-1-ol, (Z)-2-pentenol, 3-methyl-2-buten-1-ol, hexanol, (Z)-3-hexenol, 3-octanol, cyclohexanol, 1-octen-3-ol, octanol                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Aliphatic aldehydes</b>        | Hexanal, (E)-2-hexenal, (E,Z)-2,4-heptadienal, (E,E)-2,4-heptadienal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Aliphatic esters</b>           | Methyl 2-methylbutyrate, (Z)-3-hexenyl acetate                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Aliphatic ketones</b>          | 3-Octanone, 3-hydroxy-2-butanone, 6-methyl-5-heptenone, 6-methyl-(E,E)-3,5-heptadien-2-one, $\beta$ -ionone, <i>cis</i> -jasmane, <i>trans</i> - $\beta$ -ionone-5,6-epoxide, methyl jasmine                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Aliphatic acids</b>            | Butanoic acid, octanoic acid, decanoic acid                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Aromatic compounds</b>         | Benzaldehyde, methyl benzoate, phenyl acetaldehyde, 1-methoxy-4-(2-propenyl) benzene, methyl salicylate, <i>p</i> -methylacetophenone, cuminaldehyde, anethol, safrole, benzyl alcohol, phenethyl alcohol, methyl cinnamate, methyl eugenol, $\alpha,\alpha$ -dimethylphenylethyl alcohol, anisaldehyde, <i>trans</i> -cinnamaldehyde, methyl cinnamate, <i>p</i> -cresol, ethyl cinnamate, eugenol, 2-isopropyl-5-methylphenol (thymol), 2-isopropyl-2-methylphenol (carvacrol), 5-isopropyl-3-methylphenol, 4-allylphenol, dillairole, <i>p</i> -methoxycinnamaldehyde                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Miscellaneous compounds</b>    | 2,6-Dimethylpyrazine, <i>c</i> -butyrolactone, myristicin (Lee <i>et al.</i> , 2005)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

plantings, the growers must take fresh seeds from the pedigree stock. Selected lines can be multiplied vegetatively by growing tender shoot tips. Good-quality planting seeds can be obtained from reputed seed companies.

### 4.3.3 Nursery practices

Plantations can be created either by raising the seedlings in the nursery and then planting them in the field or by direct sowing of seeds in the field.

#### *Direct sowing*

Experiments have shown that direct sowing is more efficient, economical and profitable than raising seedlings in a separate nursery and then transplanting them to the field. The seeds (75–250 g/ha) are mixed with dry sand to ensure an even distribution. Before sowing, the field is ploughed into long narrow furrows at a spacing of 50–60 cm; the seed is then sown in rows by hand or drilled. After the seeds are sown, the field is worked to cover the seeds. The field must be flooded with water within 24 hours of sowing, if there is no rain.

The seeds germinate within 10–15 days. After 20–25 days, when the seedlings have grown to 15–20 cm high, the first weeding and thinning or gap-filling can be carried out, if required.

#### *Nursery sowing*

An alternative method is the raising of seedlings in a nursery before transferring them to the field. The nursery seed beds should be prepared and treated with farm-yard manure. For 1 ha of land, approximately 200–300 g seeds should be planted. Once the seeds are sown, more manure, mixed with soil, should be thinly spread over the seeds. The beds should then be irrigated with a sprinkler hose. After approximately 8–12 days, the seeds will germinate, and the seedlings will be ready to be transplanted to the field around 6 weeks after planting. The plants can be sprayed with a 2% urea solution 15–20 days prior to transplanting; this helps to ensure healthy plants for transplanting.

#### *Transplanting*

Approximately 6–7 weeks after planting, the seedlings are ready to be transplanted. At this stage, the seedlings should have four to five leaves. Seedlings are usually transplanted in April; however, if the seedlings have been raised in hot beds, transplanting may be carried out in March.

Spacing of 40–60 cm<sup>2</sup> has been found to be most suitable for *Ocimum* species. For *O. basilicum*, a spacing of 60 × 60 cm was recommended by Singh *et al.* (1970) for Assam and by Gulati *et al.* (1977) for Haldwani in Uttar Pradesh, India.

When the second irrigation is performed, the seedlings are already well established. This is the stage at which gaps should be filled and any weak plants replaced, for the purposes of uniformity. During the summer, plants should be irrigated three times a month; outside of this period, irrigation should be carried out as necessary, except during the rainy season when no irrigation is required. Over the year, irrigation should be carried out approximately 12–15 times.

#### 4.3.4 The effect of manures and fertilizers

Between 20 and 25 kg of nitrogen and 10–15 kg  $P_2O_5$  per hectare ensures good vegetative growth, herb and oil yield. The application of up to 75 kg/ha of nitrogen has also been found to be beneficial for optimum herb and oil yield.

#### 4.3.5 Organic basil

The use of inorganic fertilizers should be avoided as far as possible since this may reduce the therapeutic property of basil. Organic materials such as farmyard manure, biogas slurry, compost, neem cake and other oil seed cakes, biofertilizers, green manure and cover crops can be used as substitutes for inorganic fertilizers. The use of organic manure will increase the organic matter of the soil, reducing the bulk density and increasing the water-holding capacity. Both of these factors improve the fertility of the soil, especially when the soil is sandy in texture and low in organic matter. These should preferably be incorporated into the soil at the start of summer, as their decomposition rate increases with warm weather. Moreover, the use of organic manure is preferable as it conserves nutrients by preventing leaching losses, and releases them as a continual process. Organic manures also help in reducing soil erosion, while crop rotation and green manures involving legumes add to soil fertility. For the control of pests, bio-insecticides such as tobacco extract, turmeric extract, garlic extract, garlic–neem extract, neem seed oil emulsion and neem seed kernel extract can be used.

### 4.4 Post-harvest handling and production of basil

In countries with a Mediterranean climate, three to five cuttings per year may be taken of most *Ocimum* species, with the first 90–95 days after planting, and subsequent harvesting taking place at intervals of 60–75 days. In the more northern temperate zones, however, only one or two cuttings are usually possible in a year, one early in the summer with a low yield, and one normally just prior to flowering or in full bloom, depending on the intended use (Simon, 1995).

#### 4.4.1 Post-harvest handling and storage for fresh or dried leaves

If the basil is grown for its leaves, either fresh or dried, the main cutting is taken just prior to flowering, while the leaves are still young. The plant is cut at least 10–15 cm from the ground, above the bottom two to four sets of true leaves, in order to allow for re-growth. For large-scale and commercial purposes, harvesting may be carried out with a modified sickle bar or jerry mover with an adjustable cutting height. To ensure a continuous supply of fresh leaves, the field harvest and planting dates are normally staggered (Simon, 1995).

Once harvested, the leaves should first be washed and cleaned to remove weeds and extraneous materials. For the fresh market, only the highest quality plant material should be used, i.e. that with the best colour and aroma retention. The leaves can be preserved by hanging the foliage upside down in small bunches and air drying in a warm, dry, well-ventilated room. Foliage can also be dried by spreading flat on



a drying rack under the same conditions. Once the basil is thoroughly dried, the leaves are stripped from the stems and stored whole or chopped in an air-tight container away from heat sources and bright light. In terms of colour and aroma retention, the best results are obtained when the plant is dried below 30°C under vacuum in suitable containers. The leaves can also be preserved by storing them in small plastic bags in a freezer, or in jars of oil. If the leaves and flowers are to be processed, they should be dried at below 35°C before milling or grinding to ensure the best colour retention.

Basil cannot be stored for long periods after harvest, as this will result in a reduction in quality. However, storage at 10°C in PVC packaging reduces mould and yeast colonies and can maintain the microbiological quality of basil for up to 9 days after harvesting (Franceli *et al.*, 2005). At 5°C, the post-harvest shelf-life of basil is only 3–4 days; after this the visual quality deteriorates as the symptoms of chilling injury begin to appear. Lange and Cameron (1997) showed that an extended post-harvest shelf-life of up to 7 days can be obtained by chill-hardening the plants before harvesting and packaging. Chill-hardening should be carried out at 10°C for 2 hours at the end of the light period and 2 hours at the beginning of the dark period each day for 2 days. The same study further examined the effect of chill-hardening on 4–6 week-old plants. The plants were chill-hardened over the course of a week at different periods of the day: 4–5 week-old plants chill-hardened at the beginning of the day displayed an extended shelf-life (1–1.5 days longer), but other periods of pre-harvest chill-hardening either had no effect or actually decreased the shelf-life. Furthermore, packaged basil that was chill-hardened post-harvest for 1 day at 10°C in darkness before being transferred to 5°C had a shelf-life 5 days longer than the non-chill-hardened product. Post-harvest chill-hardening is a promising, effective and convenient method of extending the post-harvest shelf-life of packaged sweet basil.

Amodio *et al.* (2005) investigated the regulatory effects of the post-harvest use of 2.5 kPa CO<sub>2</sub> and 1-methylcyclopropene (1-MCP), which inhibits ethylene action, at 0.7 µmol on accelerated senescence in basil (Kenigsbuch *et al.*, 2009). They found that a reduction in the oxygen content of the storage atmosphere over a period of 20 days led to basil leaves of higher quality than those stored in air. No significant improvement was observed on the addition of 3 % CO<sub>2</sub>, although this concentration also did not cause CO<sub>2</sub> injury.

#### **4.4.2 Post-harvest handling and storage for essential oil**

When basil is grown for its essential oil, the plant is harvested during full bloom. The time of the harvest plays an important role in the quantity and quality of oil produced. The crop is usually harvested on bright sunny days in order to achieve a good oil yield and high-quality oil; harvesting should be avoided on rainy days or the day after rains. The crop should be cut at 15–20 cm above the ground level, and only the flowers and leaves used for processing, since the oil content in the stem is negligible. In a study by Jose *et al.* (2006), it was shown that a higher yield of essential oil can be obtained if the crop is harvested between 8:00 h and 12:00 h. The leaves should then be washed and any weeds and other material removed, just as for fresh cut basil. The length and manner of storage of the crop before processing also affects

the essential oil content. Basil is usually field-dried for 1–3 days before processing. However, the longer the basil shoots are stored, the greater the decrease in the chlorophyll and essential oil content of the plant, which is accompanied by an increase in eugenol and linalool content. Jose *et al.* (2006) found that when the biomass is dried at 40 °C for a period of 5 days, the concentration of linalool is raised from 45.18 % to 86.80 %.

#### **4.4.3 Extraction of the essential oil**

The extraction of essential oils from plants is discussed in detail in the work of Baby and George (2009), as follows:

Extraction of volatile oils from plants was known to almost all ancient civilizations. The extraction techniques have undergone constant refinements throughout the ages. The most common methods now employed are hydrodistillation, steam distillation, supercritical extraction and microwave extraction. The extraction technique is a careful choice based on the nature of the plant specimen and thermal lability of the target oil constituents.

##### *Steam distillation*

Steam distillation is carried out by passing dry steam through the plant material whereby the steam volatile compounds are volatilized, condensed and collected in receivers. Steam distillation has been in use for essential oil extraction for many years. Hydrosteam distillation is carried out when the perfumery plant material is susceptible to direct steam. In this technique the plant material is supported on a screen or a perforated grid placed at some distance above the bottom of the still. Distillation is carried out with low pressure steam which replaces the volatile compounds from the intact plant material.

##### *Hydrodistillation*

Hydrodistillation is carried out by boiling the plant material with water and the volatile compounds in the plant material are carried away along with steam which is then condensed and collected in suitable receivers.

##### *Supercritical fluid extraction*

Supercritical fluid extraction uses CO<sub>2</sub> under high pressure to extract essential oils. This is an environmentally friendly process. The plant specimen is placed in a stainless steel tank and CO<sub>2</sub> injected into this tank. Under high pressure CO<sub>2</sub> liquefies and acts as a solvent to extract the essential oil from the plant specimen. When the pressure is decreased, CO<sub>2</sub> returns to the gaseous state leaving the volatile oil. Supercritical fluid extraction is gentler on plant samples compared to steam distillation and results in fresher, cleaner and crisper oils which smell similar to the natural plant aroma.

##### *Microwave extraction*

Solvent free microwave extraction (SFME) is another recently developed green technology in which a combination of microwave heating and dry distillation at atmospheric pressure results in the extraction of essential oils from plant samples.

SFME results in substantial savings in time, energy, solvents and plant material. The oil obtained by microwave extraction can be used directly for GC and GC–MS studies without further purification. Studies have shown that the oil obtained by SFME is similar in yield and quality to that obtained by hydrodistillation for several hours.

#### *Hydrodistillation and steam distillation of basil oil*

While all of the above methods may be used for the extraction of basil oil, hydrodistillation and steam distillation are most commonly used. The flowers or/and whole herbs are put into a distillation unit and hydrodistilled or steam distilled. It takes about 4 hours to complete one charge. The oil, being lighter than water, can easily be separated from the mixture. Once the oil has been removed, the distillate should be distilled a second time as a small quantity of oil may remain. Care should be taken to see that the distillation unit is clean and free from contamination with residual oils from other sources.

#### *Essential oil quality*

As discussed above, the quality and quantity of the essential oil produced may be affected by a number of factors before processing, including time of harvest and storage method. Once the oil has been extracted, further measures may be taken to ensure a high-quality oil.

Basil oil contains a large number of terpenic hydrocarbons. If these hydrocarbons are removed, the oil is terpene-free and thus fetches a better price in the market. In order to prepare terpene-free oil, basil oil is subjected to fractional distillation under vacuum in fractionating columns. The fractionating columns are also used to isolate major components present in the oil.

Over time, essential oils may darken and deteriorate as a result of contact with light, heat and air. This deterioration can be caused by oxidation, resinification, polymerization, hydrolysis of esters and the interaction of functional groups. Basil oil, along with all other essential oils, must therefore be stored in a cool dark environment, in opaque air-tight containers. It is also crucial to ensure that the oil is free from moisture and any other impurities prior to storage.

## **4.5 Main uses of basil**

### **4.5.1 Basil as a flavouring agent in foods**

Basil leaves are widely used for flavouring purposes (Niir Board, 2005) in soups, meat pies, fish dishes, certain cheeses, tomato salads, cooked cucumber dishes, cooked peas, squash, and string beans as well as vinegars and oils. Chopped basil may be sprinkled over lamb chops before cooking. Basil is an important seasoning in tomato paste products in Italy, and is often used with or as a substitute for oregano in pizza toppings, spaghetti sauces, meat balls, or in macaroni and cheese bakes.

The essential oil of *O. basilicum* obtained by distillation is used in a number of food products as a flavouring agent and is also used in perfumery thanks to its aromatic characteristics. It contains cineol, pinene, methyl chavicol, d-camphor and ocimene (Eltohami, 1997). The major aroma constituents of basil are

3,7-dimethyl-1,6-octadien-3-ol (linalool; 3.94 mg/g), 1-allyl-4-methoxy benzene (estragole; 2.03 mg/g), methyl cinnamate (1.28 mg/g), 4-allyl-2-methoxyphenol (eugenol; 0.896 mg/g), and 1,8-cineole (0.288 mg/g) (Lee *et al.*, 2005).

Examples of food products that may be flavoured with basil essential oil include confectionery, baked goods, condiments, spiced meats, ice creams, puddings, liquors and non-alcoholic beverages (Prakash, 1990). The oil may also be used as a flavouring for certain dental and oral hygiene products.

#### 4.5.2 Uses of basil in traditional medicine

The leaves and infusions of *O. basilicum* are widely used in traditional medicine. In some Mediterranean areas, such as Eastern Morocco, they are used to decrease plasma lipid content (Zhang *et al.*, 2009), while the Santhal tribe of India use sweet basil for headache, earache, cough, cold, inflammation, snake bite and rabies (Pushpangadan *et al.*, 1993). Other reported medicinal uses of basil leaves include the treatment of diarrhoea, dysentery, constipation, flatulence and worms (Simon *et al.*, 1999); as an analgesic and insect repellent (Pushpangadan *et al.*, 1993); to relieve the symptoms of bronchitis, flu, colds, coughs and sinusitis; and as a cure for rheumatism, muscle aches, gout and exhaustion. The leaves are also reported to be effective in the treatment of warts; an ointment made of basil leaves can be used as a treatment for insect bites and can be applied directly to the skin as a cure for acne (Waltz, 1999).

The juice expressed from the leaves also has a number of therapeutic uses: it relieves the symptoms of cold and cough, and those of croup, when mixed with honey. It is also used as a treatment for toothache, earache and headache and can be mixed with camphor to stop nasal haemorrhage. It is said to give lustre to the eyes, and forms an excellent nostrum for the cure of ring worms, scorpion sting and snake bite.

Basil seeds steeped in water and eaten are said to be cooling and very nourishing. The seeds are chewed as a treatment for snake bite (Kirtikar and Basu, 1935). The washed and pounded seeds are used in poultices for unhealthy sores and sinuses, and are also used in *sharbat* for the treatment of chronic constipation and in internal piles. A teaspoon full of seed infused in a tumbler of water with a little sugar, when taken daily, acts as a demulcent in genito-urinary disease; a cold infusion of seeds is said to relieve afterpains of childbirth; and an infusion of seed is also given in fever (Dastur, 1970). The aqueous extract of the seeds is used as a diuretic (Pushpangadan *et al.*, 1993). Finally, the roots of the plant are used for bowel complaints in children (Chopra *et al.*, 1956).

#### *Use in Ayurvedic medicine*

*O. basilicum* is referred to as 'barbari' by Bhavamisra. According to Ayurveda, the plant is used for diseases caused by aggravation of Kapha and Vata while the seeds are used for pacifying aggravation of Vata and Pitta. The medicinal properties of *O. basilicum* are described in a number of classical Ayurvedic texts such as the Sushruta Samhita, Charaka Samhita, Ashtangahridaya, Bhavaprakasham, Danwanthari Nighandu and Kaiyadeva Nighandu (Pushpangadan *et al.*, 1993). In Ayurveda, the whole plant is used to treat cough, asthma, bronchitis, ophthalmia, giddiness, inter-

mittent and malarial fever, catarrh, otalgia, cephalalgia, dyspepsia and spasmodic affections (Udayan and Balachandran, 2009). The plant is considered stomachic, stimulant, carminative, diaphoretic, expectorant, diuretic and antipyretic (Kirtikar and Basu, 1935).

## 4.6 Functional properties of basil

### 4.6.1 Basil as an antioxidant

Rosmarinic acid isolated from the leaves of *O. basilicum* has been found to be responsible for its antioxidant activity. The nature of the antioxidant activity of rosmarinic acid in the liposome system was examined by Jayasinghe *et al.* (2003): the results showed that one rosmarinic acid can capture 1.52 radicals and that there is a synergistic effect between  $\alpha$ -tocopherol and rosmarinic acid. Durga *et al.* (2009) showed that various concentrations (50, 100, 250 and 500  $\mu\text{g/ml}$ ) of acetone and ethanol extracts of *O. basilicum* displayed antioxidant activities, which varied by concentration. The 500  $\mu\text{g/ml}$  concentration of ethanol extract displayed 75.87 % activity, which is very close to the level displayed by a 500  $\mu\text{g/ml}$  concentration of  $\alpha$ -tocopherol (82.14 %), the reference compound. The activity of the extract increased with the increase in polar solvent, suggesting that polyphenols, flavanone and flavonoids affect the activity level. The ethanolic extract of the leaves of *O. basilicum* showed significant antilipid peroxidation effects *in vitro*, besides exhibiting significant activity in superoxide radical and nitric oxide radical scavenging in goat liver (Meera *et al.*, 2009).

### 4.6.2 Basil as an antimicrobial agent

Basil has shown strong inhibitory effect against multi-drug resistant clinical isolates from the genera *Staphylococcus*, *Enterococcus*, and *Pseudomonas* (Opalchenova and Obreshkova, 2003). In a study by Adiguzel *et al.* (2005) ethanol, methanol, and hexane extracts from *O. basilicum* were investigated for their *in vitro* antimicrobial properties. The result showed that none of the three extracts tested have antifungal activities, but they do have anticandidal and antibacterial effects. Both the hexane and methanol extracts, but not the ethanol extracts, inhibited three isolates from the 23 strains of *Candida albicans* studied. The hexane extract showed a stronger and broader spectrum of antibacterial activity, followed by the methanol and ethanol extracts, which inhibited 10, 9 and 6 % of the 146 bacterial strains tested, respectively (Adiguzel *et al.*, 2005). Chiang *et al.* (2005) used extracts and purified components of *O. basilicum* to identify possible antiviral activities against DNA viruses (herpes viruses (HSV), adenoviruses (ADV) and hepatitis B virus and RNA viruses (coxsackievirus B1 (CVB1) and enterovirus 71 (EV71)). The results showed that crude aqueous and ethanolic extracts of *O. basilicum* and selected purified components, namely apigenin, linalool and ursolic acid, exhibit a broad spectrum of antiviral activity. Of these compounds, ursolic acid showed the strongest activity against HSV-1, ADV-8, CVB1 and EV71, whereas apigenin showed the highest activity against HSV-2, ADV-3, hepatitis B surface antigen and hepatitis B e-antigen and linalool showed the strongest activity against ADV-II. No activity was noted for

carvone, cineole,  $\beta$ -caryophyllene, farnesol, fenchone, geraniol,  $\beta$ -myrcene and  $\alpha$ -thujone (Chiang *et al.*, 2005). The essential oil from *O. basilicum* and its purified compounds, especially linalool, have anti-giardial activity. Linalool (300  $\mu$ g/ml) was able to kill 100 % parasites after one hour of incubation, which demonstrates its high anti-giardial potential (de Almeida *et al.*, 2007).

Kaya *et al.*'s (2008) study used the disc diffusion method to test the antimicrobial activity of chloroform, acetone and two different concentrations of methanol extracts of *O. basilicum* L *in vitro* against 10 bacteria and four yeast strains. While no effect was observed with the chloroform and acetone extracts, the methanol extracts of *O. basilicum* proved effective against the bacteria and yeast strains tested. Inhibition zones against strains of *Pseudomonas aeruginosa*, *Shigella* sp., *Listeria monocytogenes*, *Staphylococcus aureus* and two different strains of *Escherichia coli* were observed. The volatile oils of *O. basilicum* inhibited the growth of *Klebsiella pneumoniae* at a concentration of 0.51 % in the agar; *Streptococcus viridians* and *S. albus* at 1.10 % and *P. aeruginosa* at 10.0 %. *Proteus vulgaris* was inhibited at 0.67 % by *O. basilicum* in dental isolates (Ahonkhai *et al.*, 2009).

Hossain *et al.* (2010) examined the antibacterial activity of the essential oils (10  $\mu$ L/disc of 1:5, v/v dilution with methanol) and methanol extracts (300  $\mu$ g/disc) of *O. basilicum*. The results showed that both the essential oils and methanol extracts exerted antibacterial activity against *Bacillus cereus*, *B. subtilis*, *B. megaterium*, *S. aureus*, *L. monocytogenes*, *E. coli*, *Shigella boydii*, *S. dysenteriae*, *Vibrio parahaemolyticus*, *V. mimicus* and *Salmonella typhi* murium. The zones of inhibition were 11.2–21.1 mm and the MIC values were 62.5–500  $\mu$ g/mL. Basil oil had the strongest antimicrobial activity against *Salmonella enteritidis* SE3. The composition of the oil, as revealed by GC–MS analysis, was found to be: linalool (64.35 %), 1,8-cineole (12.28 %), eugenol (3.21 %), germacrene D (2.07 %),  $\alpha$ -terpineol (1.64 %) and *p*-cymene (1.03 %). Rattanachaikunsopon and Phumkhachorn (2010) experimentally inoculated *nam*, a fermented pork sausage, with *S. enteritidis* SE3, applied basil oil and stored the product at 4°C. It was found that the oil inhibited the *S. enteritidis* in a dose-dependent fashion, as follows: a concentration of 50 ppm reduced the number of bacteria from 5 to 2 log cfu/g after 3 days of storage; a concentration of 100 ppm resulted in an unmeasurable level of bacteria after 2 days; and a 150 ppm concentration led to unmeasurable levels of bacteria after 3 days.

A further study of the antimicrobial properties of basil oil was carried out by Edris and Farrag (2003). Linalool and eugenol, two major constituents of the oil, were tested against a number of fungi that cause deterioration and severe decay in peaches during marketing, shipping and storage, namely *Sclerotinia sclerotiorum* (Lib.), *Rhizopus stolonifer* (Ehrenb. exFr.) Vuill and *Mucor* sp. (Fisher) in a closed system. Linalool alone showed a moderate antifungal activity, while no antifungal activity was observed when eugenol alone was used. When linalool and eugenol were mixed in a ratio similar to that found in basil oil, enhanced antifungal activity was observed, indicating a synergistic effect.

#### 4.6.3 Basil as a larvicidal agent

A study was carried out on the larvicidal effect of crude CCl<sub>4</sub>, methanol and petroleum ether leaf extracts of *O. basilicum* against *Anopheles stephensi* and *Culex*



*quinquefasciatus*. Petroleum ether extract was found to be most effective against the larvae of both mosquitoes, with LC<sub>50</sub> values of 8.29, 4.57; 87.68, 47.25 ppm and LC<sub>90</sub> values of 10.06, 6.06; 129.32, 65.58 against *A. stephensi* and *C. quinquefasciatus* being observed after 24 and 48 hours of treatment, respectively. These extracts are highly toxic against mosquito larvae from a range of species (Prejwltta *et al.*, 2009).

The larval toxicity and smoke repellent potential of *O. basilicum* Linn. at different concentrations (2, 4, 6, 8 and 10 %) against the different instar (I, II, III and IV) larvae and pupae of *Aedes aegypti* were also evaluated. The LC<sub>50</sub> values of *O. basilicum* for I instar larvae were 3.734, II instar 4.154, III instar 4.664, IV instar 5.124 (Murugan *et al.*, 2007). A laboratory investigation using plants such as *Vetiveria zizanioides* (Linn.) (Poaceae), *O. basilicum* (Linn.) (Lamiaceae) and the microbial pesticide spinosad against the malarial vector *A. stephensi* Liston showed 85 % mortality. The observed mortality rate suggests that the above extract can be used as a biopesticide. The LC<sub>50</sub> of second, third and fourth instar larvae of *A. stephensi* were 0.276 %, 0.285 % and 0.305 %, respectively (Aarthi and Murugan, 2010).

The direct toxicity of the essential oil *O. basilicum* L. to females of six species of predacious mites of the family phytoseiidae was tested. The phytoseiid mites tested were *Typhlodromus athiasae* Porath and Swirski, *Euseius yousefi* Zaher and El-Borolossy, *Amblyseius zaheri* Yousef and El-Borolossy, *A. deleoni* (Muma and Denmark), *A. swirskii* Athias-Henriot and *A. barkeri* (Hughes). Sweet basil oil was highly toxic to females *E. yousefi* and was relatively intoxic to females *A. swirskii*. The essential oil has a toxic effect on the predator species, *T. athiasae* and *A. barkeri*. With the exception of *A. zaheri*, females of all predacious mites tested suffered a depression in reproduction and food consumption when treated with sweet basil oil at a concentration of 2 % (Momen and Ame, 2003).

#### 4.6.4 Health-promoting properties

*O. basilicum* has a number of beneficial effects on the cardiovascular system: it may contain polar products with the ability to lower plasma lipid concentrations (Harnafia *et al.*, 2009), which could prove effective in the treatment of hyperlipidaemia, atherosclerosis and related diseases, which are becoming an increasing health concern in developing countries. Amrani *et al.* (2006) showed that *O. basilicum* extract displayed significantly stronger hypolipidaemic activity compared to fenofibrate treatments. *O. basilicum* aqueous extract displayed a very high antioxidant power (Amrani *et al.*, 2006), and was shown to produce a  $\beta$ -adrenergic effect in albino rats (Muralidharan and Dhananjayan, 2004).

In a study by Singh *et al.* (1999b), the fixed oil of *O. basilicum* was shown to display significant anti-inflammatory activity against paw edema in rats caused by carrageenan and other mediators, including arachidonic acid and leukotriene. A significant inhibitory effect was also observed in castor oil-induced diarrhoea in rats. The fixed oil of *O. basilicum* may therefore prove to be a useful anti-inflammatory agent which, thanks to its linolenic acid contents, is able to block both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism (Singh, 1998, 1999b).

Singh (1999a) showed that the fixed oil of *O. basilicum* exerted a significant anti-ulcer effect against aspirin, indomethacin, alcohol, histamine, reserpine, serotonin and stress-induced ulceration in experimental animal models. Aspirin-induced gastric ulcers and secretion in pylorus ligated rats were also shown to be inhibited. The anti-ulcer activity of the oil is likely to be the combined result of its lipooxygenase inhibiting, histamine antagonistic and antisecretory effects.

In a preliminary experiment, the toxic and mutagenic potential of essential oil from basil and pure substances, linalool,  $\beta$ -myrcene and 1,8-cineole, were tested using *S. typhimurium* TA98, TA100 and TA102, with and without S9 mix (microsomal fraction of rat liver). No mutagenic effect of basil derivatives was detected in any tested strain.

The antimutagenic effects of essential oil from basil and its pure constituents were further evaluated in the Ames test using *S. typhimurium* TA100. UVC irradiation and three chemical mutagens, 4-nitroquinoline-N-oxide (4NQO), 2-nitropropane (2-NP), and benzo(a)pyrene (B(a)P) were used to induce mutagenesis. All tested basil derivatives significantly reduced UV-induced mutations. The maximum inhibition was in the range of 64–77 %. In the presence of S9, EO and 1,8-cineole showed moderate inhibition of 2-NP induced mutagenesis, while the remaining two substances had no effect. Linalool exhibited a high co-mutagenic effect with B(a)P, 1,8-cineole showed moderate inhibitory effect against B(a)P-induced mutations, while EO and  $\beta$ -myrcene were ineffective (Olivera *et al.*, 2007). In a sample of 17 Thai medicinal plants, sweet basil oil had the highest antiproliferative activity with an IC(50) value of 0.0362 mg/ml (12.7 times less potent than Fluorouracil (5-FU)) in the P388 cell line (Manosroi *et al.*, 2006).

## 4.7 Quality issues and toxicity

### 4.7.1 Toxicity studies

Estragole is a natural constituent of basil oil. Several studies with oral, intraperitoneal or subcutaneous administration to CD-1 and B6C3F1 mice have shown that estragole is carcinogenic. The 1-hydroxy metabolites are stronger hepatocarcinogens than the parent compound. Controversial results are reported for the mutagenicity of estragole. However, the formation of hepatic DNA adducts *in vivo* and *in vitro* by metabolites of estragole has been demonstrated (Vincenzi *et al.*, 2000).

Smith *et al.* (2005) developed a guide for evaluating the safety of essential oils that are used as flavourings in foods, based on the chemical composition of the oil and on the extent to which it varies in the product. The guide classifies the chemically-identified constituents of the oil, biosynthesized by common pathways, into congeneric groups. Each congeneric group is evaluated and its safety judged on the basis of data on absorption, metabolism and toxicology from the members of the group. The guide further evaluates the intake of the group of unidentified constituents assuming that the essential oil is to be consumed in food, and on the basis of the toxicity data on the oil or an oil of similar chemotaxonomy (Baby and George, 2009).



### 4.7.2 Quality specifications

The physical and chemical characteristics of the oil vary depending on the geographic origin, variety, time of harvest and the plant parts used for extraction. The oil obtained from the inflorescence is of higher quality than the oil obtained from the whole plant. The physical constants of the European oil are reported by various authors in Tables 4.2 and 4.3. Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of *O. basilicum* are generally recognized as safe (GRAS) for their intended use.

**Table 4.2** The physical constants of European basil oil

|                           |                           |
|---------------------------|---------------------------|
| Colour                    | Light yellow              |
| Specific gravity at 15 °C | 0.895–0.930               |
| Refractive index          | 1.477–1.495               |
| Specific rotation         | –22° to –86°              |
| Ester value               | 3–15                      |
| Acid value                | 0–4                       |
| Solubility                | 1–2 parts of 80 % alcohol |

**Table 4.3** EOA standards for Oil of Basil (*Ocimum basilicum*)

| Property                               | Specifications                                                      |
|----------------------------------------|---------------------------------------------------------------------|
| Preparation                            | By steam distillation of the flowering tops or the whole plants     |
| <b>Physical and chemical constants</b> |                                                                     |
| Appearance and odour                   | Light yellow liquid with a spicy odour                              |
| Specific gravity at 25 °C              | 0.95–0.973                                                          |
| Optical rotation at 25 °C              | 0° to +2°                                                           |
| Refractive index at 20 °C              | 1.5120 to 1.5190                                                    |
| Acid value                             | Not more than 1                                                     |
| Saponification value                   | 4–10                                                                |
| Ester value after acetylation          | 25–45                                                               |
| <b>Descriptive characteristics</b>     |                                                                     |
| <b>Solubility</b>                      |                                                                     |
| Benzyl benzoate                        | Soluble in all proportions                                          |
| Fixed oils                             | Soluble in all proportions in most fixed oils                       |
| Mineral oil                            | Soluble with turbidity                                              |
| Propylene glycol                       | Soluble up to 5 % with slight haziness                              |
| Glycerine                              | Insoluble                                                           |
| <b>Stability</b>                       |                                                                     |
| Alkali                                 | Unstable                                                            |
| Acids                                  | Unstable in the presence of strong mineral acids                    |
| Containers                             | Glass or aluminium containers                                       |
| Storage                                | Store in tight full containers in a cool place protected from light |

## 4.8 References

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## 5

# Bay leaves

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**Abstract:** This chapter discusses the cultivation, production and processing of bay leaves. They are derived from the leaves of the *Laurus nobilis* L. tree, which is part of the Lauraceae family. Other topics discussed include the chemical composition and functional properties of bay leaves, their toxicity and their allergenicity. The quality aspects of bay leaves and their uses in the food processing industry have also been reviewed in this chapter, along with some important references.

**Key words:** bay leaves, *Laurus nobilis*, functional properties, toxicity and allergenicity, quality.

## 5.1 Introduction

The commodity, traded as sweet bay leaf, and true, Roman, or Turkish laurel, is derived from the leaves of *Laurus nobilis* L. (Family – Lauraceae). Because of the similarity in the leaves, several other trees are also variously known as: West Indian bay tree (*Pimenta racemosa*), Cherry laurel (*Prunus laurocerasus*), Portugal laurel (*Prunus lusitanica*), laurel of the southern states (*Prunus caroliniana*), the laurel or mountain laurel of California (*Umbellularia californica*). However, the leaves of true *L. nobilis* must not be confused with other laurels. *L. nobilis* is a native of the Mediterranean and grows spontaneously in scrubland and woods in Europe and in California. It is widely cultivated in Europe, America and in Arabian countries from Libya to Morocco (Anon., 1962; Bailey, 1963; Sangun *et al.*, 2007; Lira *et al.*, 2009).

The flavouring properties of *L. nobilis* have been known since antiquity. In biblical times, the bay was symbolic of wealth and wickedness, and in the classical world, heroes and victors were decorated with a laurel wreath. In addition to being a very well known culinary herb, the leaves and fruits of *L. nobilis* are used medicinally throughout the world. Infusions or decoctions made from these materials have diaphoretic and carminative effects and also serve as a general gastric secretion stimulant. Laurel oil or butter obtained from the fruits (berries) of *L. nobilis* is a vital ingredient of laurin ointment, a popular medicine for rheumatism and gout and for the treatment of spleen and liver diseases. It also finds application in veterinary medicine (Anon., 1962; Francesco and Francesco, 1971; Wren, 1975; Duke,

1989). Demirbas (2010) examined the potential of bay leaves as a source of biodiesel via compressed methanol transesterification. Use of bay leaf oil in some perfume compositions has also been reported (Asplund, 2008).

*L. nobilis* is an evergreen shrub, or more rarely a tree attaining a height of 15–20 m. The smooth bark may be olive green or of reddish hue. The luxurious, evergreen leaves are alternate with short stalks, lanceolate or lanceolate oblong, acuminate, 5–8 cm in length or longer and 3–4 cm wide, coriaceous, pellucid-punctate, and with revolute, entire wavy margins; the upper surface is glabrous and shiny, olive green to brown and the lower surface is dull olive to brown with a prominent rib and veins. The flowers are small, yellow in colour, unisexual and appear in clusters. The fruits (berries) are cherry-like, succulent, purple to black in colour, ovoid, coarsely wrinkled and contain a single seed with loose kernel. The dried fruits are drupaceous, ovoid, about 15 mm long and 10 mm wide. The outer surface is glabrous, shining, nearly black and is coarsely wrinkled owing to the shrinkage of the narrow succulent region beneath the epidermis. The remains of the style appear as a small point at the apex, and a small scar at the base marks the point of attachment of the fruit to the thalamus. The endocarp is thin and woody and the testa is adherent to its inner surface. The entire pericarp is about 0.5 mm thick. The kernel of the seed consists of two large plano-convex cotyledons and small superior radicle; it is brownish-yellow, starchy and oleaginous, with an aromatic odour and aromatic and bitter taste (Wallis, 1960; Bailey, 1963; Francesco and Francesco, 1971).

The cross-section of the leaf shows epidermal cells with thick cuticle; the epidermal cells in surface view are sinuous, pitted and thick walled. The lower epidermal walls are more curvilinear and distinctly beaded. The stomata are present only on the lower surface, singly or in pairs. The mesophyll of the leaf is distinctly represented by two layers of parenchymatous palisade cells and a region of spongy parenchyma containing scattered spheroidal oil reservoirs, fibrovascular and collenchymatous tissues. The leaf has characteristic fragrance when crushed and its taste is bitter and aromatic (Wallis, 1960; Bagchi and Srivastava, 1993).

## 5.2 Cultivation, production and processing of bay leaves

Sweet bay is propagated by seeds or preferably by cuttings. From a well-ripened wood, cuttings of about 7.5–10 cm length are put in sharp sand, either under bell-glasses or in glass cases. The rooted cuttings are placed in small pots containing fairly rich sandy loam with good drainage, and then can be put in a hot bed, with gentle bottom heat where they will make a good strong growth. *L. nobilis* stem cuttings produce roots better in July/August, under Mediterranean conditions, than in other seasons, although the optimal rooting period can be extended by bottom heating from May until September (Raviv *et al.*, 1983a). Ligneous, sub-apical stem cuttings of bay laurel have a higher rooting percentage than herbaceous apical cuttings, probably due to water deficit in the latter. Moisture sufficiency may be critical due to the very long rooting period of four to five months (Raviv *et al.*, 1983b).

Rapid and efficient rooting of *L. nobilis* occurs at a root medium temperature of 20–30°C, especially during the winter when, if not heated, both the medium and air



temperatures are less than 15°C in the Mediterranean region (Raviv and Putievsky, 1983). After that, they may be planted in nursery beds with rich sandy soil and good drainage. In one growing season, the plants may attain a height of 1–1.5 m. At the end of the growing season and long before the cold season the young plants together with their stakes are kept in well-lit and ventilated sheds, and temperature is kept just above freezing. These plants are kept in close rows and watered once or twice a week. The plants are taken out during the spring season and either potted or plunged in nursery. The rich peaty soil with plenty of water and congenial moist atmosphere near the sea coast are favourable conditions for fast and luxuriant growth (Bailey, 1963). *L. nobilis* also grows well under the partly shaded conditions in gardens or orchards.

The leaves of *L. nobilis* are plucked and dried under shade for use as a flavouring material in a variety of culinary preparations, especially in French cuisine. The leaves contain an essential oil of aromatic, spicy odour and flavour which can be isolated by steam distillation. A novel microwave method has been applied for the hydro-thermal extraction of essential oils from *L. nobilis* (Flamini *et al.*, 2007). The oil is a valuable adjunct in the flavouring of all kinds of food products, particularly meats, sausages, canned soups, baked goods, confectionery, etc. The oil replaces the dried leaves to great advantage because it can be dosed more exactly and therefore gives more uniform results than the dried leaves (Guenther, 1953).

Laurel berries contain about 1 % of an aromatic volatile oil and 25–30 % fat. The separated fat is the *Oleum lauri expressum* of commerce. The pure fat is of dull green colour, granular and has an aromatic odour. The expressed oil is used in stimulating liniments and in veterinary practice (Wallis, 1960).

Currently, two types of essential oils are traded internationally under the name 'bay oil', although they are entirely unrelated to each other. The West Indian bay oil or bay leaf oil is distilled from the leaves of the tree *Pimenta racemosa*, which is found on the various islands of the West Indies, but most particularly in Dominica. The Turkish bay oil or laurel leaf oil is distilled from the leaves of *L. nobilis*. The sources of the bulk culinary bay leaves are Turkey and the Balkan countries and, in small quantities, France. The annual production level of the genuine *L. nobilis* oil is only about 2 t. It is marketed mainly in Western Europe, largely in Germany and the Netherlands (ITC, 1986).

### 5.3 Chemical composition of bay leaves

A good deal of work on physicochemical characterisation and chemical composition of essential oils of different parts of *L. nobilis* has been reported. The reported values of physicochemical constants and chemical constituents identified are provided in Table 5.1. The studies carried out so far on the bay oil indicate the influence of geographical origin of variety and harvest season on the chemical composition. The chemical composition of the flower essential oil is quite different from other parts of the plant, namely leaves, stem bark and stem wood (Fiorini *et al.*, 1997). The earlier studies were mostly carried out by chemical methods (Nigam *et al.*, 1958), but recent gas chromatography–mass spectroscopy (GC-MS) and gas–liquid

**Table 5.1** Physicochemical properties and chemical constituents of essential oil extracted from different parts of *Laurus nobilis* of varying geographical origins

| S. No. | Geographical origin of the resource material    | Plant part and its essential oil content      | Physical characteristic(s) determined                                                                                                                                                                           | Chemical constituent(s) identified                                                                                                                                                                          | Reference(s)                      |
|--------|-------------------------------------------------|-----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|
| 1.     | NA                                              | NA                                            | Yellowish-brown unpleasent odour, d 0.9278 ( $\alpha$ ) <sup>22</sup> d-120 <sup>0</sup> , hd 1.4730, soluble in ethyl alcohol 1:90                                                                             | $\alpha$ -pinene, eugenol, phellandrene                                                                                                                                                                     | Rattu and Maccioni (1952)         |
| 2.     | NA                                              | Fruits, 1 %                                   | –                                                                                                                                                                                                               | Pinene, cineole, lauric acid, alcohols, and sesquiterpenes                                                                                                                                                  | Rattu <i>et al.</i> (1953)        |
| 3.     | NA                                              | Fruits                                        | N <sub>d</sub> <sup>30</sup> 1.4898, d <sub>20</sub> <sup>20</sup> 0.9218, ( $\alpha$ ) <sub>D</sub> <sup>20</sup> -18.9 <sup>0</sup> , acid no. 5.92, sap. no. 67.94                                           | Cineole (12.8 %), free alcohols (10.7 %), esters (chiefly me-cinnamate 17.9), free cinnamic acid (1.3 %) free phenols (2.0 %), terpene hydrocarbons (15.4 %), different carbonyl comopounds sesquiterpenes  | Nigam <i>et al.</i> (1958)        |
| 4.     | Idzhevanskii, Armenia, Noemberyam-skii, Armenia | Leaves                                        | d 2.5–3.3, ( $\alpha$ ) 3.8–3.1                                                                                                                                                                                 | –                                                                                                                                                                                                           | Melkumyan and Khurshundyan (1959) |
| 5.     | NA                                              | Fruits, 4.1 %                                 | N <sub>d</sub> <sup>20</sup> 1.4898, d <sub>20</sub> <sup>20</sup> c.9218, ( $\alpha$ ) <sub>D</sub> <sup>18</sup> -18.9 <sup>0</sup> . Acid value 5.92, sap. value 67–94, sap. value (after acetylation) 99.80 | Carbonyl compounds (11.48 %), alkali soluble (by vol) (9 %), $\alpha$ -pinene, citral terpeneol, me-cinnamic acid, caryophyllene, sesquiterpenes hydrocarbons                                               | Nigam <i>et al.</i> (1958)        |
| 6.     | NA                                              | Information on plant part not mentioned 2.5 % | NA                                                                                                                                                                                                              | $\alpha$ -pinene, camphene, sabinene, limonene, careen, 1,8-cineole (35 %)                                                                                                                                  | Teisserie (1966)                  |
| 7.     | NA                                              | NA                                            | NA                                                                                                                                                                                                              | $\alpha$ -pinene, camphene, $\beta$ -pinene, sabinene, 3-carene, $\alpha$ -phellandrene, myrcene, $\alpha$ -limonene, $\beta$ -phellandrene, $\gamma$ -terpinene, <i>p</i> -cymene, terpinolene and ocimene | Teisseire (1966)                  |
| 8.     | Czechoslovakia                                  | Leaves                                        | NA                                                                                                                                                                                                              | $\beta$ -pinene, camphene, myrcene, limonene, <i>p</i> -cymene, $\beta$ -phellandrine, $\beta$ -selinene, $\gamma$ , $\delta$ -cadinene                                                                     | Chow <i>et al.</i> (1965)         |



|     |                  |                                                                                     |                                                                                                                                                  |                                                                                                                                                                                                                                                                                                    |                                                            |
|-----|------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| 9.  | Kazakhstan       | Shoot, 0.5 %                                                                        | NA                                                                                                                                               | $\alpha$ -pinene, $\beta$ -pinene, camphene, 1-sebinene, $\beta$ -myrcene, $\alpha$ , $\beta$ -phellandrene, 1-limonene, <i>p</i> -cymene, 1,8-cineole, acetic, propionic, butric, caproic, caprylic, pelargonic and enanthric acid, eugenol                                                       | Goryaev <i>et al.</i> (1966)                               |
| 10. | Greece<br>Turkey | Leaves, 1.0 %<br>Leaves, 0.8 %                                                      | NA<br>NA                                                                                                                                         | $\alpha$ -pinene, camphene, $\beta$ -pinene, sabinene, myrcene, $\beta$ -phellandrene, <i>d</i> -limonene, cineole, $\gamma$ -terpinene, <i>p</i> -cymene, terpenyl camphor, linalool, $\alpha$ -terpineol, terpenyl acetate, $\beta$ -selinene, methyl eugenol, terpin-eugenol and acetyl eugenol | Giuliana and Stancher (1968)                               |
| 11. | NA               | NA                                                                                  | NA                                                                                                                                               | A-pinene, $\alpha$ -thujene, $\beta$ -pinene, sabinene, myrcene, $\alpha$ -phellandrene, limonene, $\beta$ -phellandrene, 1,8-cineole, $\gamma$ -terpinene, <i>p</i> -cymol, linalool, terpinene-4-ol, eugenol, methyl eugenol, trepenyl formate                                                   | Kekelidze <i>et al.</i> (1977)                             |
| 12. | Italy            | NA                                                                                  | NA                                                                                                                                               | A-thujene (5.9 %), $\beta$ -pinene (20.1 %) 1,8-cineole (37.3 %) <i>p</i> -cymene (traces), $\alpha$ -terpineol (2.2 %), terpenyl acetate (10.6 %), methyl eugenol (0.3 %)                                                                                                                         | Hector and Retamar (1978)                                  |
| 13. | Turkey           | Leaves                                                                              | NA                                                                                                                                               | <i>Cis-thujen</i> -4-ol (a new compound)                                                                                                                                                                                                                                                           | Novak (1985)                                               |
| 14. | Greece           | Leaves                                                                              | NA                                                                                                                                               | 1,8-cineole and $\alpha$ -terpenyl acetate (major component) pinocarvone and (E)-pinocarveol (new compounds)                                                                                                                                                                                       | Tucker <i>et al.</i> (1992)                                |
| 15. | Utrakhand, India | Fruits, 5 %                                                                         | $d_{20}^{28}$ 0.923, $n_D^{35}$ , 1.4960, [9]<br>$D^{28}$ -5.73°, acid value, 3.34 and ester value, 25.86, ester value after acetylation – 54.68 | 1,8-citole (28.4 %), methyl cinnamate, (20.1 %), $\alpha$ -phellandrene (10.1 %), $\alpha$ -pinene (9.3 %), $\alpha$ -terpenol (5.8 %), sabinene (4.9 %), $\alpha$ -thujene (3.8 %), $\beta$ -humulene (3.3 %), linalool (2.3 %), camphor (2.2 %), $\alpha$ -gurujunene (2.2 %)                    | Nigam <i>et al.</i> (1992), Appendino <i>et al.</i> (1992) |
| 16. | India            | Petroleum ether extract of fruits                                                   | NA                                                                                                                                               | 10-hydroxyoctacosanyl tetradecanoate, 1-docosanol tetradecanoate and 11-gavceramanthin, dehydrocostus lactone, costunolide, zalu zanin, sesquiterpene alcohol                                                                                                                                      | Garg <i>et al.</i> (1992)                                  |
| 17. | Toulouse, France | Flowers, 0.18 %<br><br>Leaves, 0.57 %<br><br>Stem bark, 0.68 %<br>Stem wood, 0.07 % | NA                                                                                                                                               | (E)-ocimene and sesquiterpene compound – $\beta$ -carophyllene, viridiflorene, $\beta$ -clemene, germacrene-D-4-ol, germacrene-D<br>1,8-cineole, linalool, methyleugenol, $\alpha$ -terpenyl acetate<br>1,8-cineole<br>$\alpha$ -terpenyl acetate, methyl eugenol, $\alpha$ -copaene               | Fiorini <i>et al.</i> (1997)                               |

NA = information, not available.

chromatography (GLC) analyses has made it possible to isolate and characterise a number of compounds more accurately and efficiently (Nigam *et al.* 1992; Fiorini *et al.*, 1997). The chemical structure of some of the important constituents is provided in Fig. 5.1. The presence of 1,8-cineole in appreciable amounts makes the oil of bay leaves an important perfumery item (Pruidze and Kekelidze, 1971).

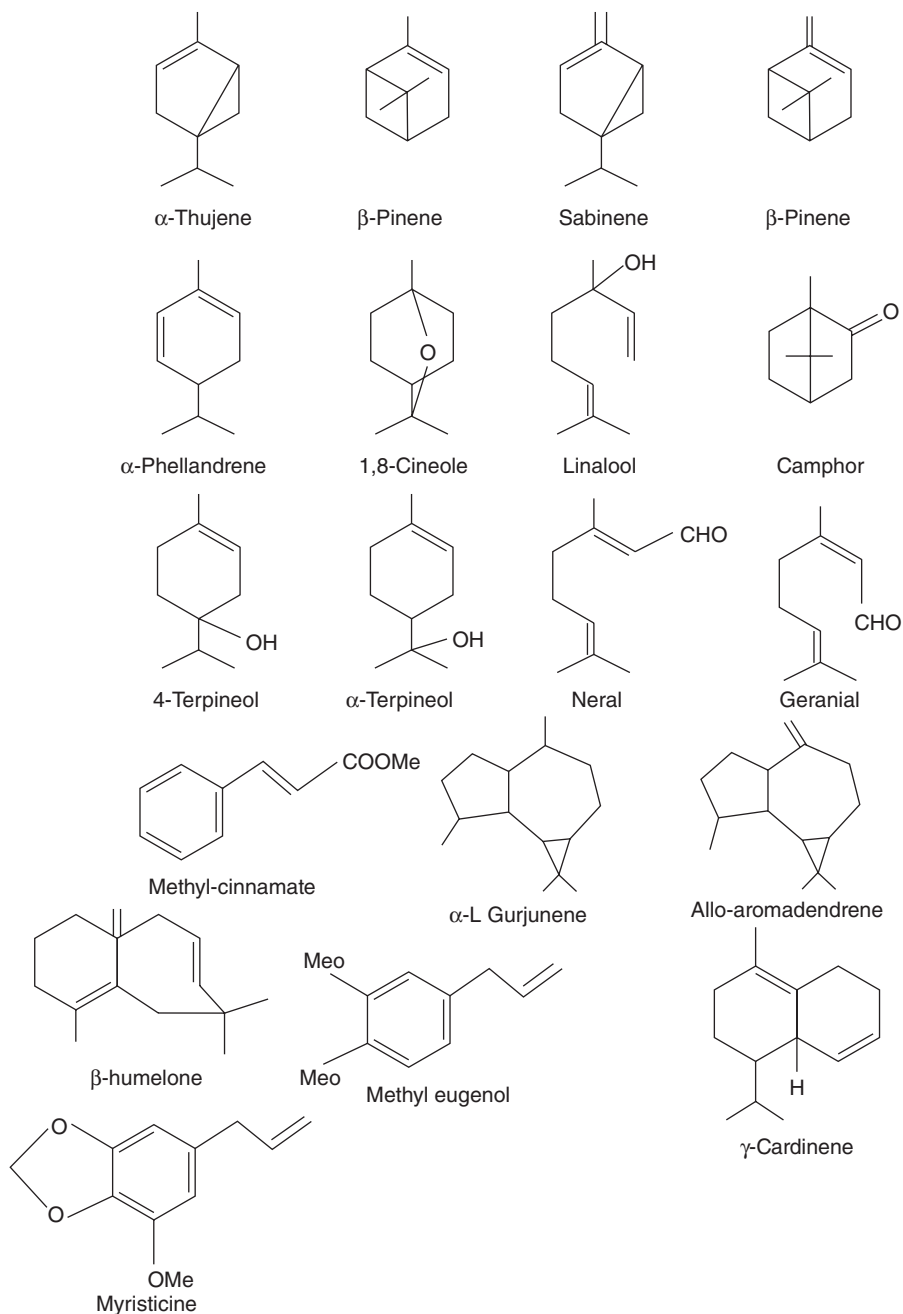


**Fig. 5.1** The chemical structure of some of the important constituents of bay leaves.

## 5.4 Functional properties of bay leaves

Although the dried bay leaves and their essential oil are mainly used as a spice and food flavouring agent, the bay oil also finds use in folk or traditional medicines of different countries, for the treatment of a number of diseases. Recent studies have shown that it has the following functional properties:

- antimicrobial and antifungal characteristics;
- hypoglycaemic properties (in the control of diabetes);
- anti-ulcerogenic properties;
- antioxidative properties;
- anti-inflammatory activity.

The essential oil of *L. nobilis* has been found to be active against *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexnerii* and *Salmonella typhi*, pathogens of the intestinal tract (Syed *et al.*, 1991; Friedman *et al.*, 2002; Chaudhry and Tariq, 2006; Ezzeldeen *et al.*, 2010; Ivanovic *et al.*, 2010). It has also been noted to possess antifungal activity(ies) (MacGregor *et al.*, 1974; Raharivelomanana *et al.*, 1989).

The hypoglycaemic activity of bay leaf extracts has also been reported (Ashaevea *et al.*, 1984). Bay leaves potentiated the action of insulin in glucose metabolism (Khan *et al.*, 1990, 2009) and reduced glucose transport (Gurman *et al.*, 1992). The administration of 200 and 600 mg/kg doses of the ethanolic extract of leaves of *L. nobilis* produced a significant decrease in blood glucose levels in diabetic rabbits (Yanardag and Can, 1994).

The possible anti-ulcerogenic activity of *L. nobilis* seeds was tested on experimentally (ethanol) induced gastric ulcers in rats. The results indicated anti-ulcerogenic activity for 20 and 40 % aqueous extracts as well as for the oily fraction of the seeds. In acute toxicity studies, the aqueous extract was found safe with LD<sub>50</sub> compared to oil LD<sub>50</sub> at 0.33 ml/kg body weight (Afifi *et al.*, 1997).

Bay has also been reported as having a number of other properties. The methanolic extract from the leaves of *L. nobilis* inhibited the elevation of blood ethanol level in ethanol-loaded rats. The bioassay-guided separation resulted in the isolation of costunolide, dehydrocostus lactone and santamarine as the active constituents. The  $\alpha$ -methylene- $\gamma$ -butyrolactone structure was found to be essential for the preventive effect on ethanol absorption. In addition, the retardation of gastric emptying seemed to be partially involved in the preventive effects (Matsuda *et al.*, 1999).

The effects of aqueous extracts of leaves and flowers of *L. nobilis* on adult snail and embryo (*Biomphalaria glabrata*) have been studied. Results obtained have shown a degree of toxicity on the embryos starting at a concentration of 125 ppm. The flower extract appeared to be more effective. Cephalic and shell malformations were found in embryos treated with both leaf (50 ppm) and flower (25 ppm) extracts. The LD<sub>90</sub> value on adult snails was estimated as 340 ppm for flower extract and 1900 ppm for leaf extract (Rey and Kawano, 1987). Cockroach repellent activity has also been found in bay leaves (Verma and Meloan, 1981).

The antioxidant properties of bay have been studied widely (Lagouri and Boskou, 1995; Kang *et al.*, 2002; Simić *et al.*, 2003; Koşar *et al.*, 2005; Elmastaş *et al.*, 2006; Hinneburg *et al.*, 2006; Devi *et al.*, 2007; Muchuweti *et al.*, 2007; Emam *et al.*, 2010; Polovka and Suhaj, 2010; Ouchikh *et al.*, 2011).

The leaf essential oil of *L. nobilis*, which has been used as an antileptic remedy in Iranian traditional medicine has been reported to have anticonvulsant activity against experimental seizures (Sayyah *et al.*, 2002). Khalil *et al.* (2007) reported the wound healing effect of the oil of *L. nobilis*. *L. nobilis* extract displayed remarkable anti-inflammatory and antitumour activity (Suhr and Nielsen, 2003; Kalleh *et al.*, 2007; Mueller *et al.*, 2010).

Al-Kalaldeh *et al.*, (2010) have reported the antiproliferative activity of *L. nobilis* against human breast adenocarcinoma cells. Spirafolide, a compound purified from the leaves of *L. nobilis* showed neuroprotective effects against dopamine-induced apoptosis in human neuroblastoma cells (Ham *et al.*, 2010). Laurosides B, a megastigmane glycoside from *L. nobilis* leaves, induces apoptosis in human melanoma cell lines (Panza *et al.*, 2011). *L. nobilis* chloroform fraction showed significant activity against cerebral ischaemia neuronal damage (Choi *et al.*, 2003).

Bioactivity and qualitative analysis of essential oil from *L. nobilis* against stored-product pests have also been reported (Papachristos and Stamopoulos, 2002; Choi *et al.*, 2003; Cosimi *et al.*, 2009). Activity of essential oil of bay leaves against aflatoxigenic fungus *Aspergillus parasiticus* has also been reported (Atanda *et al.*, 2007).

#### 5.4.1 Toxicity and allergenicity

Bay leaves and their essential oil do not appear to have any significant toxicity. However, sporadic reports have indicated that bay leaves may cause allergic contact dermatitis (Asakawa *et al.*, 1974; Cheminat *et al.*, 1984; Goncalo and Goncalo, 1991), perhaps induced by one or more sesquiterpene lactone. Certain bay leaf samples of Mexican origin had been detected to be infested with gastrointestinal disease causing *Clostridium perfringens* spores @ < 100–450 Cfu/g (Rodriguez-Romo *et al.*, 1998). A number of cytotoxic compounds have been isolated and identified from bay leaves (Fang *et al.*, 2005; Barla *et al.*, 2007).

#### 5.4.2 The use of bay leaves in food processing

Bay leaves are extensively used to increase the frozen storage stability of food products (Nuray and Goezde, 2010). The antifungal activity of volatile compounds generated by essential oils of bay leaf against fungi commonly causing deterioration of baking products completely inhibited the microorganism tested (Guynot *et al.*, 2003; Suhr and Neilson, 2003).

### 5.5 Quality issues

The effect of drying methods on the quality of essential oil of bay leaf has been studied by different workers (Diaz-Maroto *et al.*, 2002; Demir *et al.*, 2004; Harnik *et al.*, 2004; Günhan *et al.*, 2005; Amin *et al.*, 2007). Demir *et al.* (2004) performed mathematical modelling and determined some quality parameters of air-dried bay leaves. The leaves were dried at different temperatures (40°, 50° and 60°) and relative humidities (5 %, 10 % and 15 %) under sun and shade conditions. The effect of drying conditions on the colour and the amount of essential oil of fresh and dried

leaves under different conditions was observed. It was found that no significant loss of quality was observed when drying at 60 °C air temperature. Diaz-Moroto reported no loss in quality after oven-drying at 45 °C and air-drying at ambient temperature, where as freezing and freeze-drying brought about substantial losses in bay leaf aroma. Harnik *et al.* (2004) proposed a heat-based treatment for elimination of *Phytophthora ramorum* from infected bay laurel leaves without having a negative impact on its quality.

The International Organization for Standardization (ISO) has produced standards for bay leaf and bay essential oil:

- ISO 6576: 2004 – Laurel (*Laurus nobilis* L.) – Whole and ground leaves – Specification
- ISO 3045:2004 – Oil of bay [*Pimenta racemosa* (Mill.) J. W. Moore]

## 5.6 References

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# 6

## Black pepper

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**Abstract:** Black pepper is the most important and most widely used spice in the world, cultivated in over 26 countries, together producing about 315–320 000 tons of pepper (black and white). Pepper is valued for its pungency contributed by the alkaloid piperine and flavor contributed by the volatile oil. This chapter looks at production and international trade in pepper, cultivars, cultivation, post-harvest handling and grading. The chemical structure of pepper is presented, together with quality issues and techniques used in industrial processing. Finally, the functional properties of black pepper and its applications in medicine and in food are described.

**Key words:** pepper, black pepper, white pepper, *Piper nigrum*, pepper products, piperine, essential oil, pepper quality, traditional medicine, value-added products.

### 6.1 Introduction

Among the spices, black pepper is the king. It is the most important, the most popular and the most widely used spice in the world. It has extensive culinary uses for flavoring and preserving processed foods and is also important medicinally. Of the total spices traded internationally, pepper accounts for about 34 % (throughout this chapter, pepper is used to mean black pepper, unless otherwise stated). South West India, particularly the Western Ghats regions of the South India, is the centre of origin of this important spice. Black pepper was the first oriental spice to be introduced into the Western world, and it was well known among the Romans and Greeks. In the Middle Ages, pepper, assumed great importance in Europe. Its use resulted in revolutionary changes in western cooking. For a comprehensive treatment of black pepper, the reader may consult Ravindran (2000a) and Ravindran *et al.* (2006). Bezerra *et al.* recently (2009) summarized the various aspects of black pepper with special emphasis on the pharmacological and chemical properties.

### 6.2 Production and international trade of black pepper

Black pepper was once produced only in the western coastal region of India, from where its cultivation spread to most tropical countries. Now pepper is grown in

tropical zones of the Asia–Pacific region, mainly India, Indonesia, Malaysia, Sri Lanka, Thailand, China, Vietnam and Cambodia. Outside the Asia–Pacific region, the crop is distributed in a total of about 26 countries, including Brazil, Mexico and Guatemala. A rough estimate of global area under pepper is around 405 000 ha, producing around 318 000 tons of pepper annually. In 1950, 70 % of world pepper cultivation was concentrated in India, but this had gone down to 46 % by 1991. During the subsequent six decades, production figures decreased by 66 % and export value by 33 %. India's share of the world market dropped from 56 % to 23 % in the same period. At the same time other countries made remarkable progress in terms of share of the world pepper market with large areas being brought under pepper production in countries like Vietnam.

Today, Vietnam ranks first in the production of pepper, while India has been relegated to fourth position. Table 6.1 gives the production of pepper in the top ten countries during 2008–10. In terms of productivity, Thailand ranks first with 3393 kg/ha, followed by Vietnam with 1742 kg/ha (Table 6.2). In terms of productivity, India occupies a very low position, with only 322 kg/ha. More than 30 % of global pepper production comes from Vietnam, the largest producer in the world; in 2008, its share

**Table 6.1** Top ten black pepper-producing countries of the world

| Rank | Area       | Production (\$1000) | Production (MT) |
|------|------------|---------------------|-----------------|
| 1    | Vietnam    | 416 769             | 89 300          |
| 2    | Brazil     | 358 938             | 77 770          |
| 3    | Indonesia  | 342 143             | 74 131          |
| 4    | India      | 318 461             | 69 000          |
| 5    | China      | 120 923             | 26 210          |
| 6    | Sri Lanka  | 89 492              | 19 390          |
| 7    | Malaysia   | 87 692              | 20 090          |
| 8    | Thailand   | 48 087              | 10 419          |
| 9    | Mexico     | 31 633              | 6854            |
| 10   | Madagascar | 24 000              | 5200            |

Source: <http://faostat.fao.org/site/342/default.aspx>.

**Table 6.2** Productivity of black pepper in various countries

| Country    | Productivity (kg/ha) |
|------------|----------------------|
| Thailand   | 3393                 |
| Vietnam    | 1742                 |
| Malaysia   | 1615                 |
| China      | 1235                 |
| Brazil     | 1000                 |
| Madagascar | 625                  |
| Sri Lanka  | 396                  |
| Indonesia  | 346                  |
| India      | 322                  |

Source: <http://faostat.fao.org/site/342/default.aspx>.

was 34.6 %, with the total area under pepper cultivation approximately 50 000 ha producing 90 000–100 000 tons. In Indonesia, Lampung province of Sumatra is the major black pepper producing region while Bangka region produces white pepper. Production in Indonesia is in the region of 22 000–25 000 tons, contributing 9–10 % of global production. In Brazil, the major pepper-producing area is the State of Para. Its estimated production in the year 2008 was 37 000 tons which stood at 14–15 % of global production. Ninety-five per cent of Malaysia's pepper is grown in the Sarawak region and thus in the international market it is traded as Sarawak Pepper. About 14 000 ha of land is under pepper cultivation producing in the region of 20 000–25 000 tons. Malaysia contributes around 7–8 % of global production. In India, almost all the pepper is grown in the south, led by the state of Kerala which contributes 97–98 % of the total production. Sri Lanka and Madagascar are the two other major producers of pepper in the world.

According to the global figures released recently by the IPC (International Pepper Community), the balance sheet of supply–demand for 2010 indicates a shortage of nearly 22 216 tons. The IPC forecast 2011's world pepper consumption to be around 320 000–350 000 tons – significantly higher than last year's global production of 285 000 tons. According to them, global consumption – excluding China and India – had grown between 4–6 % per year. The closing stock of 2009 or the carry-forward stock for 2010 is estimated to be at around 79 124 tons while the carry-forward stocks for 2011 have been projected at 72 082 tons.

Black pepper is the major commercial product, the other being white pepper produced from the ripe fruits of the pepper plant or from black pepper by suitable processes. Of the total global production of 318 662 tons of pepper in 2009, black pepper accounted for 251 762 tons, and white pepper for 66 900 tons. In 2010, the production of black pepper was 251 980 tons and that of white pepper 64 400 tons. The projection for 2011 is 309 952 tons, of which black pepper output is expected to be around 244 632 tons and white pepper 65 320 tons. During the past decade, production in all traditional pepper-producing countries has registered a declining trend, while the new entrants like Vietnam and China registered higher output. However, this is a temporary phase as pepper diseases like *Phytophthora* rot and virus diseases are on the increase in all pepper-producing countries which in future will surely lead to declining production and productivity.

Pepper is a major agricultural exporting commodity in all pepper-producing countries, and it is the most widely traded spice too. During 2009, total pepper export was 268 386 tons, consisting of 228 797 tons of black pepper and 39 589 tons of white pepper. In 2010, the estimates are 200 300 tons of black and 37 350 of white, making a projected total of 237 650 tons. The projections for 2011 are 185 250 black and 44 460 white, making a total of 229 710 tons of pepper.

Export of pepper from leading producing countries in the first 9 months in 2010 was (in tons): Vietnam 100 000, Indonesia 23 500, Brazil 20 000, Malaysia 15 000, India 10 000 and Sri Lanka 8 000. A significant feature in 2010 was that all the producing countries, such as India, China, Indonesia, Malaysia and Vietnam, also imported significant quantities, either for domestic consumption or value additions and re-exports when they found their local prices were much higher than other producing countries. In all producing countries, except India, the major share of production is exported while in India there is a very strong internal market and

widespread consumption as spice as well as in the traditional medicine manufacturing sector (Table 6.3).

Pepper consumption has registered a gradual and significant increase over the decades, an increase which is attributed to the spread of cuisines around the world and to the phenomenal growth in the fast food culture. Per capita pepper consumptions for 1975, 1980 and 1990–5 are given in Table 6.4. This table indicates that Denmark tops the list in per capita consumption of pepper. Recently, IPC has given figures for consumption in major consuming countries (Table 6.5).

North America continues to be the major importer of pepper, followed by EU countries. Pepper consumption is very high in producing countries like India, but not in others like Vietnam, Thailand, etc. In India, large quantities of pepper are used in indigenous medicine for the manufacture of a large number of medicinal formulations. This segment in fact consumes more quantity than the food and spices sector.

The pepper industry is growing and the demand for pepper is increasing throughout the world. Global production of pepper is facing shortages on account of a shift in cultivation from pepper to more remunerative crops such as rubber in countries

**Table 6.3** Export of pepper from major producing countries (quantity in tons)

| Country         | 2009    | 2010 estimates | 2011 estimates |
|-----------------|---------|----------------|----------------|
| Brazil          | 35 648  | 34 000         | 30 000         |
| India           | 21 267  | 18 050         | 19 000         |
| Indonesia       | 44 600  | 44 000         | 23 000         |
| Malaysia        | 14 000  | 14 500         | 21 950         |
| Sri Lanka       | 6 621   | 10 100         | 10 460         |
| Vietnam         | 134 200 | 105 000        | 110 000        |
| Other countries | 12 050  | 12 000         | 15 300         |
| Total           | 268 386 | 237 650        | 229 710        |

Source: Report of IPC meeting, 2010, *Spice India*, **23** (11), 4–8.

**Table 6.4** Pepper consumption *per capita* in developed countries (in grams)

| Countries (average) | 1975 | 1980 | 1990–95 |
|---------------------|------|------|---------|
| 1 Denmark           | 102  | 128  | 194     |
| 2 Germany           | 131  | 170  | 190     |
| 3 Belgium           | 90   | 127  | 181     |
| 4 USA               | 117  | 144  | 168     |
| 5 The Netherlands   | 94   | 91   | 151     |
| 6 Austria           | 97   | 141  | 150     |
| 7 France            | 107  | 124  | 138     |
| 8 Sweden            | 90   | 96   | 122     |
| 9 Canada            | 100  | 87   | 112     |
| 10 Switzerland      | 121  | 139  | 112     |

Source: <http://www.karvycomtrade.com/downloads/karvySpecialReports>.

**Table 6.5** Consumption in the major consuming countries

|                    |              |
|--------------------|--------------|
| N. America         | 75 000 tons  |
| Europe             | 70 000 tons  |
| M. East and Africa | 33 000 tons  |
| S. America         | 15 000 tons  |
| Asia               | 123 000 tons |
| Others             | 4 000 tons   |
| Total              | 320 000 tons |

Source: Report of IPC meeting, 2010, *Spice India*, **23** (11), 4–8.

**Fig. 6.1** A part of pepper vine with spikes.

like Malaysia and India. In Indonesia, pepper-growing areas are facing threat from the tin mining industry. In many tropical countries, pepper is also being replaced with cocoa plantations. The catalyst for many such shifts in cultivation is the widespread disease incidence that makes pepper cultivation risk-prone and non-profitable. Added to this is the fluctuation in prices that makes pepper less reliable than other crops like rubber. However, demand is growing; despite the recession in the recent past, pepper consumption increased by 5%. Pepper consumption is increasing in countries like China with the spread of continental food culture, and China is importing pepper, about 18 000 tons, from Vietnam to meet the increased demand.

### 6.3 The black pepper plant and its varieties

Black pepper is a product of the mature fruits of *Piper nigrum* L., a perennial woody evergreen climber, native to the evergreen forests of the Western Ghats of South India. Under cultivation, pepper vines are trailed on supports, as columns 5–6 m tall and 1.0–2.0 m in diameter. When trailed on tall trees the vine may attain a height of 20 m or even more (Figs 6.1 and 6.2). On flowering, the pepper vine produces pendent spikes (catkins), which appear opposite the leaf on side branches, and carry numerous very small flowers that are reduced to ovary and stamens subtended by bracts. The fruit is a single-seeded drupe, but is often called a berry; it is sessile, small, usually globular, having fleshy pericarp and hard endocarp.

More than 100 cultivars are known and a few of them are still popular (Ravindran *et al.*, 2000a,b). Pepper is predominantly a self-pollinated crop. Variability in yield



**Fig. 6.2** Portion of a pepper vine climbing on a large tree.

and quality characteristics between cultivars is high. Systematic research effort over the last three decades has resulted in the release of 17 high-yielding superior lines of black pepper through clonal selection, selection in open pollinated progenies as well as through hybridization followed by selection in segregating populations (Ravindran *et al.*, 2006). Panniyur-1 is the first pepper variety to be evolved through hybridization (Fig. 6.3). The quality of black pepper is as important as the yield and depends on the contents of piperine and essential oil. Variability in the quality characters in black pepper has been investigated and cultivars were classified based on quality parameters (Gopalam and Ravindran, 1987). Evaluation studies of germ-plasm collections resulted in identifying high piperine, oil and oleoresin types (Ravindran *et al.*, 2006).

## 6.4 Cultivation of black pepper

The cultivation system of pepper varies among the different pepper growing countries. In India, pepper is cultivated mostly in homestead gardens as a mixed crop and is seldom grown as a pure plantation. In Sarawak, Thailand, Vietnam, Brazil, etc., pepper is grown mostly as a sole crop. Pepper vines are trailed on supports called standards, which are either live (e.g. trees) or non-living (e.g. concrete or wooden poles). Another technique for raising black pepper and maintaining it in bush form has developed through the use of fruiting lateral (plagiotropic) branches.





**Fig. 6.3** Spikes of the hybrid pepper var. Panniyur -1.



**Fig. 6.4** Bush pepper growing in a pot.

Such bush pepper plants under good management can yield an average of 150–300 g of dry pepper per plant/year (Fig. 6.4.).

The pepper plant starts flowering during May–June under the climatic conditions existing in India, with the onset of the south west monsoon, and harvesting is usually in December–January. Variations in maturity pattern depend on factors such as variety, rainfall, altitude, ambient temperature, etc. Pepper fruits mature about 6–8 months after flowering. The period generally coincides with dry weather in India. The flowering and harvest season of pepper varies from country to country. Harvesting is carried out when one or more berries in some spikes turn orange to red color. Entire spikes are picked when fruits are fully mature but still green. Pepper quality depends on maturity, processing and post-harvest handling. The increase in diversity



of products using pepper has necessitated the harvesting of berries at different stages of maturity, regulated depending on the various end uses.

Major production constraints all over the pepper-producing countries are diseases caused by *Phytophthora capsici* and a viral disease caused by the pepper yellow mottled virus (a Badna virus, Bhat *et al.*, 2003). Recently, an interspecific hybrid between cultivated pepper and the immune species, *Piper colubrinum*, has been developed and this gives some hope for evolving resistant highly productive pepper hybrids in future (Vanaja *et al.*, 2008).

#### 6.4.1 Post-harvest handling

Post-harvest handling is crucial to achieving a high-quality product. The harvested spikes are kept in bags for 12–24 hours or heaped and covered overnight for a brief fermentation which makes fruit separation easier. The spikes are then threshed manually by rubbing or trampling underfoot or by using mechanical threshers of various types. Mechanical threshers are used only by large growers. Recently-small-scale threshers have become popular in Sarawak and Indonesia. For a more detailed discussion on the topic, see Risfaheri and Nurdjannah (2000) and Zachariah (2000). Fruits separated by threshing are graded and then sun-dried. The ideal grading method involves using a mesh to remove the light berries and pinheads and classification based on size. A blanching process is recommended before drying, by dipping fruits (in a wire basket) in boiling water for 2 minutes. The fruits are then spread out on the floor for drying. Blanching improves color and also removes dust and adhering microbial contamination. Drying is done in the open sun in most cases. A black topped cement floor is the best for sun-drying. Mechanical, electrical and solar dryers are also used for rapid drying. Dry recovery percentage varies among cultivars and growing conditions; usually the recovery ranges from 28–38 %. After proper drying, the moisture content should be around 10 % only (for details see Ravindran, 2000a,b).

Dried pepper is also separated and graded based on size using sieves of varying meshes, a process known as garbling. The average composition of dried pepper is given in Table 6.6 (Pruthi, 1993). The dried pepper is cleaned to remove extraneous matter like dirt, grit, stones, stalks, etc.

**Table 6.6** Average composition of dried pepper

| Content                | % of composition |
|------------------------|------------------|
| Moisture               | 8.7–14.0         |
| Total nitrogen         | 1.5–2.6          |
| Volatile ether extract | 0.3–4.2          |
| Non-volatile extract   | 3.9–11.5         |
| Alcohol extract        | 4.4–12.0         |
| Starch                 | 28.0–49.0        |
| Crude fibre            | 8.7–18.0         |
| Piperine               | 1.7–7.4          |
| Total ash              | 3.6–5.7          |
| Acid soluble ash       | 0.03–0.55        |

Source: Pruthi (1993).

### 6.4.2 Grades and grading

Pepper is graded based on external quality and size through garbling (the process of separating the fresh or dried pepper berries based on size) and grading (categorization of dry pepper into various grades as provided by an approved agency). Pepper grades are important especially in international trade for:

- securing higher prices for the produce;
- increase in export of superior quality;
- reducing or even eliminating disputes regarding quality that arise through size variations;
- increasing the return of growers.

Pepper grading varies among the producing countries. The most elaborate grading system exists in India, where the following grades of pepper are recognized:

- Malabar garbled (MG) grades 1 and 2
- Malabar ungarbled (MUG) grades 1 and 2
- Tellicherry garbled special extra bold (TGSEB)
- Tellicherry garbled extra bold (TGEB)
- Tellicherry garbled (TG)
- Garbled light pepper (GL) Special grades 1 and 2
- Ungarbled light pepper (UGL) grades 1 and 2
- Pin heads (PH) Special and Grade 1
- Black pepper (non-specified) (NS).

Very small and undeveloped berries are classified as pin heads. The bulk of the pepper belongs to the average size known as Malabar (the earlier name for the south west coastal region of India, famous for pepper production and trade). Larger sized berries are grouped in the categories 'Tellicherry bold', 'Tellicherry extra bold', 'Tellicherry special extra bold', and 'giants' (Tellicherry is the name of a place, famous for pepper trading from ancient times (Zachariah, 2000)). Good garbled pepper should have a bulk density of 500–600 g per liter. Light berries should be less than 10 % and pin heads less than 4 %. Low bulk density indicates higher proportion of light berries and pin head, which results in poor-quality products. Pepper should have good aroma and a biting pungent taste. It should contain at least 1.5 % volatile oil and 3 % piperine. The chemical composition of different grades of pepper, as recognized in the Indian market, is given in Table 6.7.

## 6.5 Chemical composition of black pepper

The quality of pepper is contributed by two components:

- piperine that contributes the pungency;
- volatile oil that is responsible for the aroma and flavor.

The chemistry of pepper has been reviewed by Guenther (1952), Govindarajan (1977), Parmar *et al.* (1997) and Narayanan (2000). Menon (2000), Menon and Padmakumari (2005) and Sasidharan and Menon (2010) published analytical data on the chemical composition of pepper and the anti-microbial activity of the pepper

**Table 6.7** Grades of Indian pepper and their features

| Grades                    | Moisture (%) | Vol. oil (%) | Piperine (%) | NVEE (%) | Starch (%) | Crude fiber (%) |
|---------------------------|--------------|--------------|--------------|----------|------------|-----------------|
| Pin heads                 | 13.01        | 0.6          | 0.8          | 7.1      | 11.5       | 27.4            |
| Light pepper              | 13.0         | 2.9          | 4.1          | 13.5     | 14.6       | 27.8            |
| Malabar garbled           | 13.0         | 3.7          | 5.0          | 12.3     | 39.7       | 11.8            |
| Tellicherry garbled (EB)  | 13.0         | 2.2          | 4.4          | 9.1      | 39.7       | 11.8            |
| Tellicherry garbled (SEB) | 10.0         | 3.2          | 4.9          | 10.3     | 40.9       | 9.2             |
| Malabar ungarbled         | 12.0         | 2.8          | 5.0          | 11.4     | 41.8       | 12.5            |
| Tellicherry ungarbled     | 12.0         | 2.6          | 4.0          | 13.5     | 41.8       | 10.5            |
| High range ungarbled      | 12.0         | 2.6          | 4.0          | 11.1     | 41.8       | 10.5            |

EB = extra bold; SEB = special extra bold; NVEE = non-volatile ether extract.

Sources: Lewis (1984); Zachariah (2000).

oil. Through industrial processing three products are commonly produced: oleoresin, piperine and pepper oil. Pepper oil is extracted through steam distillation of ground pepper and the other two through solvent extraction. Oleoresin contains both the flavor (oil) and pungency (piperine) factors, and it is one of the largest traded products of pepper. As it contains both flavor and pungency, it is widely used as a substitute for pepper in many processed foods. The alkaloid piperine is the major pungent component present in pepper. In addition, five minor alkaloids possessing pungency have been identified in pepper extracts. They are piperetine, piperanine, piperylin A, piperolein B and pipericine. Parmar *et al.* (1997) listed the following alkaloids in addition to the piperine group of alkaloids mentioned above: brachymide B, guineesine, retrofractamide A, sarmentine, sarmentosine and tricholein.

The essential oil of pepper is a mixture of a large number of volatile chemical compounds. The aroma is contributed by the totality of the components. More than 80 components have been reported in pepper essential oil (Gopalakrishnan *et al.*, 1993; Narayanan, 2000; Menon and Padmakumari, 2005; Zachariah, 2008; Sasidharan and Menon, 2010). The important components are given below:

- **Monoterpene hydrocarbons and oxygenated compounds:** such as camphene,  $\delta$ -3-carene, *p*-cymene, limonene, myrcene, *cis*-ocimene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene,  $\alpha$ -pinene,  $\beta$ -pinene.
- **Oxygenated monoterpene compounds:** such as borneol, camphor, carvacrol, *cis*-carveol, *trans*-carveol, carvone, carvotanacetone, 1,8-cineole.
- **Sesquiterpene hydrocarbons and oxygenated compounds:** About 25 are present, the most important being  $\beta$ -caryophyllene followed by  $\alpha$ -*trans*-bergamontane,  $\beta$ -bisabolene,  $\delta$ -cadinene, etc.
- **Oxygenated sesquiterpenes:** such as 5,10(15) cadinen-4-ol, caryophylla-3-(12),  $\gamma$ -eudesmol, elemol, cubebol,  $\alpha$ -bisabolol,  $\beta$ -bisabolol.
- **Miscellaneous compounds:** in addition to the above groups of compounds, many others were also identified in black pepper oil, e.g. eugenol, methyl eugenol, benzaldehyde, *trans*-anethole, myristicin, safrole, piperonal, etc.
- **Phenolic components of pepper:** the phenolic components of black pepper are a mixture of the glycosides of phenolic acids and flavonol glycosides such as quercetin, isoquercetin, isorhamnetin 3- $\beta$ -D-rutinoside, kaempferol 3-arabinoside.

Gopalakrishnan *et al.* (1993) studied four improved cultivars of pepper using gas chromatography – mass spectroscopy (GC–MS) employing a methyl silicone capillary column. The oil of these cultivars possessed  $\alpha$ -pinene in the range of 5.07–6.18 %,  $\beta$ -pinene 9.16–11.08 %, sabinene 8.50–17.16 %, limonene 21.06–22.71 % and  $\beta$ -caryophyllene 21.52–27.70 %.

Menon (2000) has reported the chemical composition of essential oil extracted from fresh and dry pepper fruits. Fresh pepper aroma was isolated by amberlite column chromatography and analyzed using GC and GC–MS. The major compounds were found to be *trans*-linalool oxide and  $\alpha$ -terpeneol. On the other hand, dry pepper oil contained  $\alpha$ - and  $\beta$ -pinene, *d*-limonene and  $\beta$ -caryophyllene as major compounds. When oil is extracted by distillation, there is marked change in the composition. The content of oxygenated compounds came down from 64.4 % in original aroma of fresh pepper to 17 % in distilled oil of dry black pepper. This change explains the difference in the flavor and taste of fresh pepper and dry pepper. Sasidharan and Menon (2010) reported  $\beta$ -caryophyllene as the dominating compound in berry oil, followed by  $\beta$ -pinene, limonene,  $\alpha$ -pinene and humulene. They also found that green pepper oil contained more oxygenated compounds than dry pepper oil.

## 6.6 Quality issues

### 6.6.1 Physical quality

The IPC has prescribed certain quality criteria for pepper (Table 6.8). Apart from this, many importing countries have fixed their own quality criteria which also include, in addition to physical parameters, chemical characters and pesticide residues. Both the American Spice Trade Association (ASTA) and the European Spice Association (ESA) have established minimum standards for pepper imported into their respective regions. They have laid down procedures for testing to ascertain quality. Producing countries, especially India, have laid down elaborate standards both for internal trading and for exports.

### 6.6.2 Sensory quality evaluation of oil

The odor of pepper oil is described as fresh, dry-woody, warm-spicy and similar to that of crushed black peppercorn. Pangburn *et al.* (1970) made a sensory evaluation study of Malabar pepper oil after column chromatographic fractionation and mixtures of fractions at varying proportions. The early fractions were pepper-like and floral and the late fractions pepper-like, fresh and woody and the middle fractions falling in between. Govindarajan (1977) made extensive studies on odor analysis of pepper varieties. Using similar techniques, Gopalakrishnan *et al.* (1993) described odor evaluation of four improved high-yielding pepper varieties examined using GC–MS. They depicted the odor profile on a four-point category scale and subjected the oils to ranking tests. The mean of the scores for each odor characteristic was plotted on radiating lines representing odor characteristic in sequential odor from left to right. The desirable odors are in the upper quadrants, the undesirable ones in the lower quadrants. The aromagram developed by these authors is given in Fig. 6.5.

**Table 6.8** Quality parameters prescribed by IPC

| Quality parameter                                  | Black pepper (whole)                                                                         |                | White pepper (whole)                                                                         |          |
|----------------------------------------------------|----------------------------------------------------------------------------------------------|----------------|----------------------------------------------------------------------------------------------|----------|
|                                                    | IPC BP-1                                                                                     | IPC BP-2       | IPC WP-1                                                                                     | IPC WP-2 |
| <b>Macro</b>                                       |                                                                                              |                |                                                                                              |          |
| 1. Bulk density (g/l, min.)                        | 550                                                                                          | 500            | 600                                                                                          | 600      |
| 2. Moisture (% vol/wt, max.)                       | 12                                                                                           | 14             | 13                                                                                           | 15       |
| 3. Light berries/corns (% by wt, max.)             | 2                                                                                            | 10             | 1                                                                                            | 2        |
| 4. Extraneous matter (% by wt, max.)               | 1                                                                                            | 2              | 1                                                                                            | 2        |
| 5. Black berries/corns (% by wt, max.)             | Not applicable                                                                               | Not applicable | 1                                                                                            | 2        |
| 6. Moldy berries/corns (% by wt, max.)             | 1                                                                                            | 3              | 1                                                                                            | 3        |
| 7. Insect defiled berries/corns (% by wt, max.)    | 1                                                                                            | 2              | 1                                                                                            | 2        |
| 8. Whole insects, dead or alive (by count, max.)   | Not more than 2 numbers in each sub-sample and not more than 5 numbers in total sub-samples. |                | Not more than 2 numbers in each sub-sample and not more than 5 numbers in total sub-samples. |          |
| 9. Mammalian or/and other excreta (by count, max.) | Shall be free of any visible mammalian or/and other excreta.                                 |                | Shall be free of any visible mammalian or/and other excreta.                                 |          |
| <b>Microbiological</b>                             |                                                                                              |                |                                                                                              |          |
| 1. Salmonella (detection / 25 g)                   | Negative                                                                                     | Negative       | Negative                                                                                     | Negative |

Notes:

1. IPC BP-2 and IPC WP-2 are grades of pepper which has been partially processed (i.e. has gone through some basic cleaning processes like sieving and winnowing).

2. IPC BP-1 and IPC WP-1 are grades of pepper which has been further processed (i.e. has gone through further cleaning processes including sieving, cycloning, destoning, washing and mechanical drying).

Source: Adopted at 29<sup>th</sup> IPC Session of IPC held at Belem Hilton, Brazil, on 2 Nov. 2001.

More recently, Mamatha *et al.* (2008) analyzed sensory and flavor quality profiles using sophisticated techniques such as GC–MS and electronic nose (e-nose) and reported differences in sensory quality and flavor profile among varieties. Some varieties have the typical pepper-like, pungent, spicy and lingering herbaceous notes while varieties such as Balancotta have turmeric-like and green mango-like notes (its oil having a higher content of *p*-cymene). They also found that retronasal olfaction is more pronounced than orthonasal olfaction. GC–MS and electronic nose analysis are thus found to be excellent methods for determining the quality aroma and sensory profiles of black pepper samples (Mamatha *et al.*, 2008).

### 6.6.3 Flavor and off-flavor compounds

Jagella and Grosch (1999a, b, c) carried out some studies on the flavor and off-flavor contributing components of black pepper. Their dilution and concentration

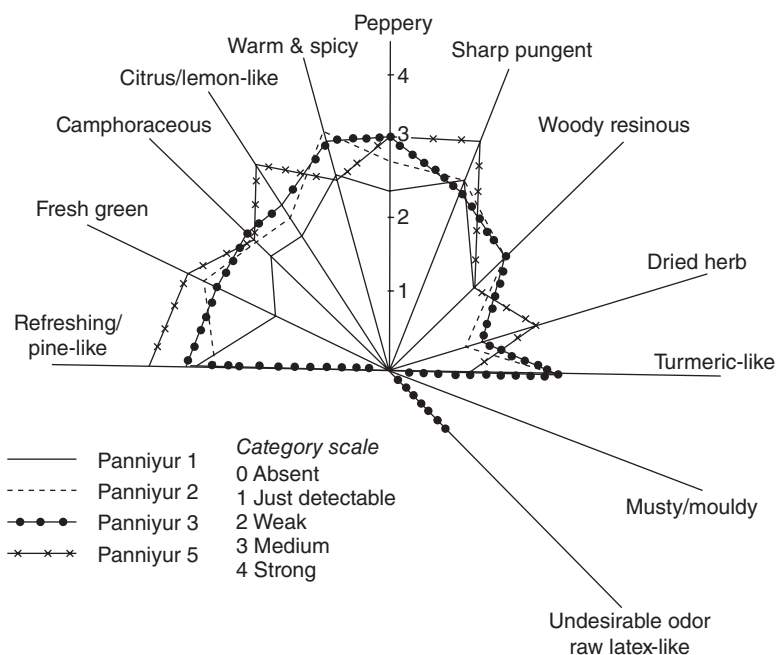


**Fig. 6.5** Aromagram developed for four black pepper cultivars, indicating the predominant notes. The vertical scale 0–4 denotes the relative strength of the various notes.

experiments as well as enantioselective analysis of optically active monoterpenes indicated that (+)-linalool, (+)- $\alpha$ -phellandrene, (–)-limonene, myrcene, (–)- $\alpha$ -pinene, 3-methylbutanol and methyl propanol as the most potent odorants of pepper. The moldy, musty off-flavor of Malaysian pepper is shown to be due to 2-isopropyl-3-methoxypyrazine and 2,3-diethyl-5-methylpyrazine. A storage experiment revealed that for ground pepper losses of  $\alpha$ -pinene, limonene and 3-methylbutanal were mainly responsible for deficits in pepper-like, citrus-like, terpene-like and methyl notes after 30 days storage at room temperature. Jagella and Grosch (1999c) developed an aroma model for pepper based on the quantification of 19 odorants and calculation of their odor activity values. Omission tests conducted by them indicated that limonene, linalool,  $\alpha$ -pinene, 1,8-cineole, piperonal, butyric acid, 3-methylbutyric acid, methyl propanal, 2- and 3-methylbutanal as key odorants of white pepper. The fecal off-flavor was caused by skatole and was enhanced by the presence of *p*-cresol.

#### 6.6.4 Adulteration

The common adulterants used in whole or ground pepper are low-quality pepper and various foreign matters. Synthetic compounds and cheap volatile sources are used to adulterate pepper essential oil (Sen and Roy, 1974). Pepper berries are adulterated with stems, chaff or similar organic extraneous matter.

## 6.7 Industrial processing and value addition

The importance of value addition in pepper has been recognized by traders, and technologies have been developed for processing pepper into a variety of products for consumer use. Once produced and dried at the farm level, the pepper is subjected to grading and further processing. The dried pepper is sent through a variety of processing equipments, such as a mechanical sifter for removal of pinheads, vegetable seeds, sand, dust and similar contaminants; and then to winnowing and destoning for removal of dust, stalks, light foreign matter and stones. These operations are now carried out in multiple-sieve cum air-classifier type of machine and gravity separators. From gravity separators pepper is conveyed to mechanical washers fitted with brushes for removal of dust, dirt, mold growth, etc., and for imparting a good shine to the product. The cleaned and washed pepper is then centrifuged to remove water, dried in a drier (usually diesel-fired or electric but with indirect heating). Finally, the dried pepper is sent through spirals for final cleaning followed by sterilization either by steam or gamma radiation and packed in suitable polythene-lined packaging.

### 6.7.1 White pepper

White pepper is an important product mainly used in food items where the dark particles are undesirable, such as salad dressings, soups, mayonnaise, light-colored sauces, etc. White pepper is prepared from fully ripe fruits by removing the outer pericarp before drying. It is produced conventionally from ripe berries by the water steeping – retting technique. Although other techniques have been tested, they have been never accepted by consumers. In the water steeping and retting technique, ripe berries and berries that are about to ripen are harvested, threshed and heaped in tanks through which water is allowed to run for 7–10 days. In Indonesia (which is the largest producer and exporter of white pepper), pepper berries are tied in gunny bags and immersed in running water in streams or rivulets. During the process of water steeping, the outer skin (pericarp) becomes rotten and can be removed easily by rubbing, and the decorticated fruits (seeds) are further washed in clean water and sun-dried. Often the decorticated fruits (seeds) are kept immersed in bleaching powder solution for a day or two to give better color to the product. The yield of white pepper will be around one quarter compared to the one-third recovery of dry black pepper. Joshi (1962) patented a chemical process for the preparation of white pepper that involved steeping of whole dried pepper in water, boiling with 4 % NaOH solution, agitating with a stirrer to remove the skin and then bleaching with 2.5 % hydrogen peroxide followed by washing in clean water and drying. However, the use of chemicals for the process was not acceptable to the market. Gopinathan and Manilal (2005) reported a bacterial fermentation process for white pepper production. For the fermentation, they employed bacteria isolated from pepper retting water (pepper skin fermenting bacteria, PSFB), belonging to the genera *Xanthomonas*, *Pseudomonas* and *Bacillus*. A cocktail of these bacteria was incubated with presoaked green or dried black pepper in a nutrient-fortified aqueous medium for 4 days at a pH of 6.7–7.0. The fermented pepper is put through a fruit pulping machine to remove the skin completely. The pulped pepper seeds are then



washed and dried. The product is creamy white, and of very good quality. The yield of white pepper varied from 60–72 %, depending upon the maturity and size of pepper berries.

### 6.7.2 Ground pepper

In Western countries the most common form of black pepper available to the consumer is ground pepper. Ground pepper is produced by grinding dried, cleaned and sterilized white or black pepper in a hammer mill having copper-tipped hammers. The ground pepper is then sieved in sieves of required mesh size and packed in airtight containers. The following points have to be kept in mind in the production of ground pepper (Ravindran *et al.*, 2000a):

- Moisture level should be kept to a minimum as high moisture will affect the storage life.
- Volatile oil content should not be affected during the grinding process.
- Particle size should be optimum so as to ensure free flow for the duration of its shelf-life.
- Packaging should be air-tight and safe.
- Microbiological cleanliness (freedom from molds and bacteria) should be ensured.

A more recent development is cryo-grinding. In this new technique, the grinding is done at low temperature to reduce the oil loss. This is done by injecting liquid nitrogen into the grinding zone, and the temperature is adjusted suitably through the control of LN<sub>2</sub> flow rate. The cryo-ground spice disperses more uniformly in spice formulations and volatile oil and flavor loss are minimized.

### 6.7.3 Pepper oil

As already mentioned, the aroma and flavor of black pepper is due to the essential oil content and this oil can be recovered by hydrodistillation or steam distillation. The essential oil contains monoterpenes and sesquiterpenes, and their oxygenated derivatives having boiling points in the range of 80–200 °C. Industrial production of pepper essential oil is by steam distillation, by passing steam through pepper powder contained in a distillation chamber. The volatile oil that comes out along with the steam is collected in the condenser and later recovered, dried and stored in air-tight containers.

### 6.7.4 Oleoresin

Oleoresin is commonly marketed as spice drops and contains the total pungency and flavor constituents of pepper. Oleoresin is produced by solvent extraction of pepper powder using a suitable organic solvent such as acetone, ethanol, ethyl acetate or ethylene dichloride. Either a one-stage or a two-stage process is employed for this. In the first case, the oil is recovered along with the resins by solvent extraction. In the second process, the oil is recovered by steam distillation followed by solvent extraction for recovering the oleoresin. Later the oleoresin and oil are



blended to meet the required specifications. The organic solvent should be recovered completely from the oleoresin, and the ISO as well as the importing countries have established maximum permissible limits for the approved solvents. The whole extraction process of oleoresin is usually done in batch extractors.

### **6.7.5 Supercritical fluid extraction (SFE products)**

SFE is the most versatile separation technology now being employed. It has high extraction selectivity from a mixture of components because of the pressure–temperature dependent solubility in the solvents. The pepper raw material is loaded into the extractor and brought into contact with the supercritical solvent at relatively high pressures of 80–350 bar, at temperatures of 35–70 °C. The solute mixes into the supercritical solvent and both are passed through a pressure-reducing valve. The pressure on the separator side is about 40–60 bar, while the temperature is lower due to sudden expansion of the supercritical solvent. These conditions lower the solubility of the pepper raw materials in the solvent. After one cycle of extraction, the gas is again compressed back to extract the material. Solvent recycling is achieved by means of a compressor (Anon., 1997).

Supercritical CO<sub>2</sub> is an ideal solvent for extraction of pepper, because it is cheap, abundant, inert, non-toxic, non-corrosive, non-inflammable and does not pollute the environment. Separation can be carried out at low temperature, residual solvent content can be reduced to near zero, solubility variation of active constituents can be easily manipulated, fractions can be extracted easily, the process consumes little energy, transfer rates are high and there are no fire hazards. Pepper extraction has been very successful with about 98 % extraction of piperine and 81 % of essential oil. The quality of the product is high compared to conventional extraction process. The extracted oleoresin is also used for the separation of piperine by centrifuging the oleoresin in a basket centrifuge. From the oleoresin, numerous secondary products have been developed having specified flavor strength and other properties. Such products include seasonings, emulsions, solubilized spices, dry soluble spices, encapsulated spices, heat-resistant spices, fat-based spices, etc.

### **6.7.6 Microencapsulated pepper**

Microencapsulation is a recent development in which the flavor material is entrapped in a solid matrix, but releases the flavor when the product comes into contact with water or on heating. Methods such as spray-drying, co-acervation, polymerization, etc. are used in microencapsulation. The process involves homogenization of the oil/water mixture in the presence of the wall material followed by spray-drying under controlled conditions. The wall materials commonly used include vegetable gums, starches, dextrans, proteins, cellulose esters, etc. A process known as CR-100 has been developed for microencapsulation which overcomes the limitations of the spray drying process (Narayanan *et al.*, 2000).

### **6.7.7 Other value-added products**

Many effort has been denoted to adding value to pepper, and a variety of products have been developed, in addition to the most widely traded products discussed above. They can be classified as:

- Green pepper-based products;
- Black and white pepper-based products;
- Pepper by-products (Ravindran *et al.* 2000a).

#### *Green pepper-based products*

- Canned green pepper in brine
- Bottled green pepper in brine
- Bulk packaged green pepper in brine
- Cured green pepper (without any covering tissue)
- Frozen green pepper
- Freeze-dried green pepper
- Semi-dried or dehydrated green pepper
- Green pepper pickle in oil/vinegar/brine
- Green pepper-mixed pickle in oil/vinegar/brine
- Green pepper flavored products
- Green pepper paste.

#### *Black and white pepper based products*

- Black pepper powder
- White pepper powder
- Other pepper products (such as soluble pepper, pepper paste)
- By-products from pepper waste.

#### *Pepper-based products*

- Many products, in which pepper is a major ingredient, have been developed such as lemon pepper, garlic pepper, sauces and marinades that have pepper as the main component.
- Spice mixtures and blends – curry powders and spice blends for various culinary uses.
- Pepper flavored products such as pepper mayonnaise, pepper cookies, pepper keropak, pepper tofu, etc.
- Products using pepper extracts – pepper candies, pepper perfume, etc.

## **6.8 Functional properties of black pepper**

Black pepper is an essential ingredient in the Indian systems of medicines – *Ayurveda*, *Sidha* and *Unani* – and is used as a curative agent for many maladies. Pharmacological studies have substantiated many of these traditional uses (Vijayan and Thampuran, 2000). Pepper has a number of functional properties, including:

- analgesic and antipyretic properties;
- antioxidant effects;
- antimicrobial properties.

Piperine, the active ingredient in pepper, exerts substantial analgesic and antipyretic effects. Lee *et al.* (1984) found that piperine reduces inflammation in carragenin-induced tests at an oral dose 50 mg/kg body weight. The anti-inflammatory

effect was substantiated by Kapoor *et al.* (1993). Piperine and its homologues are absorbed through skin and are hence capable of acting on the subcutaneous tissues as well as on nerves and blood vessels. The effect of pepper on the nervous system and on sexual organs (priapism) indicates anticonvulsive and vasodilator properties. Pepper also has an effect on lactation by increasing milk production. Pepper oil warms the skin and brings blood to the surface, stimulating circulation.

Pepper as well as piperine increases the bioavailability of medicaments, including ampicillin and synthetic drugs, as well as increasing the uptake of amino acids from food (Johri *et al.*, 1992). A commercial patented product (Bioperine) intended to enhance the bioavailability of nutrient supplements is now available in the market (US Patent 5 536 506, Anon., 2011a, b). Absorption of piperine seems to interact with the intestinal cells so as to increase cell permeability. Piperidine is noted as a CNS-depressant, insecticidal, spino-convulsant and urate solvent. The amides present in pepper have been shown to have insecticidal properties. Vijayan and Thampuran (2000) give a detailed account of the pharmacological and toxicological properties of pepper and piperine.

In Ayurveda, pepper is used in the treatment of epileptic fits and to bring about sleep. Piperine exhibited protection against penitrazole-induced seizure and also against electroshock seizure (Won *et al.*, 1979). Piperine also possesses a strong potentiating effect on hexobarbital-induced hypnosis in mice. A compound of great interest extracted from pepper is 1-(3-benzodioxol-5yl)-1-oxo-2-propenyl-piperidine, known as anti-epilepsirine, which was shown to have strong anti-epileptic properties. This is used in Chinese hospitals for the treatment of epilepsy (Ebenhoeh and Spadaro, 1992).

Both pepper and piperine exert protective action on the liver. Kaul and Kapil (1993) found that piperine reduces *in vitro* and *in vivo* lipid peroxidation and prevents depletion of GSH (gastric sulfhydryls) and total thiols. This is a very significant property, as lipid peroxidation causes free radical production which causes tissue damage. Pepper has antioxidant activity which is attributed to its tocopherol and polyphenol contents. Supercritical CO<sub>2</sub> extracts of ground black pepper have been found superior to solvent extracted ones in reducing lipid oxidation of cooked ground pork (Tipsrisukond *et al.*, 1998). The antioxidative activity of black pepper can, at least partially, be ascribed to the presence of glycosides of the flavonoids kaempferol, rhamnetin and quercetin (Vösgen and Herrmann, 1980), as well as to the phenolic amides. Nakatani *et al.* (1986) established that all the five phenolic amides present in pepper possess very good antioxidant properties, superior even to those of the synthetic antioxidants like butylated hydroxy toluene and butylated hydroxy anisole. Addition of pepper to foods increases keeping quality and prevents spoilage, due to the antimicrobial properties of pepper.

The most far-reaching attribute of piperine has been its inhibitory influence on enzymatic drug-biotransforming reactions in the liver. It strongly inhibits hepatic and intestinal aryl hydrocarbon hydroxylase and UDP-glucuronyl transferase (Srinivasan, 2007). Piperine has been found to enhance the bioavailability of a number of therapeutic drugs and phytochemicals due to this property. Piperine's bioavailability-enhancing property is also partly attributed to increased absorption as a result of its effect on the ultrastructure of the intestinal brush border. Initially, there were a few controversial reports regarding its safety as a food additive, but

none could provide any experimental evidence, and later several animal studies established the safety of black pepper and its active principle, piperine, (Srinivasan, 2007). Piperine is now known not only to be non-genotoxic, but also to possess antimutagenic and antitumor properties.

The essential oil of pepper is found to be inhibitory to *Vibrio cholerae*, *Staphylococcus albus*, *Clostridium diphtheriae*, *Shigella dysenteriae*, *Streptomyces faecalis*, *Bacillus* spp., *Pseudomonas* spp., etc. Pepper oil stopped growth and aflatoxin production by *Aspergillus parasitica* at a concentration of 0.2–1 %. Pepper leaf oil also exhibits antifungal activity.

### 6.8.1 Pepper in Indian traditional medicine

Black pepper is one of the most important and commonly used drugs in Ayurveda, Unani and Siddha, which are the Indian systems of medicine. It is used as a single drug or in combination with dry long pepper (*Piper longum*) and dried ginger (*Zingiber officinale*); the combination is popularly known as '*thrikatu*', the three acrids that cure the three disordered humors – *vata*, *pitta* and *kapha* – which, according to the ancient Ayurvedic system of Indian medicine, are responsible for all ailments. According to the ancient classical Ayurvedic texts, pepper is pungent and acrid, hot, rubefacient, carminative, dry corrosive, alternative, antihelmintic and germicidal. It promotes salivation, increases digestive power, enhances the taste of food and cures cough, dyspnoea, cardiac diseases, colic, worms, diabetes, piles, epilepsy and almost all diseases caused by the disorders of *vata* and *pitta*. Pepper is prescribed in cholera, flatulence, diarrhea and various gastric ailments. It is employed as antiperiodic in obstinate fevers, as an alternative in paraplegia and arthritic diseases, as an aromatic stimulant in cholera, weakness following fevers, vertigo, coma and as a stomachic in dyspepsia and flatulence. In traditional medicine systems, pepper is used in diseases of the spleen, pain in the liver and muscles, leucoderma, lumbago and paralysis. Externally, it is valued for its rubefacient properties and as a local application for relaxed sore throat, piles, alopecia and some skin diseases. Pepper is valued as a digestive tonic (Khare, 2007).

There is a current movement towards natural organic health supplements and medicines as substitutes for synthesized chemical drugs. The health-promoting properties of pepper (as well as other spices) are being increasingly documented. Continued research is needed in this field to confirm their reported attributes.

## 6.9 Use of black pepper in food

A spice is used in cooking for the following purposes:

- flavoring
- masking/deodorizing
- pungency
- colorant.

The spice interacts with the taste buds as well as other components of food leading to complex effects. A spice thus induces both direct and indirect (complex) effects

**Table 6.9** Direct and indirect effects of spices

| Direct effect                           | Indirect (complex) effect             |
|-----------------------------------------|---------------------------------------|
| Flavor                                  | Increased appetite                    |
| Taste (pungency, bitterness, sweetness) | Masking effect                        |
| Color                                   | Improvement of texture and appearance |
| Antifungal effect                       | Preservation                          |
| Antibacterial effect                    | Preservation                          |
| Antioxidant effect                      | Preservation                          |

as shown in Table 6.9 (Hirasa and Takemasa, 1998). Black pepper is the most widely used spice and occupies a proud place in the cuisines of both West and East, in both vegetarian and non-vegetarian cooking. Black pepper contributes towards flavor, taste, antifungal, antibacterial and antioxidant properties, the predominating ones being taste and flavor, and hence pepper is a multifunctional spice. Pepper is often used three times in the same dish before the food is eaten: first, in the kitchen as an ingredient in the dish; second, to correct or improve the overall seasoning during cooking; third, at the dining table for the diners to add more pepper to enhance the taste and flavor of a prepared dish. Both white and black pepper are used; they are used either whole, cracked, coarsely ground, medium ground or finely ground. Whole pepper corns are added as such to meat dishes, fish preparations, soups, pickles and in certain fried items such as *bonda*, *urdu vadai*, etc. Ground pepper is used in eggs, salads and gravies. White pepper is popular in sauces and in preparations where pepper flavor is wanted but without the black specks of black pepper. Ravindran and Shylaja (2006) have discussed the various aspects of the use of pepper in food.

Heath (1978) has presented a flavoring triangle to indicate the use of various spices in relation to the various food items. Here, herbs and spices are listed in terms of their flavor strength to indicate the position occupied by a spice in relation to others; the weakest spice is placed at the top and the strongest ones at the bottom. In this flavor triangle, pepper occupies a position almost at the bottom indicating that the flavor strength is strong and that it is especially suitable for mutton, game, ham and beef. For strongly flavored dishes, pepper is a choice spice, as it gives pungency, aroma, flavor and at the same time masks unfavorable flavor notes. On the other hand, the flavor intensity of pepper is mid-way between the strongest (fresh chillies) and the weakest (cardamom), thereby indicating its usefulness with all food types when a balanced flavor is desired and the feasibility of using larger quantities when stronger flavor is needed. Such generous use according to personal preferences may not be possible with spices like chillies because its flavor is so intense that the dish becomes unsuitable for consumption when used in excess.

Yet another widespread use of spices is for deodorizing and masking undesirable flavors and odors. Spices differ much in their masking and deodorizing ability. This masking effect of spice was studied sensorially by adding phased solutions of spices to a known strength of trimethylamine solution or to a solution of methyl mercaptan. Pepper has 30 % power compared to thyme (99 %), rosemary (97 %), mint (90 %), celery (44 %). The masking effect of pepper is very weak and hence its deodorizing power is low (Hirasa and Takemasa, 1998).

**Table 6.10** Antioxidant activity of pepper in comparison with other common spices

| Spice        | Ground spice POV (meq/kg) |
|--------------|---------------------------|
| Black pepper | 364.5                     |
| Ginger       | 40.9                      |
| Turmeric     | 399.3                     |
| Chillies     | 108.3                     |
| Cardamom     | 324.8                     |
| Cinnamon     | 324.0                     |
| Clove        | 22.6                      |
| Nutmeg       | 205.6                     |
| Mace         | 13.7                      |
| Rosemary     | 3.4                       |
| Sage         | 2.9                       |

POV = peroxidation value.

Source: Saito *et al.* (1976).

Black pepper is a strong antioxidant and its antioxidant property in comparison with other common spices is given in Table 6.10. The antioxidant property of pepper is exerted by compounds such as flavonoids and polyphenols and not by compounds present in essential oil fraction (petroleum ether soluble fraction) as in the case of cardamom. The antioxidant property is very important in the preservation of food materials as oxidative degradation of the lipids present in the food material is the major reason for rapid food deterioration. Use of spices such as pepper, cardamom and turmeric prevents or retards significantly the oxidative degradation, and thereby contributing substantially to the preservation of food items.

### 6.9.1 General uses of black pepper in recipes

Hirasa and Takemasa (1998) discuss the patterning theory of spice use and conclude that pepper is suitable for dishes of meat, seafood, milk, egg, grains, vegetables, fruit, beans and seeds and beverages. Pepper plays an important role in the cuisines of China, South East Asia, India, the USA, the UK, Greece, Italy and France. With regard to cooking technique, pepper is suitable for simmered, fried, steamed, deep-fried, food dressed with sauce, pickled and fresh food, but less suitable for baked food. The suitability pattern of pepper is shown in Fig. 6.6 (Hirasa and Takemasa, 1998). There is almost no difference in suitability of pepper between Eastern and Western or continental cooking, although it shows a very high suitability for American cooking.

Black pepper forms an essential ingredient in innumerable recipes. In fact, in the Western world very few meat, fish or egg dishes are produced without the addition of black (or white) pepper. Black pepper is used with main courses, light meals and snacks, brunches, starters and nibbles, side dishes, desserts, cakes and baking, drinks and cocktails and other items like curry pastes, cookies, sauces, marmalades, chutneys, etc. In Western (European and American) cooking, pepper is the only aromatic and piquant agent used in white sauces. Pepper also is a must in freshly cut vegetable salads, such as cucumber, carrot, lettuce, radish, beetroot, onions, tomatoes, etc., in

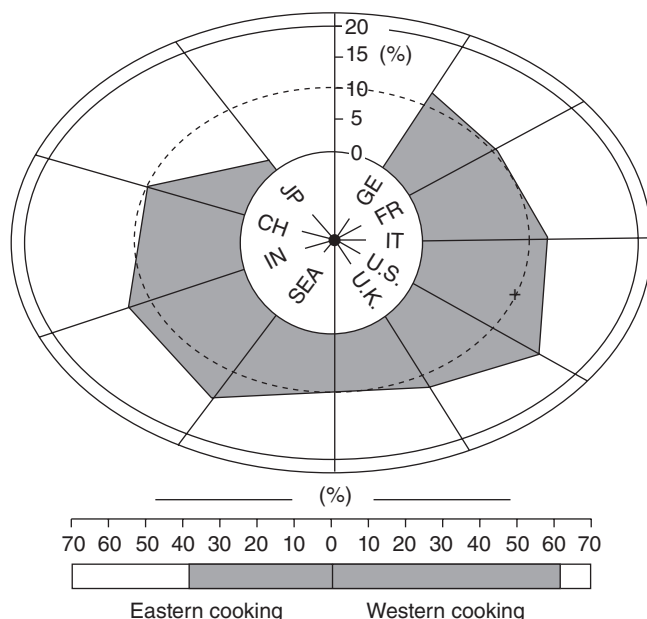


**Fig. 6.6** Suitability pattern of black pepper in eastern and western cooking.  
IN = India; CH = China; JP = Japan; GE = Germany; FR = France; IT = Italy; US = United States of America; UK = United Kingdom.

different combinations. Indian cooks use pepper and other spices in endless variations or combinations, and no two preparations have exactly the same combinations of spices. Spikes of green pepper are used in a number of dishes in households in pepper-growing areas such as Kerala and Western Karnataka in India. It is interesting to note that pepper goes into a variety of dishes, sweets and hot preserves and everyday dishes. It finds a place in exotic as well as bland preparations as in 'roghan josh' and in 'dhal soups'. Black pepper is easily the most widely-used spice, contributing pungency, flavor and taste to otherwise bland meat and vegetable dishes. There are very few food items in which pepper does not find a place, one example possibly being pure ice cream. A few types of foods in which pepper is particularly important are discussed below.

### 6.9.2 Use of pepper in curry powder and soluble seasonings

Pepper is an essential ingredient of most curry powders (masala mixes) used in cooking all over the world, but most extensively used in Indian as well as in South Asian cooking. There is an amazing variation of curry powders, to suit the hundreds of different 'curries' in the cuisines of these countries. Curry powders do play significant roles in the cuisines of other countries too. Curry powder is a mixture of coriander, cumin, turmeric, fenugreek, ginger, celery and black pepper and smaller amounts of chilli powder, cinnamon, nutmeg, clove, caraway and fennel, either with or without salt. Many countries have their own specifications for curry powder. A typical curry powder formulation is given in Table 6.11 and the federal specifications for curry powder (EE-S-631 J) are given in Table 6.12.



**Table 6.11** Typical curry powder formulation indicating the range of spices

| Ingredient          | Typical range (%) |
|---------------------|-------------------|
| Ground coriander    | 10–50             |
| Ground cumin        | 5–20              |
| Ground turmeric     | 10–35             |
| Ground fenugreek    | 5–20              |
| Ground ginger       | 5–20              |
| Ground celery       | 0–15              |
| Ground black pepper | 0–10              |
| Ground red pepper   | 0–10              |
| Ground cinnamon     | 0–5               |
| Ground nutmeg       | 0–5               |
| Ground cloves       | 0–5               |
| Ground caraway      | 0–5               |
| Ground fennel       | 0–5               |
| Ground cardamom     | 0–5               |
| Salt                | 0–10              |

*Source:* Tainter and Grenis (1993).

**Table 6.12** Federal specifications (EE-S-631 J) for curry powder

| Ingredient   | Limit (%)      |
|--------------|----------------|
| Turmeric     | 37.0–39.0      |
| Coriander    | 31.0–33.0      |
| Fenugreek    | 9.0–11.0       |
| Cinnamon     | Not < than 7.0 |
| Cumin        | Not < than 5.0 |
| Black pepper | Not < than 3.0 |
| Ginger       | Not < than 3.0 |
| Cardamom     | Not < than 2.0 |

*Source:* Tainter and Grenis (1993).

The famous oriental five spice blend (FSB) used extensively in many meat and fish preparations has the following composition:

- ground cinnamon: 25–50 %
- ground anise (or star anise): 10–25 %
- ground fennel: 10–25 %
- ground black pepper: 10–25 %
- ground cloves: 10–25 %

Soluble seasonings are spice extracts mixed with a carrier like salt or dextrose. Oleoresin is used for the preparation of soluble spice. The most commonly used carrier is salt since the size of its crystals provides good mixing action which disperses the oleoresin evenly (Tainter and Grenis, 1993). However, dextrose is preferred when a higher salt level is not desirable in the finished product. Soluble spices and seasonings are more often used in the processed foods industry, mainly because



of the convenience involved in its use compared to the extracted oleoresin or oil. For example, pepper oleoresin is a thick, green, viscous liquid, difficult to mix uniformly and not easy to pour, and it is very difficult to measure when only small quantities are required. On the other hand, soluble black pepper is a free flowing powder, easy to weigh and to add to a batch of products uniformly and accurately. Finally, waste is minimized compared to the oleoresin. When oleoresin is added, it can lead to lump formation preventing uniform mixing, while the soluble salt ensures absolute uniformity while mixing.

A typical soluble black pepper formulation consists of the following composition:

- oleoresin of black pepper: 2–5 %
- anticaking agent up to: 2 %
- salt or dextrose to make: 100 %

In the food industry, ground spice has been replaced by oils and oleoresins almost all over the world. The replacement ratios for ground spice using oils and oleoresins for pepper and some of the important spices are given in Table 6.13.

In the manufacture of foods and seasonings, food technologists calculate the spice extractive equivalent (SEE), which is a guide to the flavor strength of the spice extractive used in comparison with ground spice (Farrell, 1985). Pepper is an important ingredient in many flavoring and seasoning formulations – American, European as well as oriental. Table 6.14 gives the pepper contents in some of the well known flavoring and seasoning formulations.

Consumers look for the organoleptic quality of foods rather than their nutritive value. Even the most nutritious food is not often accepted unless it is moderately spiced. It is an art to subtly blend flavoring and seasoning in order to give distinctive tastes to the different dishes. All spices, particularly pepper, must be used with consummate skill. Even the most insipid dishes can be improved by taking advantage of the pungent taste and spicy aroma of pepper to produce savory dishes; that is why pepper is a universal favorite among the world's chefs. A list of oriental dishes where pepper is an essential component is given in Table 6.15.

**Table 6.13** Replacement ratios for ground pepper in comparison with other important spices using oils and oleoresins

| Spice     | Replace 1 # with |           |
|-----------|------------------|-----------|
|           | Oil              | Oleoresin |
| Pepper    | 0.015            | 0.050     |
| Cinnamon  | 0.025            | 0.025     |
| Clove     | 0.140            | 0.050     |
| Cardamom  | 0.030            | 0.015     |
| Celery    | 0.010            | 1.00      |
| Coriander | 0.003            | 0.070     |
| Ginger    | 0.015            | 0.035     |
| Mace      | 0.140            | 0.070     |
| Nutmeg    | 0.600            | 0.080     |

**Table 6.14** Pepper use in some of the flavoring and seasoning formularies

| Formulary                            | Content of pepper   |
|--------------------------------------|---------------------|
| Pickling mix                         | 0–10 %              |
| Poultry seasoning                    | 4.5–5.5 %           |
| Pumpkin pie sauce                    | 0–5 %               |
| Oriental five spice blend            | 10–25 %             |
| Smoked sausage seasoning             | 6.57–7.03 %         |
| Italian sausage seasoning            | 2–6 oz/100 oz       |
| Pork sausage                         | 1–4 oz/100 oz       |
| Bologna/Weiner seasoning formulation | 0.5–2 % (oleoresin) |
| Roast beef rub formulation           | 0–5 %               |
| Pepperoni seasoning                  | 1–4 oz/100 oz       |
| Hot and spicy nut seasoning          | 0–5 %               |

Source: Tainter and Grenis (1993).

**Table 6.15** Some important dishes flavored with pepper

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Beverages</b><br>1. Pepper tea<br>2. Pepper milk shake<br>3. Spicy water melon juice<br><br><b>Pickles/chutneys</b><br>4. Pickled cherries<br>5. Pickled beef or pork<br>6. Pepper spike pickle<br>7. Coconut chutney<br>8. Fresh coriander chutney<br><br><b>Sweet preparations/confections</b><br>9. Quick banana pudding<br>10. Soji halwa<br><br><b>Snacks</b><br>11. Pepper biscuits<br>12. Vegetable crispies<br>13. Bonda<br>14. Pongal<br>15. Quick hamburger onion hash<br><br><b>Vegetable preparations</b><br>16. Vegetable curry<br>17. Vegetable korma<br>18. Masala dal | <b>Soups</b><br>19. Mixed vegetable soup<br>20. Cream of vegetable soup<br>21. Clear dhal soup<br><br><b>Legume/pulse preparation to go with cereals</b><br>22. Radish sambar<br>23. Mulugutwanny (Rasam)<br><br><b>Meat dishes</b><br>24. Pepper steak<br>25. Black pepper pot roast<br>26. Pepper mutton balls<br>27. Black pepper fried chicken<br>28. Roghan josh<br>29. Korma curry<br>In fact pepper is used with most meat dishes both at the time of cooking and later at the dining table before a dish is eaten.<br><br><b>Other preparations</b><br>30. Amla preserve<br>31. French beans with coconut<br>32. Ground spice mixture |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

### 6.9.3 Masala (spice mixes)

Although some foods such as fried potatoes, lady's finger (okra), brinjal (egg plant), etc. use only two to three spices, most dishes are prepared with elaborate combinations of meticulously prepared and freshly ground spices referred to as *masala*. The masalas vary widely and each masala has a special purpose. *Garam masala*, for example, is a blend of dried and powdered spices, to be used as such or in combination with other seasonings. Pepper has an important role in *garam masala* along with cardamom, cloves and cinnamon. Premavalli *et al.* (2000) analyzed various

commercial brands of *garam masala* and *puliyodara* mixes and found black pepper to be an important ingredient in all of them.

#### 6.9.4 Condiments, sauces and seasonings

Condiments are prepared food compounds containing one or more spices, or spice extracts, which, when added to a food after it has been served, enhance the flavor of the food (Farrell, 1985). Condiments can be either simple (e.g. celery salt, garlic salt, onion salt) or compound (chilli sauce, chutney, meat sauce, mint sauce, prepared mustard, etc.). Pepper forms an ingredient in many compound condiments. Pepper powder constitutes around 0.02 % in prepared mustard formulations such as Dijon and Dusseldorf mustards, while in Swedish mustard, pepper is around 0.2 %. Pepper is an ingredient in certain Worcestershire sauce formulations. It forms about 5.2 % of the famous marinara and parmesan seasoning mixes.

Sauces are hot or cold, liquid or semi-liquid products (other than condiments) which, when added to a food as it is being served, add to its acceptance by

**Table 6.16** Sauce/seasoning and salad formulations containing pepper

| Product                      | Ingredients                                                                                                                                              |
|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Sauce/seasoning</b>       |                                                                                                                                                          |
| Cucumber cream sauce         | Lemon juice, cayenne pepper, white pepper, cream, mashed cucumbers                                                                                       |
| Cream sauce                  | Heavy cream, lemon juice, white pepper, onions, mustard                                                                                                  |
| Sour cream sauce             | Sour cream, lemon juice, white pepper, onions, egg, vinegar, dry mustard                                                                                 |
| Bearnaise sauce              | Shallots, parsley, black pepper, tarragon, chervil, vinegar, cayenne pepper, egg yolk, butter                                                            |
| Bechamel sauce               | Chicken stock, butter, white pepper, onion                                                                                                               |
| Diabale sauce                | Shallots, black pepper, white pepper, white wine, parsley                                                                                                |
| <b>Worcestershire sauces</b> |                                                                                                                                                          |
| Horseradish sauce            | Horseradish, white pepper                                                                                                                                |
| Poivrade game sauce          | Onions, carrots, shallots, garlic, bay leaf thyme, vinegar, leaf stock, red wine, black pepper, parsley, olive oil, red currant jelly                    |
| Tourangelles sauce           | Butter, onion, carrot, shallots, garlic, red wine, beef and chicken stocks, black pepper, parsley, bay leaf, thyme                                       |
| Newburgh sauce               | Mace, sherry wine, white pepper                                                                                                                          |
| <b>Salad dressings</b>       |                                                                                                                                                          |
| Chicken                      | Capers, chives, curry powder blend, white pepper, fennel, marjoram, mustard, nutmeg, onion, paprika, poppy seed, rosemary, sesame seeds, tarragon.       |
| Cabbage                      | Allspice, basil, white pepper, caraway seed, celery seed, dill, marjoram, mint, nutmeg, onion, chillies, poppy seed, paprika, rosemary, sesame, tarragon |
| Cucumber                     | Basil, capsicum, chervil, white pepper, dill, onion, paprika, tarragon                                                                                   |
| Egg                          | Celery seed, chilli powder blend, white pepper, chives, chervil, dill, marjoram, mustard, onion, parsley, paprika, tarragon                              |
| Turkey                       | Capers, chives, curry powder blend, white pepper, marjoram, onion, paprika, poppy seed, rosemary, sesame seed, tarragon                                  |

Source: Farrell (1985).

**Table 6.17** Commercial seasoning and instant gravy mixes containing pepper

| Name                                | % of pepper (white/black/oleoresin)          |
|-------------------------------------|----------------------------------------------|
| <b>Frankfurter seasoning</b>        |                                              |
| Formula A                           | 5.72                                         |
| Formula B                           | 17.03                                        |
| Formula C                           | 3.73                                         |
| Formula D                           | 3.45 g (oleoresin) per 100 oz                |
| Formula E                           | 65.71 g/1000 oz (soluble spice in salt base) |
| <b>Bologna seasoning</b>            |                                              |
| Formula A                           | 6.25                                         |
| Formula B                           | 0.37 (oleoresin)                             |
| Formula C                           | 7.14 (soluble spice)                         |
| <b>Fresh pork sausage seasoning</b> |                                              |
| Formula A                           | 10.0                                         |
| Formula B                           | 7.5                                          |
| Formula C                           | 0.43 (oleoresin)                             |
| Italian seasoning                   | 10.55                                        |
| Italian sausage seasoning           | 10.0                                         |
| Kielbase (Polish) seasoning         | 15.0                                         |
| Smoked liver sausage seasoning      | 2.8 ground                                   |
| <b>Instant gravy mixes</b>          |                                              |
| Mushroom gravy seasoning and mix    | 0.46                                         |
| Chicken gravy seasoning mix         | 0.33                                         |
| Poultry gravy seasoning mix         | 0.026                                        |
| Prawn gravy seasoning mix           | 0.46                                         |
| French onion soup seasoning mix     | 0.004                                        |
| Fish chowder seasoning mix          | 0.004                                        |
| Chicken noodle soup seasoning mix   | 0.011                                        |
| Shrimp seasoning mix                | 0.078                                        |
| Fettucine Alfredo seasoning mix     | 1.0                                          |

Source: Farrell (1985).

improving its appearance, aroma, flavor or texture. They may or may not include spices or spice extracts. Pepper is a component spice in sauces, salad dressings and seasoning formulations. The universally popular French dressing contains pepper, for example. Seasonings are compounds containing one or more spice extracts which, when added to a food, either during its manufacture or in its preparation, before it is served, enhance the natural flavor of the food and thereby increase its acceptance by the consumer (Farrell 1985). Black and white peppers are ingredients in many famous seasoning formulations and instant gravy mixes. Table 6.16 gives a sample of famous sauces and seasoning formulations being used all over the world and Table 6.17 gives a sampler of some of the famous commercial seasoning mixes, in all of which pepper is an essential ingredient.

## 6.10 Conclusion

The pepper production scenario in the major developing countries will change as a result of the socio-economic changes that these countries are facing. Farming is no

longer remunerative in the rapidly developing countries like Brazil, Malaysia, Thailand and India, where the cost of agricultural labor is on the increase, precluding economically viable production. In the coming decades, Sri Lanka is not going to be a major producer because of area limitations. China and Vietnam will play greater roles in the world pepper trade and economy as a result of the rapid area and production increase anticipated in these countries. Currently, global pepper production is facing shortages on account of a shift in cultivation from pepper to rubber in India and to crops like vanilla and cocoa in Indonesia and Brazil. More than anything else, diseases such as *Phytophthora* root rot and viruses have created a situation of instability in pepper farming; there is no guarantee that a pepper farm will remain productive even for a few years. Low production and an increasing consumption pattern may create price increase or at least price stability, and this factor may in turn motivate the farmers to turn to pepper farming. Pepper uses in the areas of cooking and human health are increasing and such factors will make pepper more expensive as well as more precious to consumers all over the globe.

## 6.11 Source of further information

For a comprehensive treatment of all aspects of black pepper refer to: RAVINDRAN P.N. (ed.) (2000) *Black Pepper*. Harwood Academic, Amsterdam.

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## Capsicum cultivars

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**Abstract:** Many different varieties of the genus *Capsicum* are widely grown for their fruits, which may be eaten fresh, cooked, smoked, as a dried powder, in a salsa, in a sauce, or processed into oleoresin (Poulos, 1993). Peppers are valued for several attributes: colour, pungency, flavour, texture, vitamin content, pain relief, repellency, and health benefits. There are five major products traded on the world market for use in food processing: paprika, oleoresin, fresh fruits, frozen fruits (whole, diced, and pureed), and dried chilli (whole, powdered, and smoked). Fermented mash is used for food processing, particularly hot sauces.

**Key words:** capsicum, chillies, paprika, cayenne, annum, capsaicin, capsanthin, capsorubin, Scoville heat unit, ASTA, vitamin A, vitamin C.

### 7.1 Introduction

#### 7.1.1 Origin

The genus *Capsicum* belongs to the family Solanaceae. Within the genus *Capsicum*, five species are commonly recognized as domesticated: *Capsicum annum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*, while approximately 20 wild species have been documented. The genus *Capsicum* shares the distinction of being the first plants cultivated in the New World with beans (*Phaseolus* spp.), maize (*Zea mays* L.), and cucurbits (*Cucurbitaceae*) (Heiser, 1973). Peppers were one of the first spices used by humans anywhere in the world. Widespread geographic distribution of *C. annum* and *C. frutescens* from the New World to other continents occurred in the 16th century via Spanish and Portuguese traders, whereas the other species are little distributed outside South America (Andrews, 1995). Most products used commercially for food processing are *C. annum*. Notable exceptions include Tabasco<sup>TM</sup> sauce (*C. frutescens*), manufactured by McIlhenny Co., Avery Island, Louisiana (Anon., 2011), and Peppadew<sup>TM</sup> peppers (*C. baccatum*), manufactured by Peppadew Int'l (Pty) Ltd, Bryanston, South Africa (Van Vuuren, 2006).

#### 7.1.2 Classification

The classification system for this genus is somewhat confusing in the literature. In Spain, the Castilian word 'pimiento' refers to any *Capsicum* species, but in the USA,



‘pimiento’ or ‘pimento’ refers only to thick-walled, heart-shaped, non-pungent fruits from the species *C. annuum*, commonly used to stuff olives. The Hungarians call all *C. annuum* fruits ‘paprika’, but paprika is defined in the world market as a ground, red powder derived from dried pepper fruits with dark red colour and acceptable flavour. The word ‘chile’ is the common name for any *Capsicum* species in Mexico, Central America and the southwestern USA. In Asia, the spelling ‘chilli’ is more common and is always associated with highly pungent varieties of *C. annuum* and *C. frutescens*, while the non-pungent sweet bell peppers are referred to as ‘capsicums’. Pungent fruits of all cultivated *Capsicum* species as a collective class are called ‘chillies’ in the Food and Agriculture Organization (FAO) Yearbook (FAO, 2011). Bird’s eye chillies are grown primarily in East Africa, but they are merely small-fruited, highly pungent forms of *C. annuum* or *C. frutescens*.

In this review, the following definitions will be used:

- **Oleoresin:** A viscous liquid derived by extraction from ground powder of any *Capsicum* species; there are three types of oleoresin: paprika (used for colour), red pepper (used for colour and pungency), and Capsicum (used for pungency).
- **Paprika:** A ground, bright red, usually non-pungent powder used primarily for its colour and flavour in processed foods; all paprika varieties are *C. annuum*; paprika fruits are used to produce paprika oleoresin.
- **Cayenne powder:** A ground, red, pungent powder used primarily for its colour and flavour in processed foods. Cayenne powder may come from several different fruit types, not just cayenne peppers.
- **Cayenne:** A pungent fruit type, named for the city of Cayenne, French Guiana, that is frequently ground into a mash and fermented to make a hot sauce. Cayennes may be used to produce red pepper oleoresin or Capsicum oleoresin. The terms chilli and cayenne are synonymous.
- **Chilli powder:** A powdered spice mix composed chiefly of chilli peppers and blended with other spices including cumin, oregano, garlic powder, and salt.
- **Pepper:** Generic term describing the fruits of any *Capsicum* species, both pungent and non-pungent, to distinguish them from black pepper (*Piper nigrum*). The misleading name ‘pepper’ was given by Christopher Columbus upon bringing *Capsicum annuum* back to Europe. At that time peppercorns, the fruit of *Piper nigrum*, an unrelated plant originating from India, were a highly prized condiment; the name ‘pepper’ was at that time applied in Europe to all known spices with a hot and pungent taste and so naturally extended to the newly discovered *Capsicum* genus.
- **Jalapeno:** A pungent fruit type native to Jalapa, Veracruz, Mexico (jalapeno means ‘from Jalapa’), characterized by a unique flavour and usually consumed green. It may be consumed fresh, diced, pickled, smoked, pureed, or stuffed.
- **Bell:** A non-pungent fruit type native to Mexico but cultivated mainly in the USA and Europe, usually consumed fresh in a variety of colours (green, yellow, orange, or red). Fruits are generally cubical in shape.

### 7.1.3 Usage

Peppers are used as a colourant, flavourant, and/or as a source of pungency, depending on the processed product. Peppers can be used fresh, dried, frozen, smoked, fermented, or as an oleoresin extract. They can be used whole, chopped, coarsely ground, finely ground, or pureed, with or without seeds. Various types of processed products containing primarily peppers include pickled fruits (whole, sliced, diced, etc.), frozen bell peppers (diced or cupped), cayenne powder, crushed red pepper flakes (with or without seeds), fermented mash, paprika, and three types of oleoresin. Hot sauces deserve special mention, as the variety of flavours and the range of heat levels is limited only by the human imagination. The world's hottest food product, 'Blair's 16 Million Reserve', contains only capsaicin crystals, and is the hottest possible capsaicin-based sauce. Other processed products that contain a significant proportion of peppers include fresh and processed salsas, curry powders, barbecue seasonings, chili powder, and many other foods (Govindarajan, 1986).

### 7.1.4 Chemistry

The main source of pungency in peppers is the chemical group of alkaloid compounds called capsaicinoids (CAPS), which are produced in the fruit. The atomic structure of CAPS is similar to piperine (the active component of white and black pepper, *Piper nigrum*) and zingerone (the active component of ginger, *Zingiber officinale*). Capsaicin ( $C_{18}H_{27}NO_3$ , *trans*-8-methyl-N-vanillyl-6-nonenamide), shown in Fig. 7.1, is the most abundant CAPS, generally around 46 % of total CAPS, followed by dihydrocapsaicin (generally around 41 %, with minor amounts of nordihydrocapsaicin (7 %), homocapsaicin (3 %), homodihydrocapsaicin (2 %), and others. Capsaicin is a white, crystalline, fat-soluble compound formed from homovanillic acid that is insoluble in water, odourless, and tasteless (Andrews, 1995). Varieties of chilli differ widely in CAPS content. The amount of CAPS in a given variety can vary depending on the light intensity and temperature at which the plant is grown, the age of the fruit, the moisture content of the soil, and the position of the fruit on the plant. The first test developed to measure pungency was the Scoville test, first developed in 1912 by Wilbur Scoville (Scoville, 1912). It measures 'heat' as Scoville heat units (SHU) in a given weight of fruit tissue. Scoville heat units can be expressed on a fresh or dry weight basis. In this article, SHU will be expressed on a dry basis unless labelled otherwise. Sweet peppers have 0 SHU, chillies with a slight bite may have 100–500 SHU, and the blistering habaneros have between 100 000 and 130 000. For many years, the title of 'World's Hottest Pepper' was held by a habanero variety called 'Red Savina', at 579 000 SHU. It was surpassed in 2007 by the Bhut Jolokia pepper, according to the Guinness World Records, topping the scale at a

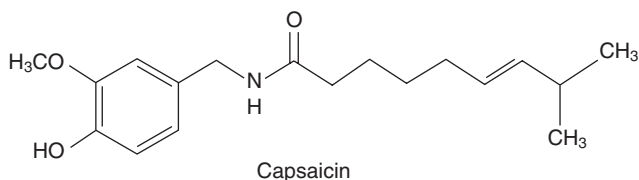


**Fig.7.1** Chemical structure of capsaicin.

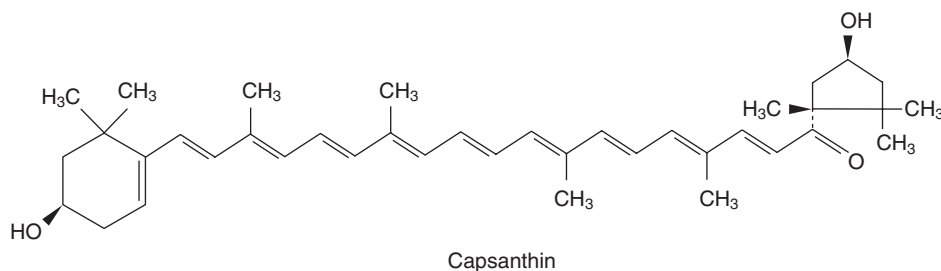


**Fig.7.2** Chemical structure of capsanthin.

whopping 1 001 304 Scoville Heat Units. In 2011, the title of ‘World’s Hottest Pepper’ passed to the Naga Viper pepper, with a blistering rating of 1 382 118 SHU.

The red colour of mature pepper fruits is due to several related carotenoid pigments, including capsanthin [(all-E,3R,3’S,5’R)-3,3’-dihydroxy-β,κ-caroten-6’-one], capsorubin [(all-E,3S,3’S,5R,5’R)-3,3’-dihydroxy-κ,κ-carotene-6,6’-dione], and β-cryptoxanthin [(3R)-β,β-caroten-3-ol], which are present as fatty acid esters. The most important pigments are capsanthin and its isomer capsorubin, which make up 30–60 % and 6–18 %, respectively, of the total carotenoids in the fruit (Govindarajan, 1985). The chemical structure of capsanthin is shown in Figure 7.2. These two pigments are unique to peppers, they are found in no other plant or animal species. The intensity of the red colour in a pepper is primarily a function of the amount of these two pigments. The Peruvian, Hungarian, and Spanish varieties used for paprika have very high amounts of capsanthin and capsorubin compared to other varieties (Govindarajan, 1985).

### 7.1.5 Stability

CAPS in oleoresins are very stable compounds and generally do not break down, even during processing at high temperatures and during long storage periods. CAPS in dry products (fruits, powder, etc.) are not as stable as in oleoresins. The temperature at which the fruits are dried affects the CAPS content. For example: drying ripe fruits at 60 °C to a final moisture content of 8 % decreases CAPS content approximately 10 % (Bensinger, 2000). If the fruits are held for extended periods of time at 60 °C after reaching 8 % moisture content as much as 50 % of the CAPS may be lost. Once the fruits are dried, they typically lose 1–2 % CAPS/month under cold (–16 °C) storage, and even more when stored under ambient conditions. Ground powder can lose as much as 5 % CAPS/month depending on the fineness of the grind and the storage temperature (Bensinger, 2000). The red colour of paprika and chilli powder, on the other hand, is not as stable as oleoresin and CAPS, and much work has been done to optimize the processing and storage conditions for dried chillies and paprika to maximize the colour intensity for the longest period of time (Isidoro *et al.*, 1990; Lee *et al.*, 1991; Garcia-Mompean *et al.*, 1999).

## 7.2 Production of capsicum cultivars

Reliable production figures for paprika and chillies are difficult to obtain because the FAO Yearbook includes semi-official or estimated data. In 2008, the FAO

reported that there were 1.83 million hectares of peppers grown. The average yield in 2008 was  $16.1 \text{ t ha}^{-1}$ , with total production estimated at 2.94 million metric tons (MMT) (FAO, 2011). The major pepper-producing nations in terms of volume are India, Ethiopia, Myanmar, China, Peru, Thailand, Pakistan, Bangladesh, Indonesia, Mexico, and Sri Lanka.

### 7.2.1 Paprika production

Paprika is produced commercially in Peru, Spain, Central Europe, Israel, southern Africa, and the USA, but Hungary is by far the most famous paprika-producing country, with approximately 2200 ha devoted to the crop (Moor, 2000; FAO, 2011). Many food historians believe that Turks and Bulgarians of the Ottoman Empire brought peppers to Hungary in the sixteenth century. Kalocsa and Szeged are the main centres of paprika production. Once the fruits are harvested, they are loosely stacked in long, narrow, cylindrical mesh bags made of red plastic and allowed to 'cure' for 3–4 weeks. Then the peppers are washed, dried, crushed and finally ground into powder. The mill master selects the proportion of seeds to be ground with the pepper pods, to produce the desired level of pungency and colour in the paprika. During grinding, the crushed peppers are heated to release the oil in the seeds, which interacts with the pigment in the fruits to produce the intense red colour. Colour has no effect on the pungency of the paprika. Bright red paprikas may be sweet or pungent. Heat causes the natural sugar content of the fruits to caramelize slightly, which affects the taste and aroma of the paprika. During the process, if the fruits are heated too much, they will scorch. If they are not heated enough, the moisture content will be too high, and both the flavour and colour will be affected. Optimum moisture content is 8 % (Moor, 2000). Only undamaged fruits less than a year old are used for the top grades of Hungarian paprika. Before non-pungent paprika varieties were available, the top grades of paprika were prepared by removing the dissepiment (ribs on the inside of the pericarp which are rich in CAPS) using special knives (Anon., 1999), but this method is no longer used (Moor, 2000). Peru is the largest paprika-producing country by area, with 21 500 ha in 2008 (FAO, 2011).

### 7.2.2 Oleoresin production

Oleoresin is a viscous liquid or semi-solid material derived by extraction from finely-ground powder, which contains the aroma and flavour of its source. Three types of oleoresin are produced. High-pungency *Capsicum* oleoresin is produced primarily in India, Africa, and China near the production areas of low-cost, very pungent chilli varieties. Medium-pungency red pepper oleoresin is produced in many regions. Non-pungent paprika oleoresin is produced in Spain, Ethiopia, Morocco, Israel, India, the USA, Mexico, and South Africa (Govindarajan, 1986). Oleoresin is extracted from finely ground chilli or paprika powder. A volatile non-aqueous solvent such as hexane, ether, or ethylene dichloride is added and allowed to thoroughly wet the material. The oleoresin enters into solution with the solvent, forming micella. After a period of time, the micella are removed, and the solvent replaced with fresh solvent to continue the extraction. The solvent is subsequently removed from the extract by evaporation at the lowest practical temperature to avoid loss of

aromatic volatile compounds. This is done in two stages, the first stage removes approximately 95 % in a standard film evaporator, and then the concentrated micella pass through a partial vacuum that removes the rest of the solvent and reduces the miscella to oleoresin. The remaining solvent held in the mass of the extracted powder is recovered by very high vacuum. Typical yield of oleoresin depends on the solvent used and ranges from 11.5–16.5 % (Govindarajan, 1986). The oleoresin pungency depends on the pungency of the original powder. Paprika oleoresin has little to no pungency, and is used for its colour and flavour properties, while Capsicum oleoresin can have CAPS levels up to 10 %, and is used primarily as a source of high pungency.

### 7.2.3 Dry chilli production

Chilli peppers are typically produced on small farms, less than 1 ha in size, in areas where cheap labour is available for harvesting. The largest producer is India, with an estimated 779 000 ha devoted to the crop in 2008 (FAO, 2011); India is also the largest exporter of dried chilli in the world. The second-largest producer is Ethiopia, which grows an estimated 290 000 ha annually, followed by Myanmar with 109 000 ha annually. Fruits are typically allowed to partially dry on the plant, then harvested and placed in well-ventilated areas receiving direct sunlight for drying. Sun-drying can result in bleached fruits, especially if rainfall is received during the drying period, and the fruits may have extraneous matter adhering to them. In more advanced regions, the use of controlled drying improves the quality of the dried fruits. The best drying temperature is 60–70 °C; this gives maximum colour values and longest colour retention time. Higher temperatures tend to caramelize the sugars present in the fruits and give them a dark colour. The optimum moisture content is approximately 10 % (Lease and Lease, 1962; Kanner *et al.*, 1977; Carnevale *et al.*, 1980).

## 7.3 Main uses in food processing

There are many uses of peppers in food processing, including as a food colourant, as a source of pungency in food, as a source of flavour, as a source of texture, as a source of pain relief for pharmaceutical use, and as a repellent. In many cases, two or more of these properties are included in the same product; for example, paprika powder may be a source of colour, texture, and flavour.

### 7.3.1 Colour

People whose diets are largely colourless starches, such as rice or maize, use peppers to add colour to their bland, achromatic diets. Paprika, paprika oleoresin, red pepper oleoresin, and dried chilli may all serve as a source of red colour in various processed products, but the primary sources of red colour are paprika and paprika oleoresin. Paprika is used in many products where no pungency is desired, but the colour, flavour, and texture of a finely ground powder is desired. These include processed lunchmeats, sausages, cheeses and other dairy products, soups, sauces, and snacks such as potato chips. Paprika oleoresin is used as a source of colour in canned meats,

sausages, smoked pork, sandwich spreads, soups, cheeses, orange juice, spice mixtures, sauces, and sweets. In poultry feed, it is used to deepen the colour of egg yolks. Paprika oleoresin is used as a colour and flavour additive in many products where the texture is important and small particles of paprika powder would be undesirable (Govindarajan, 1986). In the USA, paprika oleoresin is listed as a colour additive 'exempt from certification' (Title 21 Code of Federal Regulations part 73) and generally classified as a natural colour. In Europe, paprika oleoresin (extract), and the compounds capsanthin and capsorubin are designated by E160c (UK Food Guide, 2012).

### **7.3.2 Pungency**

Using oleoresins to add pungency to products allows the processor to add a defined amount of oleoresin of a defined pungency level to consistently obtain a defined pungency of product. Red pepper oleoresin is used as a source of both colour and pungency in canned meats, sausages, smoked pork, sandwich spreads, soups, and in dispersed form in some drinks such as ginger ale and orange juice. Capsicum oleoresin is used as a source of pungency in many products, especially chilli sauces with extremely high SHU ratings. Many companies that want to produce salsa with different pungency levels (mild, medium, and hot) make a sweet base formula and add defined amounts of either red pepper or Capsicum oleoresin to obtain consistent, defined pungency levels in the final product (Wahl, 2005). Oleoresin has considerable advantages over dried chilli, including more stable colour retention, easier to handle compared to the rather bulky dried chilli, and the ability to mix and dilute oleoresin with other substances to produce a range of colour and/or pungency values. Dried chilli is used primarily as a source of colour, texture, and pungency, particularly in the production of crushed red pepper flakes, chilli powder, and chilli sauces.

### **7.3.3 Flavour**

Paprika is valued for its flavour in many products in addition to its colour. Dried chilli is also valued for its contribution to flavour in chilli sauces and chilli powders. The flavouring principle is associated with volatile aromatic compounds and colour. As a general rule, when the colour of paprika or chilli powder fades, the flavour also disappears. The 'green bell pepper flavour' associated with green peppers is due to 3-isopropyl-2-methoxypyrazine, one of the strongest flavour compounds known. The human nose can detect one drop of this compound in 22 million gallons of water. This compound generally disappears as bell peppers ripen from green to mature colour (red, orange, or yellow), and the sugar content of the fruits increases, creating a different flavour profile for coloured bell peppers versus green bell peppers. In many countries, coloured bell peppers are preferred for their flavour, even though they are generally more expensive than green bell peppers.

### **7.3.4 Pharmaceutical**

Capsicum oleoresin is the primary form of peppers used for pharmaceutical purposes. Here, the primary requirement is the CAPS level. Further refinement of the

oleoresin may be performed to produce pure capsaicin. At least two types of pain relief products are currently being marketed, including creams containing 0.75 % capsaicin (for example, Zostrix™), and plasters containing 3% oleoresin (for example, Vorwerk™). Several types of capsules containing cayenne powder with a range of capsaicin levels are currently being marketed. More recently, a series of compounds related to CAPS called capsinoids have been found to induce weight loss in humans. Capsinoids are a family of compounds that are analogues of capsaicin, but are non-pungent. They are approximately 1000 times less pungent in contrast to capsaicin. Capsinoids is a collective name that includes capsiate, dihydrocapsiate, and nordihydrocapsiate. They can increase thermogenesis and energy consumption, promote energy metabolism, and suppress body fat accumulation (Iwai *et al.*, 2003; Lee *et al.*, 2010b).

## 7.4 Functional properties and toxicity

Peppers are well-known for their health benefits, such as high vitamin content, and other health-enhancing effects. These include clearing the lungs and sinuses, protecting the stomach by increasing the flow of digestive juices, triggering the brain to release endorphins (natural painkillers), making your mouth water, which helps to neutralize cavity-causing acids, and helping protect the body against cancer through antioxidant activity (Andrews, 1995).

### 7.4.1 Toxicity

The acute toxicity of capsaicin has been measured in several animal species. In mice, the LD<sub>50</sub> values for CAPS depended on the mode of administration, ranging from 0.56 (intravenous) to 512 (dermal) mg kg<sup>-1</sup> body weight. Death was due to respiratory paralysis (Glinsukon *et al.*, 1980). To reach the LD<sub>50</sub> value for human oral administration, the average person would have to drink 1.5 quarts of Tabasco® sauce. The painfulness of the CAPS is a self-limiting factor in their role as a human food ingredient; you can only eat so much at one time. No death has ever been recorded due to CAPS-induced respiratory failure, and the investigators concluded that the acute toxicity of CAPS as a food additive in man was negligible (Glinsukon *et al.*, 1980). The effect of sub-chronic toxic doses has been examined in rats. Adult rats exhibited no noticeable behavioural or physiological changes when given sub-chronic doses of crude chilli extract by stomach tube for 60 days. Food consumption was significantly higher, but body weight was lower than the control group after 60 days (Govindarajan and Sathyanarayana, 1991).

### 7.4.2 Functional benefits

CAPS stimulates sensory neurons in the skin and mouth cavity, creating a sensation of warmth that increases to severe pain (type C nociceptive fibre pain) with higher doses. The CAPS sensory neuron in humans has been cloned; it is a heat-activated ion channel in the pain pathway. When exposed to CAPS, these sensory neurons produce the neuropeptide substance P (SP), which delivers the message of pain.



Repeated exposure of a neuron to capsaicin depletes SP, reducing or eliminating the pain sensation in many people (Caterina *et al.*, 1997). Thus the use of CAPS in pain relief has two modes of action: the sensation of heat, which may help sore muscles and arthritic joints feel better, and the depletion of SP, which reduces the pain sensation in the exposed area. Peppers have been reported to contain an anti-coagulant that helps prevent the blood clots that can cause heart attack (Andrews, 1995). Foods containing CAPS increase the thermic effects of food (TEF). The TEF is the slight increase in the body's metabolic rate after consumption of a meal. A meal containing foods with CAPS can increase the body's TEF up to 25 % for 3 hours (Andrews, 1995). The role of CAPS in triggering the brain to release endorphins (natural painkillers) is well known. As more CAPS is consumed, the body releases more endorphins, causing one to feel a mild euphoria – a natural high! Regular consumption has only a slight desensitizing effect.

### 7.4.3 Nutritional benefits

The Hungarian scientist Albert Szent-Gyorgyi won the 1937 Nobel Prize for isolating ascorbic acid, better known as vitamin C, from peppers in 1932. Peppers are also high in vitamin A (red peppers only), vitamin E, and potassium. One hundred grams of fresh red chilli pepper has 240 mg of vitamin C (five times higher than an orange), 11 000 IU of vitamin A, and 0.7 mg of vitamin E. Vitamin C is sensitive to heat and drying, but vitamin A is very stable, and paprika and dried chilli both contain relatively high amounts of this important nutrient (Govindarajan, 1986).

## 7.5 Quality issues

The quality parameters of paprika, oleoresin, and dried chilli focus primarily on colour, pungency, and microbial and insect contamination, but vary depending on the product and the intended end-use.

### 7.5.1 Paprika quality

Paprika in world trade always refers to a ground product prepared of highly coloured, mild, or sweet red fruits. The main quality factors are colour and pungency. There are eight grades of Hungarian paprika (Table 7.1). The condition of the fruits at harvest, and to some extent the manner in which they are processed, determines which grade of paprika will be made from them. Fruits are graded at harvest for colour, pungency, and aroma. The Grade 1 fruits are used to make the best grades of paprika (Special, Capsaicin-free, and Delicatessen), while Grade 2 fruits are used to make lower grades of paprika (Fine sweet, Semi-sweet). Fruits from later harvests and those rejected from higher grades are used for Rose, while spotted fruits not belonging to any other grade are used for Pungent, which is the lowest grade. Spanish paprika is divided into three types [sweet (dulce), semi-sweet (agridulce), and pungent (picante)] by pungency, and each type is divided into three grades (extra, select, and ordinary) by colour, ash content, and moisture content. The best Spanish paprika is sweet, extra grade, with no pungency, bright fiery red colour, and



**Table 7.1** Grades of Hungarian paprika, from best (special) to worst (pungent)

| Grade                          | Quality characteristics     |                         |                     |                      |               |                     |                   |                                       |
|--------------------------------|-----------------------------|-------------------------|---------------------|----------------------|---------------|---------------------|-------------------|---------------------------------------|
|                                | Colour                      | Pungency                | Aroma               | H <sub>2</sub> O (%) | Total ash (%) | Acid-insol. ash (%) | Ether extract (%) | Powder fineness <sup>1</sup> (sieve#) |
| 1) Special                     | Bright, fiery red colour    | None or very little     | Pure, very aromatic | 10.0                 | 5.0           | 0.3                 | 12.0              | 0.45                                  |
| 2) Capsaicin-free (mild table) | Bright red                  | None or some bitterness | Pure, sweet         | 10.0                 | 5.5           | 0.4                 | 13.0              | 0.50                                  |
| 3) Delicatesse (table)         | Bright red, darker or light | Barely detectable       | Typical pure aroma  | 10.0                 | 6.0           | 0.45                | 14.0              | 0.50                                  |
| 4) Hot table <sup>2</sup>      | Bright red, darker or light | Less pungent            | Typical pure aroma  | 10.0                 | 6.0           | 0.45                | 14.0              | 0.50                                  |
| 5) Fine sweet                  | Dark or yel lowish red      | Less pungent            | Aromatic            | 10.0                 | 6.5           | 0.5                 | 16.0              | 0.50                                  |
| 6) Semi-sweet                  | Darker to yel lowish red    | Pungent                 | Less typical aroma  | 10.0                 | 7.0           | 0.7                 | 17.0              | 0.63                                  |
| 7) Rose (pink)                 | Dull red to pale yellow     | Markedly pungent        | Typical aroma       | 10.0                 | 8.0           | 0.8                 | NS                | 0.63                                  |
| 8) Pungent                     | Dark red                    | Very pungent            | NS                  | 10.0                 | 10.0          | 1.2                 | NS                | 0.63                                  |

<sup>1</sup> 100 % of the powder can pass through sieve no.

<sup>2</sup> Similar to Delicatesse, but with a CAPS minimum of 25 mg 100 g<sup>-1</sup>.

NS = Not specified.

Source: Govindarajan (1986).

only 8.0 % moisture (Govindarajan, 1986). Microbial contamination by bacteria such as *Bacillus cereus*, *B. subtilis*, and *Clostridium perfringens* (Smith, 1963; Seenappa and Kempton, 1981; Baxter and Holzapfel, 1982), yeasts, and aflatoxin-producing moulds such as *Aspergillus flavus*, *A. glaucus*, and *A. niger* (Flannigan and Hui, 1976) have been reported. A total bacterial plate count below 10000/g is desirable, with yeast, mould, and coliforms below 1000/g (Weber, 1980). Major health hazard organisms such as *E. coli*, *Salmonella*, and *Shigella* must be negative. Control by fumigation with ethylene oxide is generally recognized as safe by many countries, as long as fumigant residues do not exceed international standards (WHO, 1972). Ethylene oxide is toxic and requires special vacuum equipment and technical skill to administer, but it vapourizes rapidly from the material, leaving little residue, and it has no effect on colour, pungency, or flavour, so it is generally considered the most effective method (Weber, 1980). Paprika that is properly processed and stored generally does not have problems with insect contamination (Govindarajan, 1985).

### 7.5.2 Oleoresin quality

The quality specifications for the different types of oleoresin are given in Table 7.2. Three types of oleoresin are specified, based on the pungency and colour values. Capsicum oleoresin has very high pungency and low colour, and is used as a source of pungency where colour is not important. Red pepper oleoresin has both moderate pungency and colour, and is used where both traits are important. Paprika oleoresin has very high colour and little or no pungency. Importers of Capsicum

**Table 7.2** Essential Oils Association standards for oleoresins

| Trait               | Type of oleoresin                                   |                                        |                                        |
|---------------------|-----------------------------------------------------|----------------------------------------|----------------------------------------|
|                     | Capsicum                                            | Red pepper                             | Paprika                                |
| Number              | EOA no. 244                                         | EOA no. 245                            | EOA no. 246                            |
| Preparation         | Solvent extraction of dried ripe fruit <sup>1</sup> | Solvent extraction of dried ripe fruit | Solvent extraction of dried ripe fruit |
| Appearance          | Clear, red or light amber, viscous                  | Deep red                               | Deep red                               |
| Odour/taste         | Very pungent, aromatic                              | Pungent, aromatic                      | Aromatic                               |
| SHU                 | >480 000                                            | >240 000                               | 0                                      |
| Colour <sup>2</sup> | <4000                                               | <20 000                                | 40 000–100 000                         |
| Soluble in:         |                                                     |                                        |                                        |
| Benzyl benzoate     | Yes                                                 | Yes                                    | Yes                                    |
| Alcohol             | Partly, with oily layer                             | Partly, with oily layer                | Partly, with oily layer                |
| Fixed oils          | Yes                                                 | Yes                                    | Yes                                    |
| Propylene glycol    | No                                                  | No                                     | No                                     |

<sup>1</sup> Residual solvent limits: <25 ppm hexane, <30 ppm ethylene chloride or acetone, or <50 ppm isopropyl or methyl alcohol for all oleoresins.

<sup>2</sup> Colour is determined by measuring the absorbance of a 0.01 % solution of oleoresin in acetone at 258 nm. The absorbance value is multiplied by 61 000 to convert to total colour units. Source: EOA (1975).

oleoresin prefer a pungency value in the range of 6–10 % CAPS (1 000 000–1 600 000 SHU) (Govindarajan, 1986). Oleoresin that is properly processed and stored has few problems with microbial or insect contamination.

### 7.5.3 Dried chilli quality

Like the other forms of peppers used in food processing, colour and pungency are the major quality factors, as well as aroma. Factors that affect the colour of dried chillies include the cultivar, the stage of maturity at harvest and subsequent curing, fruit drying conditions, and the final moisture content. At less than 10 % moisture, the colour appears bleached, while at levels greater than 10 % there is darkening, possibly caused by non-enzymatic browning. The colour of crushed or ground chilli powder deteriorates faster than whole chillies, due to the auto-catalyzed degradation of carotenoids. The major factor influencing colour retention during storage is the temperature, followed by the moisture content. The effect of air, light, and type of container is minimal. The optimum storage conditions for chilli powder colour retention are  $-16^{\circ}\text{C}$ , and 10–11 % moisture (Guzman *et al.*, 1973; Malchev *et al.*, 1982). The American Spice Trade Association (ASTA) has set standards for measuring colour in pepper products that are widely followed. Samples are extracted in acetone and the absorbance is read by a spectrophotometer at 460 nm (ASTA, 1997). Values are expressed as ASTA units; a value greater than 200 is considered a very deep red colour. Commercial samples of chilli powder normally range from 100–200 ASTA units, and a premium may be paid by processors for lots with extractable colour greater than 140 units.

The standard for measuring pungency in all forms of peppers is Scoville Heat Units (SHU). The concept was introduced in 1912, when Wilbur L. Scoville, a pharmacologist with Parke Davis, developed the Scoville Organoleptic Test (Scoville, 1912). A panel of five people is used as heat samplers. The panel analyzes a solution made of exact weights of dried chillies dissolved in alcohol and diluted with sugar water. The pungency of the peppers is rated in multiples of 100 SHU. A majority of three samplers has to agree on one value before any sample is given a value. This method is subjective, especially as it depends on the taster's palate and sensitivity to pungency. Also, the human palate quickly becomes desensitized to CAPS after tasting more than a few samples within a short period of time. It has largely been replaced by high-pressure liquid chromatography (HPLC) (Woodbury, 1980), which is relatively rapid and reliable compared to the Scoville Organoleptic Test (Collins *et al.*, 1995). Results are reported as ppm CAPS, which can be converted to SHU or other units by the following scale:  $1 \text{ ppm} = 16 \text{ SHU} = 0.0001 \% = 0.001 \text{ mg g}^{-1}$ . Pure capsaicin = 16 000 000 SHU.

Microbial contamination is also a problem in dried chilli, and the same standards used for paprika also apply to dried chilli. In addition, insect contamination can be a problem, particularly the universal drugstore beetle, *Stegobium paniceum* (Linn.) and the cigarette beetle, *Lasioderma serricorn* (Fab.) (Muthu and Majumder, 1974). Insect damage is three-fold, first by the physical loss as frass, second by the loss of quality due to broken pods and loose seed, and third by mould growth and entry of mites due to insect holes in the fruit walls. Control can be achieved by fumigation with insecticidal chemicals, although their use must be carefully monitored and fumigant residues

must be within the limits set by international standards (Monro, 1961). The control method used depends on the quantity, the technological capability, and the cost.

#### **7.5.4 Fresh fruit quality**

Pepper fruits are defined as non-climacteric, meaning they produce little or no ethylene and there is no large increase in CO<sub>2</sub> production during ripening (Lee *et al.*, 2010a). Non-climacteric fruits tend to maintain whatever quality they had at harvest without many beneficial changes. Therefore harvesting fruits at the proper stage for their intended end-use is critical. Fruits that are at the proper stage of maturity, in excellent condition, and are handled properly after harvest will have a longer shelf-life compared to fruits that are immature or over-mature. Care should be taken during the production cycle to ensure that fruit quality is high at harvest time. This includes proper nutrition of the crop, proper control of insects and diseases, and proper amounts of water. If the crop is grown in some form of protected culture (greenhouse or plastic house), this includes proper management of the growing temperature and light intensity as well. After harvest, fruits should be cleaned if necessary and stored under optimum temperature conditions. For bell peppers, this is generally 7–10°C. Full ripe or red peppers are less chilling sensitive than mature-green fruits of the same cultivar.

#### **7.5.5 Adulteration**

Several types of adulteration are possible and have been reported at various times. Whole dried chillies may be adulterated by adding sub-standard dried fruits, which are darkened or bleached, by adding moisture to increase the weight of a load, or by coating with mineral oil or synthetic dyes such as Sudan I or Para Red (paranitraniline red) to improve the colour and appearance (Govindarajan, 1986). Discovery of Sudan I in Worcester sauce in the UK in 2005 led to the recall of almost 450 food products at an estimated cost of £100 million (sterling) (FSA, 2005).

A more difficult problem is the mixture of fruits of varieties or harvests that vary in CAPS and colour but have similar shape, size, and visual colour. Only testing of representative samples and sub-samples can determine if a lot is homogeneous. Chilli powder may be adulterated in many ways, some of which are very difficult to detect without sophisticated tests such as HPLC analysis, and 'pure' standards must always be used for comparison. Chilli powder may be adulterated by adding extra amounts of bleached pericarp, seeds, calyx, and peduncle to increase bulk without visibly affecting appearance. Other reported adulterants include artificial dyes, almond shell dust, and dried red beet pulp. Filth, such as insect fragments, rodent droppings and hairs, and fungal spores is an indication of poor handling and storage. Heavy metals and chemical residues from pesticides represent another adulteration problem. Pesticide residues reported in chilli powder include chlorinated hydrocarbons, DDT, dieldrin, endrin, and lindane, but generally in very low levels. Oleoresin may be adulterated by adding synthetic saturated acid vanillylamides such as pelargononic vanillylamide. Detection of these adulterants can be done by sophisticated gas chromatography of the saponified extract (Govindarajan, 1986) or by thin-layer chromatography coupled with HPLC (Bensinger, 2000). Due to the difficulty in

detecting adulterants in powders, many companies prefer to buy whole dried fruits and process them into powder in their own facilities.

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## Cardamom

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**Abstract:** Cardamom is one of the most expensive spices in the world. India's warm humid climate, loamy soil rich in organic matter, distributed rainfall and special cultivation and processing methods all combine to make Indian cardamom truly unique in terms of aroma, flavour, size and parrot green colour. Cardamom has well-established medicinal and culinary values, and is used in a wide range of sweets and confectionery. In this chapter, we have consolidated scientific information available on genetic improvement, varieties, horticultural technologies and post-harvest processing. Cardamom's uses in traditional medicine, pharmacological properties and applications in food industry are also discussed.

**Key words:** cardamom, *Elettaria cardamomum*, trade, botany, genetic improvement, varieties, nutrition management, harvest, post-harvest processing, value addition, essential oil, oleoresin, oil composition, uses, quality standards, grade specifications, adulteration issues.

### 8.1 Introduction

Cardamom of commerce, popularly known as 'Queen of Spices', is the dried fruit of the herbaceous perennial *Elettaria cardamomum* Maton, belonging to the Zingiberaceae family. It is a shade-loving plant cultivated at an altitude of 600–1200 m above mean sea level (MSL) with an annual rainfall of 1500–4000 mm and a temperature range of 10–35°C. It is used in Ayurvedic medicine preparations because of its healing effect and other properties. It is also used in processed food, perfumes, oleoresins and many other applications. The genus *Elettaria* is one of the few compact natural groups of plants whose origin is the evergreen rainforests of South India and Sri Lanka (Purseglove *et al.*, 1981), from where it spread to other tropical countries. India was the main producer and exporter of cardamom until the 1980s, with Guatemala emerging later as a keen competitor to India in the international cardamom market. Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, Cambodia and Papua New Guinea are also cardamom-growing countries. Cardamom was introduced to Guatemala in 1920, either from India or Sri Lanka (Lawrence, 1978). After World War II, cardamom production in Guatemala increased on account of high demand and a rise in the international price. The cultivation of cardamom has also grown in popularity in the virgin forest lands of Papua New Guinea, though it is restricted to private estate owners (Krishna, 1997). Cardamom is also cultivated in the East Usambara Mountains (4°48'132–5°13' S and 38°32'–38°48' E) of Tanzania (Myers



*et al.*, 2000). Cardamom cultivation grew considerably in Sri Lanka during the 1960s, with the Knuckles region becoming the country's highest cardamom-producing area (Goonewardene, 2005).

### 8.1.1 History and importance

Cardamom is known to have been used in India since ancient times. It is known as *Ela* in Sanskrit and references to this can be found in ancient Sanskrit texts. Assyrian doctors and chemists were known to use many herbs, including cardamom (Parry, 1969). It was also mentioned that the ancient king of Babylon (721–701 BC) grew cardamom among other herbs in his garden. According to Watt (1972) it was one of the most popular oriental spices in Greek and Roman cuisine and, because of its importance, cardamom was listed as an item liable to duty at Alexandria in 176 AD.

Linschoten, in his *Journal of Indian Travels* (1596), describes two forms of cardamom used in South India (Watt, 1872). However, in general, early references to the use of cardamom are sparse in comparison to other spices, namely, black pepper, cinnamon and cassia.

The Arab states were the original major traders of Indian spices, including cardamom. Later, in the sixteenth century, the Portuguese started collecting and exporting pepper, ginger and cardamom directly to Europe. Only at the beginning of the nineteenth century were plantations established for cardamom cultivation, and even then they were only a secondary crop in coffee plantations. German settlers introduced cardamom to Tanzania in the 1890s. In 1954, Amani Botanical Garden in the Usambaras region distributed some seedlings to farmers, who vegetatively propagated and supplied to other farmers. After a decade, the cardamom trade began to flourish. Watt (1872) also briefly described cardamom cultivation in South India. The system of collecting cardamom from naturally growing plants continued until at least 1803 but, in later years, as demand increased large-scale organised cultivation began in India and Sri Lanka (Ridley, 1912). An example of this is the government of Travancore, India, who took up active cultivation of cardamom in 1823. Further information about cardamom cultivation can be found in the Madras Manual (Watt, 1872).

### 8.1.2 Production and trade in India and other countries

The present cardamom-growing tract in India lies between 8°30' and 14°30' N latitude and longitude of 75–70°E. It is an elongated tract from north to south extending over 2000 km, from Sirsi of the Karnataka region to Thirunelveli of the Tamil Nadu region. east to west, it is a narrow belt of land distributed over the Western Ghats (Madhusoodanan *et al.*, 1994). The important areas of cultivation include Nelliampathy, Wynad and Idukki, which are in the Travancore Cochin (*Malabar*) region of the Kerala state; Uttar Kannada, Shimoga, Hassan and Chickmagalur, which are in the hills of the Kodagu (Coorg) district in Karnataka; and the northern and southern foothills of Nilgiris, Didigul, Theni, Salem and Tirunelveli, which are part of the Coimbatore district in Tamil Nadu.

In India, the amount of cardamom cultivated has fluctuated over the years (Table 8.1). Production rose from 2900 mt during 1987–8 to 10075 mt in 2009–10. During



**Table 8.1** State-wise area and production of cardamom in India

| States     | 1970-1 |            | 1980-1 |            | 1990-1 |            | 2000-1 |            | 2006-7 |            |
|------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|------------|
|            | Area   | Production | Area   | Production | Area   | Production | Area   | Production | Area   | Production |
| Kerala     | 55 190 | 2130       | 56 380 | 3100       | 43 826 | 3450       | 41 288 | 7580       | 41 200 | 8500       |
| Karnataka  | 28 220 | 1000       | 28 220 | 1000       | 31 605 | 800        | 25 947 | 2100       | 24 000 | 1000       |
| Tamil Nadu | 8 070  | 235        | 9 350  | 300        | 6 123  | 500        | 5 085  | 800        | 4 400  | 400        |
| Total      | 91 480 | 3365       | 93 950 | 4400       | 81 554 | 4750       | 72 320 | 10 480     | 69 600 | 9900       |

*Note:* area in ha, production in MT.

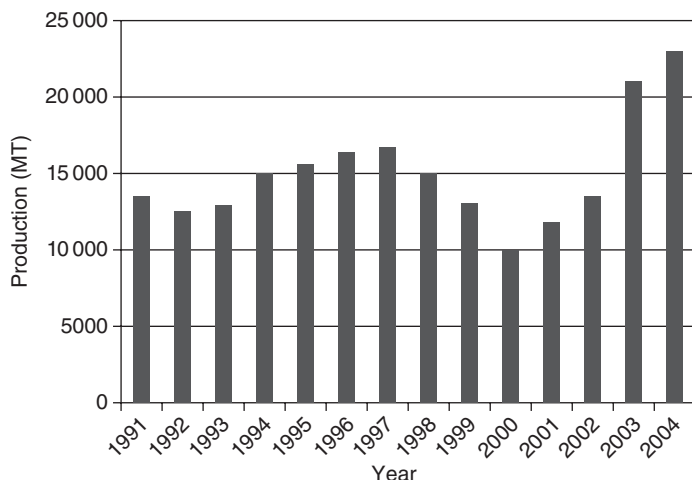


**Fig. 8.1** Cardamom production in Guatemala (various publications of Banco De Guatemala and Spices Board, India).

this period, productivity increased from almost 41 kg/ha to 201 kg/ha. This productivity increase is due to the use of high-yielding varieties and improved agro techniques. However, the demand for exported cardamom reached a low point during the same period. The maximum export recorded (3272 mt) was during 1985–6, whilst the lowest amount exported (173 mt) was during the year 1989–90. During 2009–10, India exported 1975 mt, valued at Rs. 165.70 crores, in comparison to 500 mt, valued at Rs. 24.75 crores, exported in 2007–8.

Saudi Arabia accounts for 1117.7 mt (69 %) of Indian exports (2009–10) followed by UAE (296 mt), Kuwait (92.2 mt) and Egypt (71 mt). This can be accounted for by increased competition from other cardamom-producing countries, as well as a rise in domestic consumption (Joseph, 2010).

Presently, 90 % of the cardamom traded in the world comes from Guatemala (Fig. 8.1). During 2004, the production of cardamom in the country was 23000 mt. In the Usambaras area of Tanzania, the main cardamom growers are the local Washambaa people and immigrants from the nearby highlands (Teija *et al.*, 2006). The average annual cardamom yield in the East Usambaras mountains is 80 kg/ha (Masayanyika 1995). The production levels in Tanzania and Sri Lanka are around 125 mt and 75 mt, respectively, per year (Ravindran, 2002).

## 8.2 Classification of cardamon

Cardamom (*Elettaria cardamomum* Maton) belongs to the natural order Scitaminae under the family Zingiberaceae. The genus *Elettaria* consists of a small number of spices distributed in India, Sri Lanka, Malaysia and Indonesia (Holtum, 1950; Wills, 1967; Mabbberley, 1987). Two botanical varieties have been distinguished based on the size of the fruits, one for the wild taxon and one for the cultivated forms:

1. *Elettaria cardamomum* var *major* Thw. comprising the 'wild' indigenous cardamom of Sri Lanka, also known as greater oblong cardamom or long cardamom.
2. *Elettaria cardamomum* var *minor* Watt (syn *Elettaria cardamomum* var *minuscule* Burtkill) comprising all the cultivated groups. This cultivated cardamom can be grouped into many cultivar groups, the two most important ones being *Malabar* and *Mysore* (Wardini and Thomas, 1999).

In addition to the two main varieties, a few more have been recognised. All the varieties and races are interfertile and the observed variations are probably due to natural crossing. Based on adaptability, nature of the panicle, shape and size of fruits, three types of cultivated small cardamom have been identified, viz., *Malabar*, *Mysore* and *Vazhukka* (Madhusoodanan *et al.*, 1994). Two more varieties, *mysorensis* and *laxiflora*, have been recognised based on some morphological characteristics.

A lot of confusion prevails over the botanical identity of Sri Lankan wild cardamom and the Indian cardamom varieties mentioned above. Various authors have named cardamom varieties differently:

- Sri Lankan wild cardamom: *Elettaria ensal* Abheywickrama (*E. major* Thawaites) (Madhusoodanan *et al.*, 2002);
- Malaysian and Indonesian: *Elettaria longituba* (Ridl.), (Holtum, 1950).

The vernacular name 'cardamom' is also often also used for many other taxa, particularly *Amomum* species.

### 8.2.1 Botany

Cardamom is an herbaceous perennial (2–5 m in height) with underground (subterranean) rhizomes, with aerial pseudo stems (tillers) made of leaf sheaths. Studies of vegetative growth indicate that suckers continue their growth for a period of about 18 months from time of emergence. The rate of linear growth is at its maximum during June and July, when the suckers have attained an age of about one year. The development of reproductive buds (panicles) can be seen in 89 % of them, indicating that the suckers require about 10–12 months to attain maturity. It takes almost ten months for a vegetative bud to develop and about a year for the panicle to emerge from the newly formed tillers (Sudharsan *et al.*, 1988). Kuruvilla *et al.* (1992) carried out a round-the-year study on the phenology of the tiller and panicle in three varieties of cardamom. They found that it took around 90–100 days for the emergence of the first flower from the panicles, irrespective of the variety.

The inflorescence is a long panicle with racemose clusters rising from the underground stem, but coming up above the soil. Pattanshetty and Prasad (1976) and Parameswar (1973) have made detailed observations on panicle production; its growth, duration of flowering, etc. The linear growth of the panicles extends over a period of about seven months. The rate of growth is at its maximum during April, and is slower during the earlier and later stages. The growth habit of the panicles and the shape and size of the capsules vary in different cultivated varieties/types of cardamom (Table 8.2). These panicles either grow erect (*Mysore*), prostrate (*Malabar*) or in a semi-erect manner (*Vazhukka*). Multiple branching (compound panicle) of panicles appears in certain cultivars. In such cases, the central peduncle

**Table 8.2** Characteristic features of three cardamom types

| Characters                 | <i>Malabar</i>                   | <i>Mysore</i>                      | <i>Vazhukka</i> (natural cross between <i>malabar</i> and <i>mysore</i> ) |
|----------------------------|----------------------------------|------------------------------------|---------------------------------------------------------------------------|
| Adaptability               | Lower altitudes<br>600–900 m MSL | Higher altitudes<br>900–1200 m MSL | Wide range                                                                |
| Areas of cultivation       | Karnataka                        | Kerala and parts of<br>Tamil Nadu  | Kerala                                                                    |
| Plant vigour               | Medium                           | Robust                             | Robust                                                                    |
| Panicles                   | Prostrate                        | Erect                              | Semi-erect                                                                |
| Capsules                   | Round or oblong                  | Bold, elongated                    | Round to oblong                                                           |
| Leaf petiole               | Short                            | Long                               | Long                                                                      |
| Capsule colour at maturity | Pale/golden/yellow               | Green                              | Green                                                                     |

undergoes further branching of secondary and tertiary branches producing compound panicle types.

Flowers appear on the panicle after four months and flowering continues for a period of six months. Each flower cluster is a cincinnus (Holttum, 1950). The characteristics of the flowers are listed below:

- The flowers are bisexual.
- The bracts are linear, oblong and persistent.
- There are three racemose sepals.
- They have three petals, which are unequal and have a longer lip with three violet carpels.
- There is one style.
- The ovary is trilocular and is of axile placentation.
- There are numerous ovules in each carpel.

Pai (1965) has concluded from anatomical evidence that the labellum is a double structure, whereas Parameswar and Venugopal (1974) are of the view that the labellum is made up of three modified anthers.

Normally, flowering in cardamom is seen throughout the year on panicles produced during the current year, as well as on panicles produced during the previous year. The peak flowering is spread over a period of six months between May and October. The time required from flower/bud initiation to full bloom stage ranges from 26 to 34 days. Capsule development takes about 110–20 days from the full bloom stage (Krishnamurthy *et al.*, 1989).

The early hours of the day are when the maximum number of flowers open. Anthesis follows immediately. In the Mudigere region of Karnataka, flowering commences at 3.30 am and continues until 7.30 am. The dehiscence of anthers takes place immediately followed by anthesis at 3.30 am which continues up to 7.30 am. The maximum pollen bursting occurs between 5.30 am and 6.30 am. The pollen grains are round and mostly found individually. They measure on average 87.6  $\mu$  in diameter. By appearance, 85.2 % of the pollen grains are fertile, but a maximum of

70.1 % germinate on an artificial medium, containing 20 % sucrose and 1 % agar. Studies of the viability of pollen grains indicate that only 6.5 % remain viable after 2 hours of storage. After 6–8 hours of storage, the percentage of viability was practically zero (Krishnamurthy *et al.*, 1989). Pollen fertility is maximum at full bloom stage, and low at the beginning and end of the flowering periods (Venugopal and Parameswar, 1974). With respect to pollen morphology, the three varieties of cardamom are round in shape and the pollen mass appears as a creamy powder. The largest pollen grains are observed in *Mysore* variety, while the smallest are found in *Vazhukka* variety.

Even though cardamom has bisexual flowers and is self-compatible, cross-pollination is common. Self-pollination is hindered due to the slight protrusion of the stigma above the stamens. In cardamom, cross-pollination is mediated by the activity of bees (*Apis cerana*, *Apis indica* and *Apis dorsata*) who act as pollinators. Cardamom flowers remain in bloom for 15–18 hours per day. Stigma receptivity and pollen viability are at maximum during the morning hours. Receptivity is at maximum between 8 am and 10 am, when 72 % of the opened flowers set fruit. After 10 am the stigma receptivity decreases gradually and only 24 % of the flowers opening at 4 pm set fruit. It is reported that receptivity of the stigma is highest between 8 am and 12 noon (Krishnamurthy *et al.*, 1989; Kuruvilla and Madhusoodanan, 1988). Parvathi *et al.* (1993) and Belavadi *et al.* (2000) noticed that the peak receptivity around 12 noon coincides with peak pollinator activity. The active foraging of bees is mainly seen in the morning hours; this activity is instrumental in increasing the fruit set in cardamom.

### 8.2.2 Cytology

The chromosomal numbers for cardamom are  $2n = 48$  (Gregory, 1936; Sharma and Bhattacharyya, 1939) and  $2n = 52$  (Chakravarti, 1948). Cardamom is considered to be a balanced tetraploid. Allied genera such as *Globa*, *Balbifera*, *Phoemaria*, *Amomum* sps and *Alpinia* sps also possess  $2n = 48$  and are considered to be evolved from a common basic number,  $x = 12$ . The *Mysore* and *Malabar* varieties of cardamom possess  $2n = 50$  and  $2n = 48$  chromosomes, respectively, and aneuploidy as well as structural alterations in the chromosome have contributed to the varietal differentiation (Chandrasekhar and Sampathkumar, 1986). Earlier researchers (Chandrasekhar and Sampathkumar, 1986) have reported that cardamom is of amphidiploid origin from wild species, and the two species considered to be the putative parents are the Sri Lankan cardamom *E. major* and the Malaysian species *E. longituba*.

## 8.3 Genetic improvement and varieties

Six research organizations in India – viz., Indian Institute of Spices Research (ICAR), Cardamom Research Centre, Appangala, Karnataka; Indian Cardamom Research Institute (ICRI), Myladumpara and its Regional Research Station, Sakleshpur, Karnataka; Regional Research Station, Mudigere (UAS, Bangalore) and

Cardamom Research Station, Pampadumpara (KAU, Kerala) – are presently engaged in research for the improvement of cardamom. Regular surveys are being undertaken by these institutions, gathering the variable germplasm and exploiting the desirable genes of these accessions through various crop improvement techniques. The collections of a large number of indigenous lines are being conserved in different centres. In 1994, a detailed descriptor on cardamom was published by the International Plant Genetic Resources Institute (IPGRI), presently Bioversity International, Rome, Italy (IPGRI, 1994). Among the different cardamom-growing countries, India has a very strong research base.

Breeding methods such as selection, hybridisation, mutation breeding and polyploidy breeding have been employed in cardamom improvement. The major objectives of the crop improvement program in cardamom (IISR, 2007) are:

- to develop varieties having wider adaptability to different agro-ecological conditions;
- to evolve high-yielding genotype with superior capsule characters;
- to breed varieties having resistance to biotic stress such as, thrips, root grub, nematodes, fungal (rhizome rot) and viral diseases (*katte* and *kokke kandu*);
- to evolve drought-tolerant high-yielding varieties.

### 8.3.1 Clonal selection

The improved varieties have been evolved by selecting superior plants with desirable characteristics such as higher yield and superior capsule characteristics from land race populations. The highly heterozygous nature of the crop and the ability to multiply selections by clonal propagations has contributed to this success. Selections are made '*in situ*' in planters, fields and forests. The selected plants are multiplied clonally, subjected to preliminary evaluation and subsequently evaluated in a comparative yield trial and a multi-location trial to confirm their superiority and adaptability. Selection of cardamom is highly location-specific to their agro-ecological requirements. The improved selections are much superior to the local clones with regard to yield and capsule characteristics (Venugopal and Prasath, 2003; Prasath *et al.*, 2009a). Salient features of varieties in vogue are summarised in Table 8.3.

### 8.3.2 Intervarietal hybridization

Since cardamom is amenable to both sexual and vegetative propagation, hybridisation is a very useful tool for crop improvement. Research at various institutes has led to the isolation of high-yielding recombinants and heterotic hybrids. Bhat *et al.* (2010) reported a novel hybridisation technique, known as the straw tube technique, for simple, easy and effective hybridisation. The diallel cross-combinations were more vigorous compared to the parental lines (Krishnamurthy *et al.*, 1989). Madhusoodanan *et al.* (1998, 1999) reported few high-yielding heterotic recombinants. The elite selections were compatible with each other; however, the degree of compatibility varied with the parents selected for hybridisation (Venugopal and Padmini, 1999). Padmini *et al.* (2001) observed hybrid vigour in seedlings of cardamom with regard to plant height, number of leaves per plant,

**Table 8.3** Cardamom varieties released for various useful traits in India

| Variety    | Year of release | Breeding method | Pedigree/parentage and plant type                              | Important traits                                                                                                                                                                                                                                                                                                  | Institute/ university                        |
|------------|-----------------|-----------------|----------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|
| Mudigere 1 | 1984            | Selection       | Clonal selection from <i>Malabar</i> type                      | Erect and compact plant, short panicle, pale green, oval bold capsules, suitable for high-density planting, moderately tolerant to thrips, hairy caterpillar and white grubs, pubescent leaves. Contains oil 8.0 %, 1,8-cineole 36.0 %, $\alpha$ -terpenyl acetate 42.0 %, dry recovery 20.0 % with 275 kg dry/ha | Regional Research Station, UAS, Mudigere     |
| Mudigere 2 | 1996            | Selection       | Clonal selection from open pollination of <i>Malabar</i> type  | Early maturing, suitable for high-density planting, round/oval and bold capsules. Contains oil 8.0 %, 1,8-cineole 45.0 %, $\alpha$ -terpenyl acetate 38.0 % with 475 kg dry/ha                                                                                                                                    | Regional Research Station, UAS, Mudigere     |
| PV 1       | 1991            | Selection       | A selection from Walayar collection, a <i>Malabar</i> type     | An early maturing type, short panicle, elongated slightly ribbed light green capsules. Contains oil 6.8 %, 1,8-cineole 33.0 %, $\alpha$ -terpenyl acetate 46.0 %, dry recovery 19.9 % with 260 kg dry/ha                                                                                                          | Cardamom Research Station, KAU, Pampadumpara |
| PV 2       | 2001            | Selection       | A selection from OP seedlings of PV-1, <i>Malabar</i> type     | Early maturing, unbranched lengthy panicle, long bold capsules, high dry recovery percentage (23.8 %), essential oil 10.45 %, field tolerant to stem borer and thrips. Suitable for elevation range of 1000–1200 m above MSL with 982 kg dry/ha                                                                   | Cardamom Research Station, KAU, Pampadumpara |
| ICRI 1     | 1992            | Selection       | Selection from Chakkupallam collection, <i>Malabar</i> type    | An early maturing variety, medium sized panicle with globose, round and extra bold dark green capsules. Contains oil 8.7 %, 1,8-cineole 29.0 %, $\alpha$ -terpenyl acetate 38.0 %, dry recovery 22.9 % with 325 kg dry/ha                                                                                         | ICRI (Spices Board), Myladumpara             |
| ICRI 2     | 1992            | Selection       | Clonal selection from germplasm collection, <i>Mysore</i> type | Performs well under high-altitude and irrigated condition, medium long panicles, oblong bold and parrot green capsules, tolerant to azukkal disease. Dry recovery 22.5 % with 375 kg dry/ha                                                                                                                       | ICRI (Spices Board), Myladumpara             |



**Table 8.3** *Continued*

| Variety        | Year of release | Breeding method | Pedigree/parentage and plant type                                               | Important traits                                                                                                                                                                                                                                                  | Institute/ university            |
|----------------|-----------------|-----------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| ICRI 3         | 1993            | Selection       | Selection from <i>Malabar</i> type                                              | Early maturing, non-pubescent leaves, tolerant to rhizome rot disease, oblong, bold parrot green capsules, suitable for hill zone of Karnataka. Contains oil 6.6 %, 1,8-cineole 54.0 %, $\alpha$ -terpenyl acetate 24.0 %, dry recovery 22.0 % with 440 kg dry/ha | ICRI (Spices Board) Sakleshpur   |
| ICRI 4         | 1997            | Selection       | Clonal selection from Vadaraparai area of lower pullenys, a <i>Malabar</i> type | Early maturity, medium-sized panicle, globose bold capsules. Contains oil 6.4 %. Suitable for low-rainfall areas, relatively tolerant to rhizome rot and capsule borer with 455 kg dry/ha                                                                         | ICRI (Spices Board), Myladumpara |
| ICRI 5         | 2006            | Hybridization   | MCC260 X MCC 49                                                                 | First hybrid variety; early maturity, moderately tolerant to drought, high yield under intensive management (responds well to intensive management). Capsule size – bold 68 % (with more than 7 mm), oil – 7.13 %; dry recovery – 23.15 %                         | ICRI (Spices Board), Myladumpara |
| ICRI 6         | 2006            | Selection       | Clonal selection                                                                | High yield, medium maturity, relatively tolerant to drought, high percentage of bold capsules and volatile oil content. Capsule size – 71 % with more than 7 mm; volatile oil – 7.33 %; dry recovery – 19.0 %                                                     | ICRI (Spices Board), Myladumpara |
| IISR Suvashini | 1997            | Selection       | Clonal selection from OP progenies of Cl.37                                     | <i>Malabar</i> type, early maturing. The variety has an average yield of 745 kg/ha. In few high-production plots, yield as high as 1775 kg/ha has been achieved                                                                                                   | IISR, CRC, Appangala             |
| IISR Avinash   | 1999            | Selection       | Clonal selection from OP progenies of CCS-1                                     | <i>Malabar</i> type, tolerant to rhizome rot, shoot and capsule borer, average yield of 847 kg/ha (potential yield of 1483 kg/ha). It has an extended flowering period.                                                                                           | IISR, CRC, Appangala             |
| IISR Vijetha   | 2001            | Selection       | Clonal selection from Natural <i>katte</i> escapes (NKE 12)                     | <i>Malabar</i> type, resistant to <i>katte</i> (Cardamom mosaic virus). It has an average yield of 643 kg/ha. The potential yield of this variety is 979 kg/ha.                                                                                                   | IISR, CRC, Appangala             |

leaf length and breadth. The hybrids MHC 18, MHC 10 and MHC 12 showed vigour with regard to yield and yield-contributing characteristics (Pradipkumar *et al.*, 2002). Appreciable heterosis was recorded over the standard parent, with desirable yield characteristics and high-yielding mosaic-resistant hybrids were identified (Prasath *et al.*, 2009b). Genetic analysis of diallel hybrids revealed the influence of dominance and over-dominance in the manifestation of quantitative characteristics (Prasath and Venugopal, 2002). The resistance of the cardamom plants to CdMV (cardamom mosaic virus) is quantitative, with possibly two major factors, and is dependent on gene dosage with completely dominant gene action (Prasath *et al.*, 2010).

### 8.3.3 Polycross breeding

Chandrappa *et al.* (1998) carried out studies on the impact of selection in a polycross progeny population. Since cardamom is a cross-pollinated crop, the polycross method of breeding is ideal to evolve superior types. Elite clones, having predominantly desirable characteristics, are planted together on an isolated plot. Beehives are maintained in the plot for assured pollination so that maximum fruit set and a high number of seeds per capsule can be obtained.

### 8.3.4 Intergeneric hybridisation

Although a few improved high-yielding varieties of cardamom have been evolved, combining yield and cardamom mosaic resistance has not been possible. To achieve this objective, intergeneric crosses were made using *Ammomum subulatum*, *Alpinia neutans*, *Hedychium flavascene* and *Hedychium coronarium* as male parents. A few fruits have been obtained in the cross involving *Alpinia neutans*; in other crosses no seeds were set (Parameswar, 1997). Therefore, the results of intergeneric crosses are not encouraging (Krishnamurthy *et al.*, 1989, Madhusoodanan *et al.*, 1990). Compatibility barriers prevented the formation of fruits in these cross-combinations (Madhusoodanan *et al.*, 1990).

### 8.3.5 Mutation breeding

To develop clones tolerant to the cardamom mosaic (*katte*) virus, drought seeds and rhizomes of cardamom have been subjected to x-rays, nitrosomethyl urea (NMU), diethyl sulphate (DES) and ethyl methane sulphate (EMS). No desirable mutant has so far been obtained. However, sterility and lack of macromutations in the M1 generation (ICRI, 1987) were also reported.

### 8.3.6 Polyploidy breeding

Polyploids were induced in cardamom by treating the sprouting seeds with 0.5 % aqueous solution of colchicine. The tetraploid lines exhibited an increase in the layers of epidermal cells, a thick cuticle and a thicker wax coating on the leaves. These characteristics are generally associated with drought tolerance (Sudharsahan, 1989).

## 8.4 Production of cardamom: horticultural technologies and nursery management

Cardamom is propagated either through seeds or vegetative means and, being a cross-pollinated crop, the seedling population is not uniform. Only 35 % of the plants are good yielders in a plantation raised from a seedling population (Krishnamurthy *et al.*, 1989). Hence, vegetative propagation is normally adopted for multiplication of elite clones. Vegetative propagation can be either through suckers or tissue culture.

The seeds are collected from fully ripe capsules, preferably from the second to third round of harvest in September. The seeds are then either washed in water and sown immediately or mixed with wood ash and dried for 2–9 days at room temperature. Storage of seeds results in loss of viability and delay in germination. Immediate sowing results in maximum germination. Germination has been shown to be at its highest (71.8 %) when sown in September (Pattanshetty and Prasad, 1973; Pattanshetty *et al.*, 1978). Cardamom seeds sown immediately after harvest (September) germinate uniformly, and seedlings are ready for transplanting at the end of the tenth month. The ideal sowing season has been reported to be November–January for Kerala and Tamil Nadu, and September for Karnataka.

Germination is significantly correlated with maximum and minimum temperature prevalent in the area (Gurumurthy and Hegde, 1987), as the seeds fail to germinate at a temperature less than 15 °C and greater than 35 °C. The germination is optimum at 30 °C (70.9–73.0 % RH) with the highest percentage during September–October (Siddagangaiah *et al.*, 1993). The optimal light requirement for a cardamom nursery was found to be 55 % of the normal (Ranjithakumari *et al.*, 1993). This intensity was found to produce better growth and development of seedlings in the nursery. Mulching of seedbeds also influenced the germination of seeds. Mulching with coir dust, paddy straw and dry sal leaves enhanced germination (Sulikeri and Kololgi, 1978).

Seedlings at the four to five leaf stage from the primary nursery beds are transplanted to the secondary nursery, and set at a distance of 20–25 cm or in polythene bags (20 × 20 cm). In Karnataka, where seeds are sown during August–September, transplanting in the secondary nursery is done during November–January. In Kerala and Tamil Nadu, seedlings from primary beds are transplanted to secondary nursery beds at a spacing of 20 × 20 cm during June–July. The rate of mortality can be minimized by transplanting at the four–five leaf stage (Ankegowda, 2008).

Seed treatment with *Trichoderma* is desirable as a prophylactic measure for managing nursery rot diseases. At the time of preparation of the beds, incorporation of vesicular arbuscular mycorrhiza (VAM) multiplied in an organic medium is recommended. The potting mixture, 3:1:1 soil rich in organic matter, well-rotted cow dung or vermicompost and sand, is prepared for raising polybag seedlings. VAM and *Trichoderma* can also be added to the potting mixture (250 g mixed with 25 kg of well-rotted cow dung). Spraying with vermiwash once a month is desirable to enhance seedling growth. The diseases in the nurseries are best managed by regular surveillance and by adopting phytosanitary measures. Restricted application of Bordeaux mixture 1 %, may be done to control root rot disease at the initial stage.

Changing the nursery site can be of benefit to ward off pests and diseases, and it can also encourage vigorous growth of seedlings (Anon., 1998).

High-yielding varieties are multiplied in isolated clonal nurseries. The virus-free high-yielding plants are selected and subcloned for further multiplication. Rapid clonal multiplication is standardised in cardamom for large-scale multiplication (Korikanthimath, 1997).

## 8.5 Production of cardamom: planting and aftercare

### 8.5.1 Site selection and shade requirement

The shade canopy provides a suitable environment by maintaining humidity and evaporation at suitable levels. The shade requirements vary from place to place and depend on the lay of land, soil type, rainfall pattern and crop combination, etc. Cardamom yield level is highest under 65–70 % light intensity (Sulikeri, 1986), whereas total biomass, phytochemical activity, chlorophyll and protein contents are adversely affected under full light (100 %), when compared to medium light (45–55 %) (Ravindran and Kulandaivelu, 1998). Plant growth has been shown to be reduced by 40 % under full light, compared to those exposed to medium light. The photosynthetic efficiency appears to be greater under low light intensities (30.6–106.63  $\mu\text{E}/\text{m}^2/\text{sec}$ ) (Vasanthkumar *et al.*, 1989). In Tanzania, farmers leave 75–100 shade trees/ha to provide shade for optimum production of their crop (Masayanyika, 1995).

### 8.5.2 Main field preparation

Cardamom is generally cultivated in forest areas with an overhead umbrella canopy to take advantage of filtered shade. If the land is slanted, it is advisable to start cleaning from the top and work downwards. For slopes, adequate soil and water conservation measures are necessary whilst preparing the land for planting. Planting in trenches across the slopes in low rainfall areas, diagonal planting and soil mulching result in better soil and water conservation. Nair (2006) has presented a comprehensive review of various aspects of the agronomy and economy of cardamom.

### 8.5.3 Spacing

Spacing is decided based on variety and duration of crop. The *Mysore* and *Vazhukka* varieties are vigorous and need wider spacing, whereas *Malabar* types need closer spacing. High yield was recorded in cardamom seedlings planted at  $2 \times 1$  m in hill slopes and  $2.1 \times 2.1$  m in flat lands (Korikanthimath and Venugopal, 1989). The trench method of planting with a spacing of  $2 \times 1$  m resulted in better growth and yield (Siddagangaiah *et al.*, 1998). In general, *Mysore* and *Vazhukka* types are planted at a spacing of  $3 \times 3$  m or  $2.4 \times 2.4$  m in high rainfall or irrigated areas. A spacing of  $1.8 \times 1.8$  m or  $2 \times 2$  m is suitable for the cardamom-growing areas of Karnataka.

### 8.5.4 Method of planting

The trench method of planting (60 × 30 cm) with a spacing of 2 × 1 m resulted in the maximum growth and yield, with a greater moisture retention than the pit system of planting. *Mysore* and *Vazhukka* types are usually planted in the pits of 90 × 90 × 45 or 90 cm, whereas 45 × 45 × 45 cm pits, which are generally opened during April–May after pre-monsoon showers, are used for planting *Malabar* types. Pits are filled with a mixture of topsoil, compost or well-rotted farmyard manure (FYM) and 40 g of rock phosphate.

### 8.5.5 Planting season

Planting season is based on topography and rainfall pattern, and planting is normally done in June–July. In areas receiving a heavy south–west monsoon (July–September), planting is either completed before July or is taken up in August–September in order to avoid heavy rains in July. Early planting gives better establishment and growth than late planting. Planting in low lying areas (such as valleys) should be done only after heavy rains in July. Better establishment and growth of seedlings have been recorded in August planting in Mudigere, Karnataka (Pattanshetty and Prasad, 1972). Cardamom suckers are planted from June–August on the surface or 15–20 cm below the surface. During planting, a minimum of 320 mm rainfall along with temperature range of 15.5–17.5°C (minimum) and 19.5–25.0°C (maximum) is required for better establishment.

### 8.5.6 Planting

Cardamom seedlings 10–18 months old are planted in pits by taking a small portion of filled soil and adding rock phosphate (40 g). Soil is then replaced, taking care that the roots are distributed in their normal position, and well pressed around the base of the clump. It is advisable to plant just above the ground level to avoid rotting during heavy rains. Seedlings are normally planted in a pit at an acute angle to promote shoot production. Light root pruning is required once the seedlings reach 18 months old. In the case of suckers, they should be planted in a slanting position and with the base of the rhizome covered with soil. After planting, cross-staking is required immediately to avoid wind damage. After transplanting, care should be taken to offset the transplanting shock and to save the seedlings from heavy rains.

### 8.5.7 Mulching

Mulching is done to reduce evapotranspiration and rain damage. The fallen leaves from the shade trees can be used for mulching. Sufficient mulching should be undertaken during November–December to overcome drought during summer. Demulching is also equally important during May or after the pre-monsoon showers to facilitate honeybee movement for better pollination and capsule setting. It also provides better aeration and minimises incidences of clump rot or rhizome rot. The practice of uncovering the panicles shortly after the commencement of flowering improves fruit set in cardamom.

### 8.5.8 Weeding and thrashing

Clean weeding is to be limited to the plant bases (50 cm), the inter-rows are to be maintained by slash weeding. The weeded materials can be used for mulching, as can trashed materials and fallen leaves. The dry leaves and leaf sheaths should be trashed and the old suckers and rhizomes removed once a year, after completion of the final harvest.

These waste materials can then be used, for composting. Trashing thrice a year and cleaning the plant bases during monsoon months improves the chances of pollination, by eliminating breeding sites for sucking pests. This also reduces incidence of rhizome rot.

### 8.5.9 Irrigation management

Cardamom is generally grown as a rain-fed crop, and the cardamom tracts of India experience a dry spell of about 5–6 months. Increased denudation of forests and deterioration in forest ecology, coupled with erratic trends of rainfall, can lead to aridity, adversely affecting cardamom production (Rethinam and Korikanthimath, 1985). Since cardamom is a shallow-rooted crop, the moisture in the root zone is maintained by irrigating the crop from January to May (Sivanappan, 1985). Irrigation at 75 % available soil moisture (ASM) recorded favourable growth parameters compared to 25 % ASM (Raju, 1981). Cardamom plants irrigated at 25 % ASM and 75 % ASM produced 2.01 and 2.14 times more capsules, respectively, than those without irrigation. Irrigation at 75 % ASM combined with high levels of light resulted in the highest yield of 2618 kg/ha.

Sprinkler irrigation, equivalent to 4 cm rainfall at every 12–15 days, would be quite sufficient for cardamom crop (John and Mathew, 1977; Saleem, 1978). Daily drip irrigation, at 8 L per plant from 15 January, recorded a significantly higher yield and it was followed by sprinkler irrigation once every 12 days (IISR, 2004). In low-to medium-rainfall areas (1200–2000 mm), the trench system of planting was found to conserve soil moisture. In areas with a slope of more than 7 % all along the contour, bench terracing of about 1.5–1.8 m width is required for planting cardamom. Agronomical practices such as shade regulation, trenching, use of terrace systems, provision of shelter belts, mulching, earthing up, vegetative barriers and intercropping all aid better growth and development of cardamom, besides effective soil and water conservation measures. Although cardamom requires high moisture levels, it is sensitive to a high water table and the resultant waterlogged situations (Sulikeri and Kololgi, 1978).

### 8.5.10 Shade regulation

Shade requirement studies indicated that cardamom does not tolerate direct sunlight but, at the same time, too much shade affects its metabolic activities. Removal of excess shade is also essential to allow sufficient light penetration. Shade has to be regulated based on the topography of the land and moisture content, among other things, in order to achieve about 50 % filtered sunlight for proper growth and flowering. Any excess shade should be removed during the summer months (March–April) in newly planted areas, and after the summer showers (May–June) for an

existing plantation. It is desirable to maintain a mixed population of medium-sized shade trees that facilitate shade regulation and to maintain more or less optimum conditions throughout the year. Balangi (*Artocarpus fraxinifolius* Wt), Nili (*Bischofia javanica* Blum), Jack (*Artocarpus heterophyllus* Lamk), Red cedar (*Cedrela toona* Roxb), Karimaram (*Diospyros ebenum* Koenig), Karna (*Vernonia monocis* C.B. Darke), Nandi (*Lagerstroemia lanceolata*) and Spanish cherry (*Mimusops elengi*) are desirable as shade trees for cardamom (Rai, 1978). George *et al.* (1984) reported 40–50 % higher cardamom yield under *Diospyros ebenum* than under Jack, Nandi and Spanish cherry.

The desirable characteristics of shade trees are leguminous species, defoliation during the rainy season, self-pruning habit and flowering during summer. Most shade trees shed leaves between September and December; highest leaf shed was noticed in *Syzygium cumini* followed by *Terminalia arjuna*. As far as major nutrients are concerned, *Erythrina indica* recorded higher contents of nitrogen and phosphorous, while *Lagerstroemia lanceolata* contained more potassium (Dinesh Kumar and Babitha, 2006).

The survey and economic analysis of various cardamom plantations adopting mixed cropping systems in Kerala and Karnataka indicated that the inclusion of black pepper, coffee and areca nut was highly remunerative, giving higher cost–benefit ratios (Srinivasan *et al.*, 1999). Mixed cropping of cardamom in areca nut gardens give a higher income than areca nut monocropping (Korikanthimath *et al.*, 1997a,b).

### 8.5.11 Pollination management

The number of honeybee colonies required for effective pollination of cardamom is a minimum of four colonies (about 5000 foragers per colony) per hectare (3000 plants). Bee pollination resulted in a 9–13 % yield increase and better quality capsules of uniform size and shape. Mulching during flowering hinders bee pollination, resulting in a 33–44 % reduction in fruit set. Thus, de-mulching is recommended after the pre-monsoon showers to facilitate better honeybee movement and capsule setting. Promoting the use of beekeeping for pollination of cash crops will be of benefit to both the beekeeper, who will receive money for the pollination services of his honeybees as well as harvesting more honey, and the farmer whose income will be increased through boosting crop productivity as a result of the pollination services of the bees.

### 8.5.12 Nutrient management

Cardamom is generally grown in the rich fertile soils of the forest ecosystem. Being perennial, steady absorption and utilization of plant nutrients take place throughout the lifecycle of cardamom and hence a regular application of nutrients should be followed for higher yields (Korikanthimath, 1984). Application of compost or farm-yard manure, 5 kg/plant/year during May–June, is recommended. Application of 3–4 tonnes of well-rotted cattle or sheep manure apart from 30 kg each of N, P<sub>2</sub>O<sub>5</sub> and 40 kg K<sub>2</sub>O/acre can greatly enhance the yield (Shanmugavelu and Madhava Rao, 1977). Thimmarayappa *et al.* (2000) reported that integrated nutrient management with 25 % organic manure + 75 % inorganic fertilisers and 50 % organic manure + 50 % inorganic fertilizers recorded yields on a par with 75 % organic manure + 25 %



inorganic fertilizers. Application of the recommended NPK nutrients as organic fertilizers (50 % each as FYM and neem cake + 50 % P each as bone meal and rock phosphate + 50 % K as wood ash) has been shown to be as effective in increasing the yield and quality of cardamom (Sadanandan and Hamza, 2006).

Application of organic manures such as neem cake (1 kg) or poultry manure/FYM compost/vermicompost (2 kg) per plant is recommended once in a year during May–June. Nearly 70 % of cardamom roots are confined to a shallow depth of 5–40 cm and 30–50 cm radius (Khader and Sayed, 1977; Nair, 1988). Therefore, it is necessary to apply the fertiliser at a radius within 50 cm. Being a surface feeder, deep placement of manure is not advisable for cardamom.

Three species of VAM fungi (*Glomus macrocarpum*, *G. fasciculatum* and *Gigaspora coralloidea*) have been recorded in the roots of cardamom plants. Percent colonization in roots varied from 40–100 % in pre-monsoon samples and 63–94 % in post-monsoon samples. Seedlings inoculated with the VAM fungi grew taller and had more leaves and tillers and greater seedling biomass and uptake of nutrients than control seedlings. Among the various mycorrhizal fungi tested, seedlings inoculated with *Gigaspora margarita* and *Glomus monosporum* exhibited significantly greater growth with increased uptake of nutrients.

## 8.6 Harvesting and post-harvest processing

Cardamom plants start bearing two years after planting. Panicles appear from the base of the plant from January onwards; flowering is between April and December, and may extend further. Peak flowering is observed during June–August and fruits mature in about 120 days after flowering. Fruits have trilocular capsules containing 15–20 seeds and, on maturity, the seeds turn black in colour.

Due to staggered and prolonged flowering, cardamom capsules mature and are ready for harvesting successively at 10–15 day intervals over an extended period of 8 months (August–March). Harvesting is carried out at an interval of 15–30 days and is completed in eight to nine rounds. Fruits (capsules) that are fully matured have a dark green coloured rind (peel) with black coloured seeds, indicating that they are ready for harvesting. Harvesting at an over-ripe stage leads to a loss of the green colouring in the rind, and the capsule is at risk of splitting during curing. Percent recovery of dry capsules varies from 20–24 % in ripe capsules, 18–20 % at the physiologically mature stage and 14–16 % at the immature stage. Hence, it is ideal to harvest cardamom at the physiologically mature stage (green coloured rind and black coloured seeds) (Leela *et al.*, 2008). After harvesting, the capsules are cleaned and cured to reduce the moisture content from 80 % to 8–12 % in an optimum environment for retaining green colour to the maximum extent.

The colour of processed cardamom is an important factor in the consumer market. Different chemical treatments have been tried to retain the green colour of harvested capsules because such a product fetches premium price in the market. Soaking green capsules immediately after harvest in 2 % sodium carbonate solution for 10 minutes results in retention of the green colour during subsequent drying and storage (Natarajan *et al.*, 1968). Immature capsules also retain greater intensity of green colour.



### 8.6.1 Curing

On maturity, the dark green capsules are harvested and dried within 24–36 hours, in order to avoid quality deterioration. Cardamom curing is the process in which the moisture content of freshly harvested cardamom capsules is reduced from 70–80 to 11–12 % at an optimum temperature of 45–55 °C to retain its maximum green colour and volatile oil (Pruthi, 1993). The widely adopted system is a slow, passive process stretching up to 24–30 hours with an initial temperature around 45 °C.

Maturity of capsules and curing temperature influence the colour and quality of processed (cured) cardamom. During the process of curing, if temperature exceeds the threshold levels, capsules tend to develop brownish streaks as a result of heat injury. High temperature also results in volatile oil loss from seeds. Maintaining an optimum temperature of 40–45 °C in all the four stages of curing helps in retention of green colour. Curing at a temperature of 55 °C and 60 °C significantly increases the percentage of yellow and split capsules. The husk of raw capsules contains about 8 % water, which has to be removed completely during the process of drying. Maximum loss of chlorophyll occurs in the initial 6 hours of curing, and hence a lower temperature is maintained in the initial stages. Some established drying methods for cardamom are described below.

#### *Natural (sun drying)*

The capsules are dried directly in sunlight for a period of 5–6 days or more, depending upon availability of the sunlight. Frequent turning of the capsules is required during drying, which can lead to splitting. Cloudy weather and frequent rain during drying hinder the process. This method is commonly used in Karnataka (Vadiraj, 2004). The sun-dried capsules are not preferred for the export market.

#### *Flue pipe dryer (kiln drying)*

Kiln drying is one of the best methods of drying to obtain high-quality green cardamom. These curing houses are masonry structures, which are fabricated to meet the requirements. The dryer structure consists of walls which are made of brick or stone with a tiled roof. A furnace is on one side of the chamber and the heat energy is generated by burning firewood. The heated air current is passed on to the drying chamber through a pipe. The fire is regulated to maintain the temperature at 45–55 °C, and by this method quality green cardamom can be obtained in 24–30 hours. A drying chamber of dimension 4.5 × 4.5 m is sufficient for a plantation producing 2 tonnes of fresh cardamom (Kachru and Gupta, 1993). The major disadvantages of this method are the high construction costs and the large quantity of firewood required.

#### *Melccard dryer*

A melccard dryer consists of a fully-insulated (bricks with mud coating) oven kept at 3 m below the dryer. The hot flue gas from the oven is passed to an iron tank through insulated pipes. The four iron pipes carry the flue gas from the smoke tank to the dryer. Two chimneys are provided for an exhaust. The surface of the smoke tank and the flue pipes transfer the heat energy to the dryer. A central opening in the ceiling of the dryer (with an exhaust fan) ensures removal of excess heat. Roofing is also insulated with thick glass wool. The dryer can be loaded easily by opening the front doors (Palaniappan, 1986). This is a firewood-operated dryer commonly used in a few places in Tamil Nadu.

### 8.6.2 Improved curing methods

Research has been undertaken to improve cardamom curing methods. Some alternative techniques are detailed in the paragraphs that follow.

#### *Electrical dryer*

Though different types of electrical dryer are available, the one most commonly used has dimensions of  $90 \times 84$  cm. The aluminium trays ( $81 \times 40$  cm) for drying can be arranged one above the other with a 2 cm gap in between. Green capsules are uniformly spread on the trays and arranged in the dryer. Uniform distribution of heat is ensured by means of fans. This method results in good green-coloured cardamom when the temperature is maintained between 45 and 50°C. The major limitations of this method are availability of electricity and high cost. Furthermore, the splitting rate of capsules is high when compared to the conventional system.

#### *Bin dryer*

The bin dryer was designed by the University of Agricultural Sciences, Bangalore, Karnataka. The drying unit consists of an electrical heating unit and a blower with a motor and drying chamber. The blower is a backward curve vane type connected to a 373 kW motor, with 2820 revolutions per min. The volume of air driven through the dryer can be adjusted to 1.5–8 cm<sup>3</sup>/sec. The dryer is made of mild steel, asbestos sheet and wood. Aluminium or steel trays,  $0.4 \times 0.6$  m, can be arranged one above the other, with the cardamom capsules uniformly spread on them. The required amount of hot air is circulated around the trays by means of a centrally located flue pipe. Good-quality cardamom can be produced by drying the capsules at 55°C by maintaining the volume of air at 3.7 m<sup>3</sup>/s (Gurumurthy *et al.*, 1985).

#### *Solar cardamom dryer*

The direct type solar dryer developed by the Central Plantation Crops Research Institute, Kasargod, Kerala, for coconut can also be used for cardamom. The drier has an area of 1 m<sup>2</sup> drying surface comprising a wire mesh tray placed over a corrugated GI sheet inclined at 12.5°. Aluminum foil reflectors of 1.5 m<sup>2</sup> are provided from three sides of the drier. Material load density can be three times more than that used in an open drying system. Complete drying of cardamom can be achieved within 3 days using this dryer in comparison to 5 days under the open sun.

#### *Mechanical cardamom dryer*

This drier was developed by the Regional Research Laboratory, Trivandrum, Kerala and consists of a centrifugal blower, an electric furnace, uniform hot air flow and a drying chamber. It can be used for drying 120 kg of fresh cardamom per batch. It takes 22 hours for complete drying at a temperature of 50°C. The final product is superior in green colour, flavour and appearance (Kachru and Gupta, 1993).

Other types of improved driers include the kerosene stove dryer, the liquefied petroleum gas (LPG)-based dryer and the diesel dryer (Vadiraj *et al.*, 2002).

### 8.6.3 Bleaching of cardamom

Bleached cardamom is creamy white or golden yellow in colour. Bleaching can be done either with freshly harvested capsules or with dried cardamom capsules. The

fresh capsules are soaked in 20 % potassium metabisulfite solution containing 1 % hydrogen peroxide for 1 hour to degrade the chlorophyll. Drying of these capsules yields golden yellow coloured bleached cardamom.

#### *Sulphur bleaching of dry capsules*

This process involves fumigation using sulphur with alternate periods of soaking and drying. Capsules are soaked in 2 % bleaching powder (20 g/l of water) for 1 hour and spread on wooden trays which are arranged inside air-tight chambers. Sulphur dioxide (SO<sub>2</sub>) is produced by burning sulphur (15 g/kg of capsules) which is passed over the trays. The process of soaking and drying is repeated three to four times depending on the intensity of white colour required.

#### *Potassium metabisulphite bleaching*

In this method, capsules are treated with 2 % potassium metabisulphite containing 1 % hydrogen Chloride (HCl) for 30 minutes. Following this, they are transferred to a 4 % hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution for 6 hours.

#### *Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) bleaching of dry capsules*

H<sub>2</sub>O<sub>2</sub> at a low concentration (4–6 %, pH 4) can bleach capsules in 6–8 hours of soaking. These capsules are then dried to a moisture content of 10–12 %. Bleached capsules contain sulphur which protects cardamom from pests. However, bleaching also results in loss of volatile oil.

### **8.6.4 Packaging**

Cardamom is a high-value spice and all care should be given to efficient packing. The cured capsules are graded using sieves of 8, 7.5, 7 and 6 mm which are manually operated. After grading, cardamom needs to be stored over a period of time; it is normally kept in double-lined polythene bags. Storage rooms should be free from insect damage. Studies have shown that drying cardamom and maintaining it at or below 10 % moisture helps to retain the original parrot green colour without mould growth (Govindarajan *et al.*, 1982). It is advisable to make use of dried cardamom capsules preferably within 12 months of harvesting.

## **8.7 Other value-added products from cardamom**

### **8.7.1 Cardamom seeds**

Cardamom seeds are obtained by decorticating the capsules. Decortication is achieved using a flourmill or plate mill, also known as a disc mill. Normally the ratio of seeds to husk is 70:30.

### **8.7.2 Cardamom powder**

Cardamom in its powder form gives the maximum flavour to the food products, but the disadvantage with powder is that it loses aroma quality by rapid loss of volatiles. Hence, the powder needs more protection than whole capsules or seeds. Koller

(1976) found that vacuum-packed ground cardamom stored at 5°C retained flavour for long periods.

### 8.7.3 Cardamom oleoresin

Oleoresin is made of two components, *viz.*, the volatile oil and the resin. Volatile oil represents the aroma while the resin is made up of non-volatile matter, such as colour, fat, pungent constituents, waxes, etc. The total flavour effect of a spice is obtained only after blending the oil and resin. Volatile oil is obtained by steam or hydrodistillation while the resin is obtained by solvent extraction. The demand for cardamom oleoresin is slowly increasing, probably due to its mellower and less harsh flavour characteristics (Sankarikutty *et al.*, 1982). Sensory differences have also been noted between cardamom oleoresin and cardamom oil (Govindarajan *et al.*, 1982)

### 8.7.4 Cardamom oil

Cardamom oil is obtained by distillation of powdered seeds of cardamom. Steam distillation is the common method employed for the production of oil. The quality of the oil depends on the variety, rate and time of distillation. Volatile oil yield from seeds of Alleppey green, Coorg green and Saklesphpur bleached was 10.8, 9.0 and 8.0 %, respectively (Lewis *et al.*, 1966). High-grade cardamom is not economical for distillation, since it fetches a better price as whole cardamom in the trade. Lower grades which do not fetch a higher value when sold whole are ideally suited for distillation. The husk is almost devoid of any volatile oil. The flavour of cardamom is mainly due to 1,8-cineole, terpinyl acetate, linalyl acetate and linalool (Lawrence, 1978).

### 8.7.5 Extraction techniques

The oil is isolated either by the traditional hydrodistillation or by steam distillation. The major disadvantages of these methods are the loss of volatile components, longer periods of extraction and thermal or hydrolytic degradation of unsaturated or ester compounds (Tuan and Hangantileke, 1977; Khajeh *et al.*, 2004). A steam-distilled essential oil of cardamom was characterised by a high proportion of monoterpenes (87.6 %). The main monoterpene was 1,8-cineole (35.6 %) followed by  $\alpha$ -terpenyl acetate (27.1 %),  $\alpha$ -terpineol (4.9 %), linalool (4.1 %) and thujyl alcohol (Kaskoos *et al.*, 2006).

The volatile oil composition of cardamom seeds using supercritical CO<sub>2</sub> extraction shows the main components as follows:  $\alpha$ -terpinyl acetate, 42.3 %; 1,8-cineole, 21.4 %; linalyl acetate, 8.2 %; limonene, 5.6 %; and linalool, 5.4 %. The extract obtained using hexane shows strong compositional differences, mainly of the following: limonene, 36.4 %; 1,8-cineole, 23.5 %; terpinolene, 8.6 %; and myrcene, 6.6 % (Marongiu *et al.*, 2004). A comparison with the hydrodistilled oil obtained at a yield of 5.0 %, did not reveal any consistent difference.

Recent methods for extraction of natural products using microwave energy, *i.e.*, solvent free microwave extraction (SFME) and also using supercritical CO<sub>2</sub>

extraction have been successfully employed in the case of cardamom (Lucchesi *et al.*, 2007). SFME is based on the combination of microwave heating and distillation and is performed at atmospheric pressure. The composition of extracted oil varies with time, moisture content and irradiation power. The extraction time must be optimised to maximise the yield of the extraction without affecting the quality of the oil. When utilising microwave treatment, moisture content is critical since water is an excellent absorber of microwave energy. This energy subsequently provides a rise in temperature and ruptures the essential oil cells through the *in situ* water. Irradiation power is directly related to the sample size. The power must be sufficient to reach the boiling point of water (100°C).

Six major compounds of the cardamom essential oil have been identified, namely: 1,8-cineole,  $\alpha$ -terpinyl acetate, linalool, linalyl acetate,  $\alpha$ -terpineol and terpin-4-ol, in order of importance. These six compounds represent almost 90 % of the aromatic compounds of the essential oil from cardamom and all of them are oxygenated compounds. A comparison of the SFME method and hydrodistillation indicated the difference in the yields of the two major aromatic components *viz*; 1,8-cineole and  $\alpha$ -terpinyl acetate (Lucchesi *et al.*, 2007). Hydrodistillation is characterized by a long extraction time (six hours) and a high humidity level (~99 %). Overall, 1,8-cineole fraction seems to decrease with time, power and moisture, whereas  $\alpha$ -terpinyl acetate seems to increase.

Monoterpene hydrocarbons are less valuable than oxygenated compounds in terms of their contribution to the fragrance of the essential oil. In the case of SFME, substantially higher amounts of oxygenated compounds are seen, when compared with hydrodistillation. This is probably due to the diminution of thermal and hydrolytic effects during SFME, compared with hydrodistillation. The more polar the compounds, the more readily microwave irradiation is absorbed with better interaction between wave and matter, resulting in higher aromatic components. This corresponds with the higher levels of 1,8-cineole, which is more polar than  $\alpha$ -terpinyl acetate.

Grinding is a very important step in the processing of cardamom as it involves the additional problem of volatility and loss of the aroma-giving essential oil present within it, which is the sole criterion of quality for this spice. Low-temperature grinding is the practical way to achieve fine grinding without loss of flavour principles. The process of cold grinding (2–5°C) enhances the volatile oil content and the quality of the oil in terms of its aroma constituents (Omanakutty and Joy, 2007).

Marongiu *et al.* (2004) studied the effect of supercritical CO<sub>2</sub> extraction (SC-CO<sub>2</sub>) conditions on the yield and composition of the resulting cardamom volatile oil by testing two pressure values, 9.0 and 11.0 MPa; two temperatures, 40 and 50°C; two flow rate values, 0.6 and 1.2 kg/h; and two particles size values, 250–425 and >850  $\mu$ m. The extraction conditions that gave the highest yield, Y (grams of extract per gram of seeds), of 5.5 %, were as follows: pressure, 9.0 MPa; temperature, 40°C; CO<sub>2</sub> flow,  $\phi$  = 1.2 kg/h; and particles sizes in the range of 250–425  $\mu$ m. Waxes, recovered as traces, were entrapped in the first separator set at 9.0 MPa and –10°C. The oil was recovered in the second separator working at 1.5 MPa and 10°C. The main components were  $\alpha$ -terpinyl acetate, 42.3 %; 1,8-cineole, 21.4 %; linalyl acetate, 8.2 %; limonene, 5.6 %; and linalool, 5.4 %.

### 8.7.6 Storage

Quality parameters of cardamom oil obtained by supercritical CO<sub>2</sub> extraction and stored at 0°C or at ambient temperature (28 ± 3°) have been compared with those of commercially steam-distilled oils at ambient temperature (Gopalakrishnan, 1994).  $\alpha$ -Pinene, sabinene and limonene are the major terpene hydrocarbons which undergo remarkable changes during storage. These hydrocarbons show 35–50 % reduction during 90 days of storage at 0°C, in CO<sub>2</sub> extracted oil.  $\alpha$ -Pinene and sabinene together are reduced from 7.1 % to 0.4 % at ambient temperature. Similarly,  $\alpha$ -limonene is reduced from 2.3 % to 0.5 % during the same period of storage. The above hydrocarbons are reduced from 14.4 % to 6.8 % in the commercial oil stored at ambient temperature. Cineole content decreases in the 0°C stored samples, from 27 % to 21.8 %, and in the ambient temperature stored samples, from 27 % to 14.7 %, and from 38.8 % to 27.8 % in commercial oil. Reductions in percentage proportion of these values are, respectively, 19 %, 45 % and 28 %. Changes also take place in the terpene alcohols but are not prominent. In the CO<sub>2</sub> extract distilled oil stored at 0°C,  $\alpha$ -terpinyl acetate content increases during 90 days of storage. In other samples, a remarkable increase of this ester content is noted by 45 days. The two minor esters, geranyl acetate and linalyl acetate, also undergo minor changes in their contents in all of the samples during storage.

### 8.7.7 Effect of $\gamma$ -irradiation in volatile oil composition

$\gamma$ -Irradiation is currently used for the decontamination of spices, but its effect on essential oil composition is controversial and contradictory in cardamom (Ljubica *et al.*, 1983; Klaus and Wilhelm, 1990; Maija *et al.*, 1990). The parent yield of volatile essential oil isolated from non-irradiated (NI) and irradiated (I) samples of cardamom do not reveal any significant difference. Gas-liquid chromatography (GLC) profiles of the two samples of non-irradiated and irradiated oil do not show any variation in the retention time, but the relative percent distribution of the major constituents in the oil exhibits clearcut quantitative differences (Variyar *et al.*, 1998).

### 8.7.8 Encapsulation

The cardamom flavour is incorporated in processed foods, mainly by using the hydrodistilled cardamom oil or the solvent-extracted cardamom oleoresin (Govindarajan *et al.*, 1982). Solvent extraction of ground seeds of cardamom gives a greenish oleoresin containing about 70 % volatile oil. It has the full flavour of the spice. At an elevated temperature, changes may occur in the volatile oil constituents. Cardamom volatile oils consist of terpenoids, which are generally unstable under detrimental conditions like acid, light, oxygen or heat. There is an increase in *p*-cymene, a terpene with petroleum-like aroma, at the expense of the major constituent,  $\alpha$ -terpinyl acetate, which contributes to the desirable flavour of this spice (Brennand and Heinz, 1970). These problems are overcome by microencapsulation, which is defined as the technique of packing minute particles of a core material within a continuous polymer film that is designed to release its contents in a predictable manner under a predetermined set of conditions (Beristain *et al.*, 2001). Microencapsulation of cardamom oleoresin using gum Arabic could entrap the aroma for

six weeks (Krishnan *et al.*, 2005). This technique will be highly useful in the preparation of many pastry products that utilize cardamom at higher oven temperatures (from 149–205°C) and beverages at lower ranges of pH.

## 8.8 Chemical structure and characteristics

### 8.8.1 Volatiles

The volatile oil components in cardamom are summarised by Guenther (1975). The first detailed analysis of the oil was reported by Nigam *et al.* (1965). The oil has few mono- or sesquiterpenic hydrocarbons and is dominated by oxygenated compounds, all of which are potential aroma compounds. While many of the identified compounds (alcohols, esters and aldehydes) are commonly found in many spice oils (or even volatiles of many different foods), the dominance of the ether, 1,8-cineole, and the esters, -terpinyl and linalyl acetates, in the composition, makes the cardamom volatiles a unique combination (Lewis *et al.*, 1966; Salzer, 1975; Korikanthimath *et al.*, 1997c). The major components in cardamom oil are given in Table 8.4, while the trace components are grouped in Table 8.5.

The volatile oil, the most functionally important constituent of cardamom, varies in level from 6.6–10.6 % in seeds for cv. *Mysore* and *Malabar* grown in India (Krishnamurthy, 1964; Krishnamurthy *et al.*, 1967; Korikanthimath *et al.*, 1999). The oil content is low in the immature capsules (in the order of 4–5 %), while the husk oil level is reported to be 0.2 %, the husk oil being reported as having similar properties to the seed oil (Rao *et al.*, 1925). Large differences can be found between the

**Table 8.4** Main components of volatile oil in small cardamom

| Components                 | Total oil |
|----------------------------|-----------|
| $\alpha$ -Pinene           | 1.5       |
| $\beta$ -Pinene            | 0.2       |
| Sabinene                   | 2.8       |
| Myrcene                    | 1.6       |
| $\alpha$ -Phellandrene     | 0.2       |
| Limonene                   | 11.6      |
| 1,8-cineole                | 36.3      |
| $\gamma$ -Terpinene        | 0.7       |
| <i>p</i> -Cymene           | 0.1       |
| Terpinolene                | 0.5       |
| Linalool                   | 3.0       |
| Linalyl acetate            | 2.5       |
| Terpinen-4-ol              | 0.9       |
| $\alpha$ -Terpineol        | 2.6       |
| $\alpha$ -Terpinyl acetate | 31.3      |
| Citronellol                | 0.3       |
| Nerol                      | 0.5       |
| Geraniol                   | 0.5       |
| Methyl eugenol             | 0.2       |
| <i>trans</i> -nerolidol    | 2.7       |

Sources: Lawrence (1978); Govindarajan *et al.* (1982).



**Table 8.5** Trace components in cardamom volatile oil

| Hydrocarbons              | Acids                      | Carbonyls                           |
|---------------------------|----------------------------|-------------------------------------|
| $\alpha$ -Thujene         | Acetic                     | 3-methyl butanal                    |
| Camphene                  | Propionic                  | 2-methyl butanal                    |
| $\alpha$ -Terpinene       | Butyric                    | Pentanal                            |
| <i>cis</i> -ocimene       | 2-methyl butyric           | Furfural                            |
| <i>trans</i> -ocimene     | 3-methyl butyric           | 8-acetoxy carvotanacetone           |
| Toluene                   | Alcohols and phenols       | Cuminaldehyde                       |
| <i>p</i> -Dimethylstyrene | 3-methyl butanol           | Carvone                             |
| Cyclosativene             | <i>p</i> -methyl-3-en-1-ol | Pinole                              |
| $\alpha$ -Copaene         | Perillyl alcohol           | Terpinene-4-yl-acetate              |
| $\alpha$ -Ylangene        | Cuminyll alcohol           | $\alpha$ -Terpinyl propionate       |
| $\gamma$ -Cadinene        | <i>p</i> -cresol           | Dihydro- $\alpha$ -terpinyl acetate |
| $\Delta$ -Cadinene        | Thymol                     |                                     |

Sources: Lawrence (1978); Govindarajan *et al.* (1982).

**Table 8.6** Concentration of 1,8-cineole and  $\alpha$ -terpinyl acetate in cardamom oils from different origins

| S. No | Oil kind                         | Percentage  |                            |
|-------|----------------------------------|-------------|----------------------------|
|       |                                  | 1,8-cineole | $\alpha$ -Terpinyl acetate |
| 1.    | Guatemala I                      | 36.40       | 31.80                      |
| 2.    | Guatemala II                     | 38.00       | 38.40                      |
| 3.    | Guatemalayan <i>Malabar</i> type | 23.40       | 50.70                      |
| 4.    | Guatemalayan I                   | 39.08       | 40.26                      |
| 5.    | Guatemalayan II                  | 35.36       | 41.03                      |
| 6.    | Synthite (Commercial grade)      | 46.91       | 36.79                      |
| 7.    | Mysore type (Ceylon)             | 44.00       | 37.00                      |
| 8.    | Malabar type (Ceylon)            | 31.00       | 52.50                      |
| 9.    | Mysore I                         | 49.50       | 30.60                      |
| 10.   | Mysore II                        | 41.70       | 45.90                      |
| 11.   | Mysore                           | 41.00       | 30.00                      |
| 12.   | Malabar I                        | 28.00       | 45.50                      |
| 13.   | Malabar II                       | 43.50       | 45.10                      |
| 14.   | Ceylon type                      | 36.00       | 30.00                      |
| 15.   | Alleppy I                        | 38.80       | 33.30                      |
| 16.   | Alleppy green                    | 26.50       | 34.50                      |
| 17.   | Coorg green                      | 41.00       | 30.00                      |
| 18.   | Mangalore I                      | 56.10       | 23.20                      |
| 19.   | Mangalore II                     | 51.20       | 35.60                      |
| 20.   | Papua New Guinea                 | 63.03       | 29.09                      |
| 21.   | Cardamom oil (India origin)      | 36.30       | 31.30                      |

Source: Govindarajan *et al.* (1982).

concentrations of 1,8-cineole in the oils of var. *Malabar* and var. *Mysore*. In var. *Mysore*, linalool and linalyl acetate are markedly higher. This, along with a low content of 1,8-cineole, makes var. *Mysore* the largest selling Indian cardamom grade, Alleppey Green (Table 8.6). Variability in the in the oil content and the composition of the two main components (1,8-cineole and  $\alpha$ -terpinyl acetate) in the

germplasm collections at Indian Institute of Spices Research – Cardamom Research Centre, Karnataka are as indicated in Table 8.7.

The aroma differences in different sources of cardamom are attributed to the proportion of the esters and 1,8-cineole (Wijesekera and Jayawardena, 1973; Kori-kantimath *et al.*, 1999). The flavour characteristics of some important volatile components of cardamom are given in Table 8.8 and the chemical structures in Fig. 8.2.

### 8.8.2 Variability in oil composition

The value of cardamom as a food and beverage additive depends on the aroma components in the volatile oil. These are subject to variation according to variety, maturity, processing, extraction techniques and storage.

**Table 8.7** Chemical quality profile of cardamom germplasm at Indian Institute of Spices Research, Calicut, Kerala

| Acc. No. | Essential oil (%) | 1,8-cineole (% of oil) | $\alpha$ -Terpinyl acetate (% of oil) |
|----------|-------------------|------------------------|---------------------------------------|
| APG7     | 5.5               | 51                     | 29                                    |
| "12      | 8.6               | 37                     | 43                                    |
| "20      | 9.4               | 48                     | 34                                    |
| "23      | 9.4               | 39                     | 39                                    |
| "25      | 7.5               | 38                     | 43                                    |
| "27      | 6.9               | 34                     | 33                                    |
| "32      | 5.6               | 38                     | 44                                    |
| "44      | 7.1               | 34                     | 38                                    |
| "48      | 6.3               | 41                     | 33                                    |
| "54      | 6.8               | 44                     | 34                                    |
| "65      | 7.5               | 32                     | 45                                    |
| "69      | 8.3               | 49                     | 28                                    |
| "71      | 7.3               | 36                     | 31                                    |
| "87      | 5.7               | 42                     | 32                                    |
| "98      | 6.3               | 33                     | 30                                    |
| "106     | 10.0              | 43                     | 39                                    |
| "112     | 6.6               | 45                     | 34                                    |
| "117     | 6.3               | 38                     | 43                                    |
| "134     | 6.0               | 40                     | 38                                    |
| "135     | 9.9               | 45                     | 40                                    |
| "158     | 6.6               | 40                     | 38                                    |
| "175     | 9.8               | 43                     | 40                                    |
| "178     | 5.6               | 22                     | 39                                    |
| "180     | 6.8               | 31                     | 32                                    |
| "183     | 7.8               | 24                     | 39                                    |
| "187     | 8.0               | 22                     | 48                                    |
| "193     | 8.3               | 28                     | 47                                    |
| "215     | 6.0               | 23                     | 55                                    |
| "217     | 6.0               | 25                     | 51                                    |
| "218     | 7.8               | 24                     | 52                                    |
| "221     | 7.8               | 37                     | 40                                    |
| CCS-1    | 8.6               | 42                     | 36                                    |

Source: Zachariah and Lukose (1992).

**Table 8.8** Flavour characteristics of important volatile components in cardamom

| Components                 | Flavour description                                                                       | Flavour effect and use                                                             | Use level concentration (%) | Range of cardamom oil |
|----------------------------|-------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------|-----------------------|
| <b>Esters</b>              |                                                                                           |                                                                                    |                             |                       |
| $\alpha$ -Terpinyl acetate | Mildly herbaceous, sweet spicy, Variation in odour, warm, mild spicy taste.               | To stretch cardamom, herbal spice, imitation of citrus, cherry and peach flavours. | 1–15 ppm                    | 34.6–52.5             |
| Linalyl acetate            | Sweet, floral, fruity odour and taste, poor tenacity, but stronger than terpinyl acetate. | Fresh, sweet modifier in perfume and berry flavours.                               | 2–15 ppm                    | 0.7–6.3               |
| <b>Ethers</b>              |                                                                                           |                                                                                    |                             |                       |
| 1,8-cineole                | Fresh, camphoraceous, cool odour and taste, very diffusive and poor tenacity.             | Refreshing effect and lift; extensively used in perfume and flavours.              | 1–15 ppm                    | 23–51                 |
| Alcohol linalool           | Floral, woody with citrus note; creamy floral taste at low levels.                        | Lift to heavy perfume; peculiar pleasant taste effect at low levels.               | 2–10 ppm                    | 1.4–4.5               |
| $\mu$ -Terpineol           | Delicately floral, sweet, lilac like.                                                     | Citrus and spice compositions.                                                     | 5–40 ppm                    | 1.4–3.3               |
| <b>Others</b>              |                                                                                           |                                                                                    |                             |                       |
| Methyl eugenol             | Musty tea like, mildly spicy, warm, slightly earthy.                                      | Tenacious, dry, herbaceous spicy effect.                                           | 5–15 ppm                    | 1.3                   |

Source: Bernhard *et al.* (1971).

Extracts of cardamom cultivated in Costa Rica have been analysed by gas chromatography–mass spectroscopy (GC–MS) on various columns. Among the 122 compounds found, 56 represented over 99 % of the total volatile oil fraction, and 74 % were reported for the first time. Their distribution is similar to that described for other samples of *E. cardamomum* var. minor, grown in other countries (Noleau *et al.*, 1987). Traded (exported) cardamoms from India, Sri Lanka and Guatemala have been characterised based on the physical and biochemical parameters and molecular techniques (Elizabeth *et al.*, 2006). Indian cardamom was found to be superior in terms of most of the physical quality parameters and for the biochemical traits. The GC profile of the oil of Indian cardamom also indicated a high quantity of  $\alpha$ -terpinyl acetate and 1,8-cineole which imparts aroma and flavour to the cardamom, thus reinforcing the legendary belief in the high intrinsic quality of the Indian cardamom.

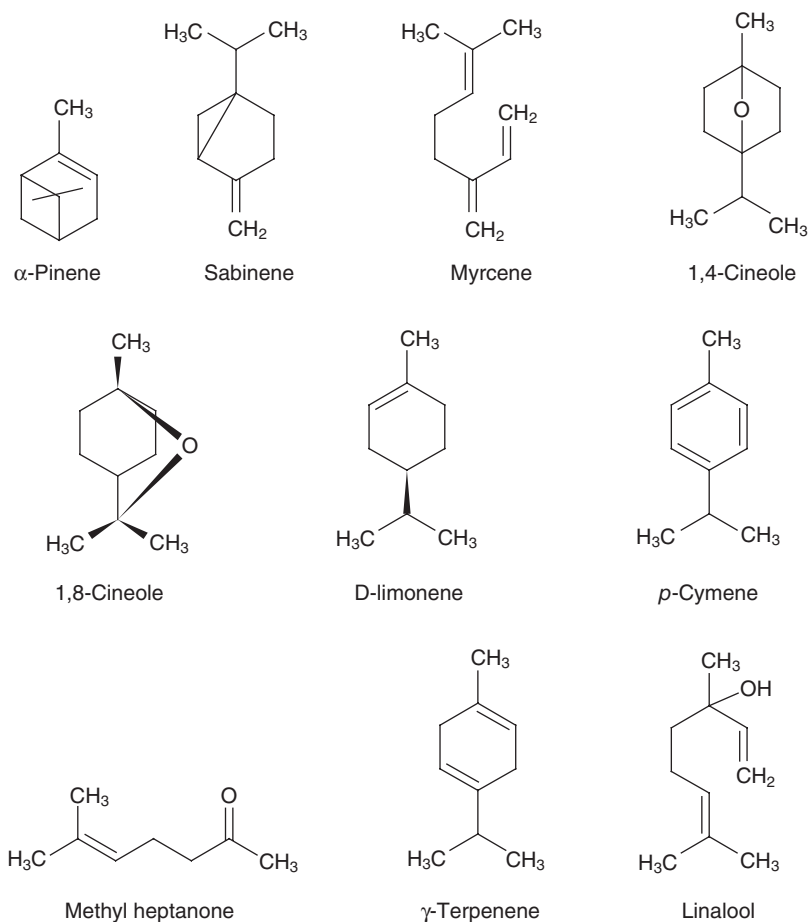
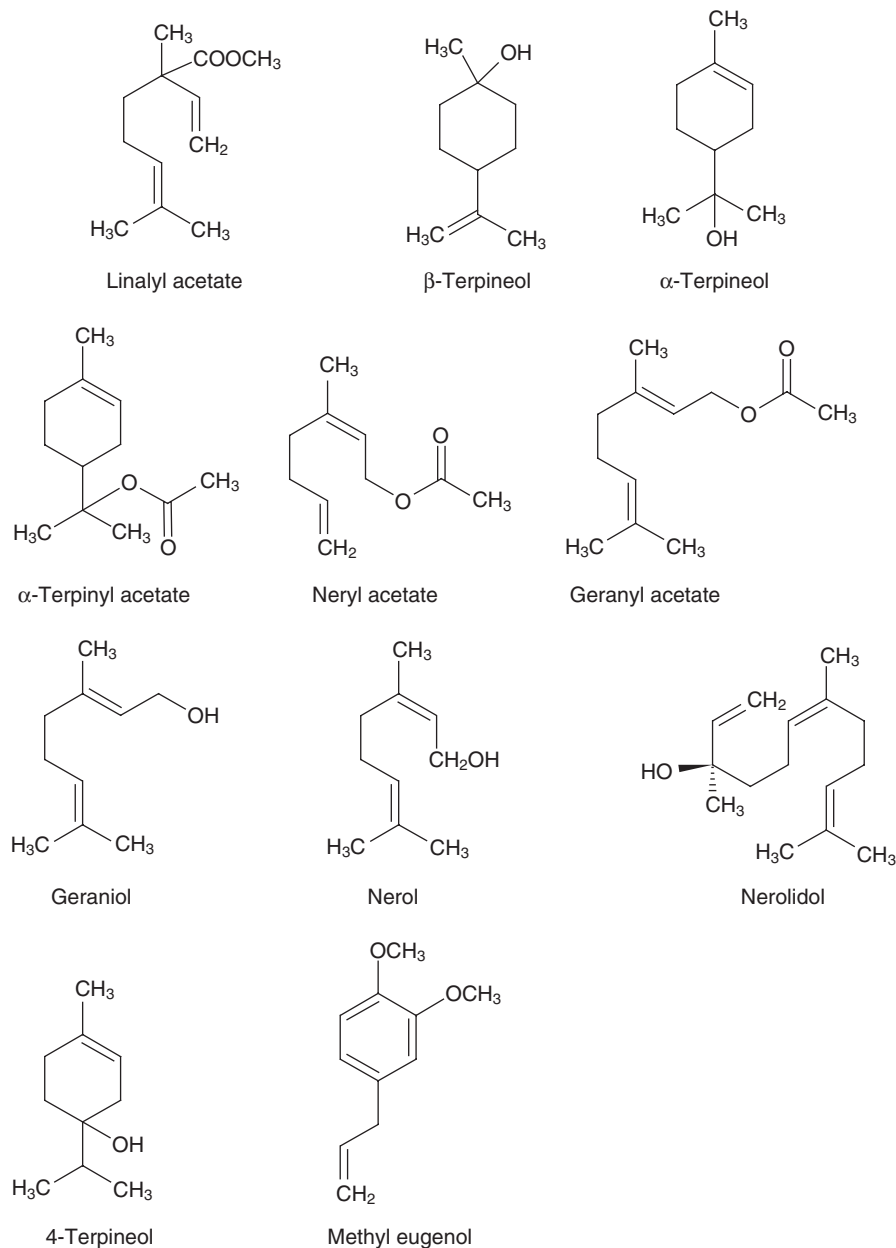


**Fig. 8.2** Major essential oil components in small cardamom.

## 8.9 Major uses of cardamom

### 8.9.1 Traditional medicinal uses

The major medicinal properties of cardamom essential oil are its antiseptic, carminative, digestive, diuretic, stimulant, stomachic, tonic and antispasmodic, anti-microbial and anti-inflammatory activities (Al-Zuhair *et al.*, 1996; de Pradier, 2006). It is also used as an aphrodisiac; it is helpful in countering the irritation experienced during premenstrual tension; and it works well on the respiratory system to ease coughs and to warm the body. The seeds of cardamom are considered cooling and stimulating and a carminative, stomachic, diuretic, cardi tonic and abortifacient. They have been used to treat bronchitis, haemorrhoids, stangury, renal and vesical calculi, anorexia, dyspepsia and gastropathy (Wanrrier *et al.*, 1994). Powdered cardamom seeds mixed with ground ginger, cloves and caraway have been used mainly for combating digestive ailments. Use of cardamom checks nausea and vomiting.

**Fig. 8.2** *Continued*

Cardamom seeds can lessen inflammation, as well as being useful for headaches (Govil, 1998). They are chewed to prevent bad breath and to prevent pyrosis (excessive watering in mouth). Powdered seeds of cardamom, boiled with tea-water, impart a very pleasant aroma to the tea, which can be used as medicine for scanty urination, diarrhoea, palpitation of the heart, exhaustion due to overwork

and depression among other things. Gargling with an infusion of cardamom and cinnamon is thought to cure pharyngitis, sore throats and hoarseness, during the infective stage of flu. It is believed that eating a cardamom capsule daily along with a tablespoon of honey improves eyesight, strengthens the nervous system and thus improves the general health of the person. Cardamom is considered capable of destroying Kapa and poison, helps to promote a good skin complexion and to relieve itching, pustules and akotha (Chempakam and Sindhu, 2008). It is also used to help relieve the symptoms of asthma, burning sensations, colds and coughs, diseases of the bladder and kidney, flatulence, heart weakness and piles (Dey, 1994).

### **8.9.2 Pharmacological properties**

Cardamom has antimicrobial activity and the seeds have an inhibitory effect against microbes (Agaoglu *et al.*, 2005). The terpenoid constituents are responsible for the antifungal and antibacterial effects. The volatile oil of cardamom seeds is more effective in inhibiting the growth of the microbial species examined than fixed oil, and the inhibitory effect against some pathogenic fungi is shown to increase in correlation with the increasing concentration of volatile oil. It also has a highly inhibitory effect on the selected pathogenic bacteria. Moreover, it exhibits highly cytotoxic and anticarcinogenic activities against human tumour cell lines. The volatile oil of cardamom can be used as a potential grain protectant, by killing various life stages of the stored product insects attacking the wheat, through direct contact and fumigant action (Huang *et al.*, 2000).

### **8.9.3 Applications in the food industry**

The major use of cardamom worldwide is for culinary purposes in the whole or ground form. In India, cardamom seeds are widely used for flavouring during cooking of food. It is an essential ingredient in 'garam masala'. In the Islamic Republic of Iran, cardamom is used in making confectionery, bakery and meat preparations to add flavour and aroma to the products. In European countries and North America, cardamom is used mainly in ground form by food industries as an ingredient in curry powder, a few sausage products, soups, buns, breads, cookies, desserts, wine and, to small extent, in flavouring tobacco and cigarettes. In Scandinavia, it is used widely in bakery products (Rosengarten, 1973).

## **8.10 Quality standards and grade specifications**

### **8.10.1 Major specifications**

For the most part, grades are based on physical parameters such as colour, size, weight per specific volume and freedom from microbial, insect and filth contaminants. The Bureau of Indian Standards (BIS) and The Directorate of Marketing and Inspection (DMI), Government of India have prescribed fairly well-defined grades for cardamom. The Indian specifications or standards are based on important

quality factors such as colour, weight per unit volume, size and percentage of 'empties', malformed, shrivelled and immature capsules (Tables 8.9–8.13).

The importance attached to the different dimensions of quality varies with the primary producer, the intermediary collector, the trader and exporter, the importer, the processor, the distributor and the consumer. The extraction, volatile oil and ingredients are valued by the processor; the interest of the distributor and consumer is in sensory quality and cost.

### 8.10.2 Adulteration issues

Decorticated seeds can be adulterated with seeds from lower grades, and also with seeds of large cardamom, as they are of similar shape, size and colour. Pale brown coloured seeds can be indicative of immature cardamom, which is poor in quality, low in volatiles and intensity. The seeds from large cardamom have a lower volatile oil content and an entirely different composition and aroma. Gross adulteration with seeds from large cardamom will show higher 1,8-cineole and higher terpene hydrocarbons, which are determinable by gas or thin-layer chromatography. However, examination of the surface of the seeds with a hand lens shows distinct differences, while the seed coat surface of true cardamom has clear furrows and ridges, the large cardamom has an almost smooth surface. Adulteration of cardamom powder is possible with almost any material powdered to similar size. Cereal and pulse flours and extracted ginger have been reported as adulterants. These can be detected using microscopy by the very different size and structure of the starch

**Table 8.9** Specifications for Indian cardamom (based on physical characters)

| Grade               | Description | Size (mm) | Weight (g/l)min | Colour                | General characteristics                                |
|---------------------|-------------|-----------|-----------------|-----------------------|--------------------------------------------------------|
| AG (Alleppey Green) |             |           |                 |                       |                                                        |
| AGB                 | Extra Bold  | 7         | 435             | Green                 | Kiln dried, 3 cornered and with ribbed appearance      |
| AGS                 | Superior    | 5         | 385             |                       |                                                        |
| AGS 1               | Shipment    | 4         | 320–350         | Light green           |                                                        |
| AGL                 | Light       | 3.5       | 260             |                       |                                                        |
| CG (Coorg Green)    |             |           |                 |                       |                                                        |
| CGEB                | Extra Bold  | 8         | 450             | Golden to light green | Round ribbed or smooth skin                            |
| CGB                 | Bold        | 7.5       | 435             |                       |                                                        |
| CG-1                | Superior    | 6.5       | 415             | Light green           |                                                        |
| CG-2                | Mota, Green | 6         | 385             | Green                 |                                                        |
| CG-3                | Shipment    | 5.5       | 350             | Cream                 |                                                        |
| CG-4                | Light       | 3.5       | 280             | Brown                 |                                                        |
| Bleached            |             |           |                 |                       |                                                        |
| BL-1                |             | 8.5       | 340             | Pale                  | Fully developed round/3 cornered ribbed or smooth skin |
| BL-2                |             | 7         | 340             | Creamy                |                                                        |
| BL-3                |             | 5         | 300             | Dull white            |                                                        |

Source: Indian Standard specification for cardamom. IS: 1907–66. Indian Standards Institution, New Delhi-1.



**Table 8.10** AGMARK specifications of Coorg clipped and bleachable white cardamoms

| Variety                    | Grade designation                              | Empty and malformed capsules by count (max) (%) | Unclipped capsules by count (max) (%) | Immature and shrivelled capsules (%) by weight | Size (mm) | Weight g/l (min) |
|----------------------------|------------------------------------------------|-------------------------------------------------|---------------------------------------|------------------------------------------------|-----------|------------------|
| Coorg clipped cardamoms    | Bold                                           | 5.0                                             | 0.0                                   | 0.0                                            | 8.5       | 435              |
|                            | Coorg green or Mota green Shipment             | 5.0                                             | 3.0                                   | 4.0                                            | 6.0       | 385              |
|                            | Light                                          | 3.0                                             | 5.0                                   | 7.0                                            | 4.0       | 350              |
| Bleachable white cardamoms | Mysore/Mangalore bleachable cardamom clipped   | 1.0                                             |                                       | 0.0                                            | 3.5       | 260              |
|                            | Mysore/Mangalore bleachable cardamom unclipped | 1.0                                             |                                       | 0.0                                            | 7.0       | 460              |
|                            | Bleachable bulk cardamom clipped               | 2.0                                             |                                       | 0.0                                            | 4.3       | 435              |
|                            | Bleachable bulk cardamom unclipped             | 2.0                                             |                                       | 0.0                                            | 4.3       | 435              |
|                            |                                                |                                                 |                                       |                                                |           |                  |

**Table 8.11** AGMARK specifications of Alleppey and Mangalore cardamom seeds

| Variety                  | Grade designation | Trade name | Extraneous matter (%) by wt. | Light seeds (%) by wt. | Weight (gm/l) min. |
|--------------------------|-------------------|------------|------------------------------|------------------------|--------------------|
| Alleppey cardamom seeds  | AS1               | Prime      | 1.0                          | 3.0                    | 675                |
|                          | AS2               | Shipment   | 2.0                          | 5.0                    | 460                |
|                          | AS3               | Brokens    | 5.0                          |                        |                    |
| Mangalore cardamom seeds | MS1               | Prime      | 1.0                          | 3.0                    | 675                |
|                          | MS2               | Shipment   | 2.0                          | 5.0                    | 460                |
|                          | MS3               | Brokens    | 5.0                          |                        |                    |

**Table 8.12** Whole cardamom: chemical and physical specification

| Specifications                         | Suggested limits |
|----------------------------------------|------------------|
| <b>ASTA cleanliness specifications</b> |                  |
| Whole dead insects, by count           | 4                |
| Mammalian excreta, by mg/lb            | 3                |
| Other excreta, by mg/lb                | 1.0              |
| Mould, % by weight                     | 1.0              |
| Insect defiled, infested, % by weight  | 1.0              |
| Extraneous, % by weight                | 0.5              |
| FDA DALs                               | None             |
| Volatile oil                           | 3 % min.         |
| Moisture                               | 12 % max.        |
| Ash                                    | 10 % max.        |
| Acid insoluble ash                     | 2 % max.         |
| <b>Average bulk index (mg/100 g)</b>   |                  |
| Bleached                               | 320              |
| Green                                  | 250              |

DALs = deficit action levels.

**Table 8.13** Ground cardamom: chemical and physical specification

| Specifications                                                   | Suggested limits          |
|------------------------------------------------------------------|---------------------------|
| FDA DALs                                                         | None                      |
| Volatile oil                                                     | 3 % min.                  |
| Moisture                                                         | 12 % max                  |
| Total ash                                                        | 10 % max                  |
| Acid insoluble ash                                               | 2 % max                   |
| Military specification (EE-S-631J, 1981) (Decorticated Cardamom) |                           |
| Volatile oil (ml/100 g)                                          | 3 % min.                  |
| Moisture                                                         | 12 % max                  |
| Total ash                                                        | 7 % max                   |
| Acid insoluble ash                                               | 3 % max                   |
| Granulation                                                      | 95 % min through a USS 40 |
| Bulk index (ml/100 g)                                            | 190                       |

granules. Cardamom starch grains, unlike those of cereal and other starches, are very small (2–4  $\mu$ m). Whole cardamom powder can be distinguished from the cardamom seed powder by microscopy. The former can be recognized by the yellowish colour, abundance of pitted fibres, spiral cells of the vascular bundles, empty parenchymatous cells and scattered resin cells with brownish clumps (Melchior and Kastnev, 1974).

## 8.11 Conclusion

Cardamom is an important spice crop, which has been prominent in world trade since ancient times. Cardamom gained particular popularity during the second half of the twentieth century. Since the 1990s, world cardamom production and demand have increased greatly. Although the aroma and flavour of cardamom are often used in the manufacturing of many foods, consumption in the developed world is low. A concerted effort is required to promote cardamom use in traditional and non-traditional countries.

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## Chives

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**Abstract:** The plant known as chives is a perennial of the Liliaceae family, *Allium* species, distributed from the Arctic regions to Asia, Europe and North America, and grown in East Asia since ancient times. This chapter looks at the chemical composition and nutritional value of chives as well as their varieties, botany and morphology. Flavour compounds, thiosulphinates, flavonoid glycosides and kaempferol glycosides are presented. Cultivation and production methods are discussed, including propagation, organic farming practice and pest management. The chapter then finishes with a description of post-harvest processing, fresh and dried, and culinary applications.

**Key words:** chive, *Allium schoenoprasum*, thiosulphinates, glycosides, glucosides, flavonoids.

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### 9.1 Introduction

The plant known as chives is a perennial of the *Liliaceae* family, *Allium* species. Chromosome number  $2n = 16, 24, 32$ . Wild chives are widely distributed from the Arctic regions to Asia, Europe and North America (Xu and Kamelin, 2001; McCollum, 1976). There were no written records of domesticated chives from the Mediterranean region until the 16th century in Europe, whereas in East Asia chives have been domesticated since ancient times (McCollum, 1976). At present chives are cultivated as vegetables or seasoning herbs all around the world, especially in the Northern Hemisphere.

Because chives are very adaptable, tolerant of cold and hot temperatures, and grow rapidly, they can be cultivated and harvested many times throughout the year. They are also easy to propagate, either from seeds or from division of clumps all year round. Chives are grown mainly for their long, cylindrical leaves, used for culinary purposes. Their flowers can also be used for salad dressings and sometimes for decorative purposes.

Since ancient times, herbs of the *Allium* species have been considered a healthy food for their special spicy flavour. Unlike the pungent flavour of garlic and onions, the flavour of chives is milder and more delicate, and more easily acceptable to the

palate. Chives also contain many vitamins and mineral nutrients (Rubatzky and Yamaguchi, 1997) as well as flavonoid compounds, which are antioxidants (Justesen, 2000). Among all *Allium* species, chives have the highest content of vitamin C and beta-carotene. Chives have green leaves that are soft in texture and are easily kept fresh and transported using drying and freezing techniques. Techniques are presently, being developed to apply chives in the processing of dairy, meat, and snack products.

## 9.2 Chemical composition and nutritional value

Chemically, chives are composed mainly of carbohydrates, proteins and amino acids, as well as many other vitamins and minerals. The unique spicy flavour comes mostly from volatile sulphur and glucosides, etc. Studies into the chemistry of *Allium* flavour began in the 18<sup>th</sup> century. So far, much has been learned about *Allium* chemistry, although many questions still remain (Block, 1992). We have learned that the flavour is the result of a multifaceted interaction among many different compounds, and we have also begun to understand the factors that affect the quality and intensity of the flavour of *Allium*. The flavour substances of various *Allium* species depend on the quantitative differences in the S-alk(en)yl cysteine sulfoxides (ACSOs). The S-alk(en)yl cysteine sulfoxides (ACSOs), when hydrolyzed by the enzyme allinase, give rise to the flavour and pungency characteristic of the *Allium* plants (Randle and Lancaster, 2002). When the tissues of *Allium* are disrupted, the enzyme allinase hydrolyzes the flavour precursors. Sulphur compounds are found in the cytoplasm of *Allium* cells, physically separated from allinase (Lancaster and Collin, 1981).

In *Allium* species, four different ACSOs have been identified (Bernhard, 1970; Freeman and Whenham, 1975; Yoo and Pike, 1998; Randle and Lancaster, 2002). 2-methyl-2-butenal, 2-methyl-2-pentenal, methyl-propyldisulphide, and dipropyldisulphide have been found in the green leaves of chives. There is also evidence for the presence of propenyl-propyldisulphide in chives, while allyl disulphide is definitely absent (Wahlroos and Virtanen, 1965). The major thiosulfinate from chive are n-propyl groups, methyl and 1-propenyl groups. The data of Table 9.2 are both qualitative and semi-quantitative (Block *et al.*, 1992).

Of the thiosulfates found in chives, 77 (75) % contain the n-propyl group, 10 (12) % contain the methyl and 12 % contain the 1-Propyl group. Total thiosulfates is 0.19  $\mu\text{mol/g}$  wet (fresh), about the middle level of the edible species of *Allium*. The

**Table 9.1** Approximate chemical composition of chives (6) (7)\*

| %<br>Water | Calories | CND | Pro | Fat | Fib | Ash | A    | C  | B1   | B2   | Niacin | Ca | P  | K   | Na | Mg | Fe  | S* | Volatile<br>o: 1* |
|------------|----------|-----|-----|-----|-----|-----|------|----|------|------|--------|----|----|-----|----|----|-----|----|-------------------|
| 92.0       | 26       | 3.9 | 2.8 | 0.6 | 0.9 | 0.8 | 6400 | 70 | 0.10 | 0.12 | 0.60   | 82 | 46 | 250 | 6  | 55 | 1.2 | 93 | 25–26             |

Source: Adapted from Rubatzky and Yamaguchi, 1997.

\* Epmakov, 1961.

**Table 9.2** Thiosulfates and sulfines from extracts of chive as determined by Si-HPLC (concentrations in mole percent of total)

| Compound no. | Compound* (response factor)** | Onions     | Chives  |
|--------------|-------------------------------|------------|---------|
| 5            | n-PrSS(O)Propenyl-(E)         | 9–12       | 2.5     |
| 6,8          | nPrS(O)SPropenyl-(Z<E)        | 10–12      | 16      |
| 7            | EtCH=S=O                      | ++         |         |
| 9            | n-PrS(O)SPr-n                 | 4–13       | 58      |
| 10           | MeSS(O)Propenyl-(E)           | 22–23      | *       |
| 11           | AllS(O)SMe                    |            |         |
| 12,15        | MeS(O)SPropenyl-(Z,E)         | 25 (24)–31 | 1(5.5)  |
| 13           | MeSS(O)Pr                     | 1–4        | 5.9     |
| 14           | MeS(O)SPr                     | 1(I)–4     | 15(10)  |
| 16           | AllSS(O)Me                    |            |         |
| 17           | OSCH(CHMe)2CHSO               | 2–11       |         |
| 18           | MeS(O)Sme                     | 0–14       | 1.8     |
|              | Total % MeS                   | 28–38      | 13      |
|              | Total % AllS                  |            |         |
|              | Total % 1-propenylS           | 37–47      | 10 (2)  |
|              | Total % nPr                   | 19–27      | 77 (75) |
|              | Total thiosulfates            | 0.14–0.35  | 0.19    |

Source: adapted from Block *et al.*, 1992.  
\* Chemical Abstracts names of compounds: AllSS(O)Propenyl-(E),AllS(O), SPropenyl-(Z,E), AllS(O)SAll, n-PrSS9O)SPropenyl-(E), n-PrS(O)SPropenyl-(Z,E), EtCH=S=O, n-PrS(O)Propenyl-(E), AllS(O)SMe, MeS(O)SPropenyl(Z,E), MeSS(O)Pr, MeS(O)SPr, AllSS(O)Me, OSCH(CHME)2CHSO, MeS(O)SMe.  
\*\* Molar absorption with HPLC 254-nm UV detector relative to benzyl alcohol calibrated by NMR peak integration. Amounts given in umol/gwet9fresh) weight.

**Table 9.3** m/z value of ions produced by liquid chromatography mass spectrometry of flavonoid glycosides present in chives

| (M-H)- | Product ions of (M-H) | Product ions of aglycone | Suggested name         |
|--------|-----------------------|--------------------------|------------------------|
| 463    | 301                   | ND                       | Quercetin glucoside    |
| 477    | 315                   | 300,271,151,107          | Isorhamnetin glucoside |
| 447    | 285                   | ND                       | Kaempferol glucoside   |

Source: adapted from Justesen, U. (2000).

n-propyl group in chives is more abundant than methyl, with the methyl/propyl 1:5.8. Chives have an onion-like flavour. The pungent and stimulating flavour of chives and onions is mainly due to the propyl group, but it is the quantitative and qualitative differences in the thiosulfates that give each species its own characteristic flavour. The flavour and lachrymatory properties of chives are due to the high n-propyl content, while the flavour of onions is due to the high proportion the 1-propyl it contains.  
It has been recognized that factors affecting flavour intensity and quality include genetic, ecological and cultivation techniques. Chives grown in different years, areas and different cultivars, with different cultivation techniques, may have distinct flavour intensities.



**Fig. 9.1** Chive field.

Chives also contain flavonoid glycosides, as shown in Table 9.3. The biological activities of flavonoids are mainly due to their antioxidant function. They are also known to inhibit several enzymes, including lipoxigenases and cyclo-oxygenase, etc. The green leaves of chive mainly contain kaempferol glucosides (di- and tri-glycosides), dominant as glucose and galactose. The 3-beta-D-glucosides of kaempferol, quercetin and isorhamnetin were isolated (Starke and Herrmann, 1976).

A comparison of flavonoids from chives grown under different light conditions indicates different flavonoid response to PAR and UV-B light. Total flavonoids from the chives were  $16.7 \text{ mg}/10 \text{ g}^{-1} \text{ f.w.}$  The ratio of the kaempferol glucoside, quercetin glucoside and isorhamnetin glucoside was 4:1:2. Exposure of PAR flavonoids increase 30 % of total flavonoids, and additional UV-B even by more than 80 %. (Nitz *et al.*, 2000).

Green leaves of chive mainly contain kaempferol glycosides with di- and tri-glycosides, the 3-beta-D-glucosides of kaempferol, quercetin and isorhamnetin as by-glycosides from chive. The structures of eight anthocyanins have been determined in acidified methanolic extract of the pale-purple flowers of chives. Four of them have been identified as the anthocyanin-flavonol complexes, with the other four, anthocyanins were found. The covalent anthocyanin-flavonol complexes show intermolecular association between the anthocyanidin (cyanidin) and flavonol (kaempferol) units, which influences the colour (Fossen *et al.*, 2000).

## 9.3 Cultivation and production

### 9.3.1 Botany and morphology

Chives are a perennial plant cultivated as a biennial. However, the productive growth cycle is commonly completed within a year. Based on molecular data, chives belong to the subgenus *Rhizideum* (Hanelt *et al.*, 1992). Bulbs are oval shaped and often clumped together. The skin of the bulbs has a grayish brown colour, with yellow or purplish tints, and the texture of carton paper. The development of elongated rhizomes and of false bulbs are advanced character states (synapomorphies), usually clustered, ovoid-cylindric, 0.5–1 cm in diameter; tunic brown or tinged with

yellow, papery, lacinate, sometimes fibrous at apex. The leaves grow in clumps of 2–5, slightly shorter than scape, 2–6 mm wide, terete, fistulose, smooth or scabrous denticulate. Chives branching results from where lateral initiation occurs after the development of every two or three leaves (Poulson, 1990), and thus plants develop clusters of shoots. Scape 10–50 (60) cm, terete, covered with leaf sheaths for 1/3–1/2 its length, smooth or scabrous-denticulate (Xu and Kamelin, 2000).

After seeding, chives flower in the second year and each year afterwards. The long and thin flower scape is cylindrical in shape, hollow and smooth. Umbel sub-globose, are densely flowered. The perianth is purple-red to white (or pale pink), tepal twice the length of filaments; polymorphous spires. Six needle shaped petals are of the same height. The pistil does not protrude out of the petals. (Xu and Kamelin, 2001). The flowers do not produce much pollen and seldom produce viable seeds. Chives are generally an out-crosser, and flowers are insect pollinated, but selfing frequently occurs as well (Rubatzky and Yamaguchi, 1997). Chives can be cultivated from seeds or from division.

Wild chives are confined mainly to meserophytic habitats; meadows, forests and high mountain zones (Hanelt *et al.*, 1992). Wild chives grow continuously all year round with no apparent dormant stage, and low winter temperatures only slow this down (Cheremushkina, 1985, 1992; Pistrick, 1992). Chives exhibit a monopodial growth habit, and only become sympodial after the formation of the first generative meristem. Thereafter, *Allium* plants produce renewal bulbs and flowers every year (Kamenetsky, and Rabinowitch, 2002). Temperatures play the most important role in normal scape elongation and flowering of *Allium* plants, although light conditions can markedly affect this process. Flowering usually does not occur if temperatures are above 18°C (Rubatzky and Yamaguchi, 1997). Like other major cultivated *Allium* crops, cold exposure is required for floral induction in chives. (Poulsen, 1990).

In chives male sterility is conditioned by genetic male sterility (GMS), which is controlled by a single nuclear gene with recessive inheritance (Engelke and Tatioglu, 2000a,b,c). An alternative cytoplasmic male sterility (CMS) depends on the interaction between the cytoplasm (S) and a single nuclear fertility restoration locus (X) (Tatioglu, 1982). Fertility of some male-sterile plants, however, can be regained under favourable environmental conditions. Hence, exposure to a constant temperature of 24°C resulted in production of viable pollen (Tatioglu, 1985). This temperature sensitivity is controlled by a single dominant allele (*t*) (Tatioglu, 1987).

*A. Schoenoprasum* contains several closely related species, in part of polyploid nature, with partly unclear species status. In such situations, molecular markers could bring some clarification that is difficult to obtain by other means. A cladistic tree of the *Schoenoprasum* RAPD data has been constructed (Friesen and Blattner, 2000).

### 9.3.2 Culture and production

Chives are very adaptive to different environments. Tolerant of cold temperature, chives can germinate slowly when daily temperature averages 3–5°C, while its most suitable temperature ranges from 15–20°C. Because of its shallow root system, care must be taken to maintain soil moisture, especially to prevent flooding. Chives grow

best in well-drained, fertile soil with medium acidity. Optimum growing temperatures are between 17–25 °C, cold hardy, and tolerant of high temperatures (Rubatzky and Yamaguchi, 1997), and so can be grown widely distributed throughout the world. The plants will start to flower after staying dormant for a period in cold temperatures.

Chives grow all year around and can be cultivated and harvested in batches throughout the four seasons. Propagation is usually with seed or division. For mass production, seeding in spring or autumn is suitable. Seedlings can be planted once 15 cm high. Each 20 × 10 cm pocket can accommodate 4–6 seedlings. They can be harvested in about two months when the plant reaches 30 to 50 cm in height. The first harvest will produce a relatively low yield. Chives can be harvested about once each month, and more frequently after the second harvest, to about 5–7 times per year in warm areas, and 2–4 times per year in colder climates. In cold areas, each harvest will yield more, to about 15 ton/ha. When processing, do not cut to the sheath (4 cm above ground). On average, reseeding or dividing the clumps every four years will keep the productivity high. In some areas, harvesting is done by plucking the plants by the roots rather than cutting.

It is important to strictly follow the guidelines of organic farming practice to grow chives. Select well drained, fertile sandy or clay soil; maintain the cleanliness of the field and weed promptly; use high quality organic compost with appropriate N.P.K. ratios. The crop is susceptible to many root diseases. Rotations are a key aspect for a sustainable agricultural production system, a rotation of at least five years is recommended. Although chives can be grown in all kinds of soils, the most suitable soils are sandy loams to loams with a fair content of organic matter and good soil structure. Soil pH of 6–6.5 is considered sufficient. Chives demand a high nutrient level. In the years following planting, the annual uptakes in yield are 185–200 kg/ha for nitrogen, 17–20 kg/ha for phosphorus, and 120–140 kg/ha for potassium in the most intensively fertilized treatment producing the highest yield. Black plastic mulch is effective in increasing yield, controlling weeds and maintaining soil moisture. Selected productive cultivars or populations produced 10–20 % higher yields than the less productive cultivars. The results show that chive is feasible for commercial production with improving cultivation techniques (Suojala, 2003).

Chives and related *Allium* crops are subject to a variety of diseases and attack by arthropod pests that can reduce crop yield and quality. Integrated pest management (IPM) is a sustainable approach to managing diseases and arthropod pests. IPM promotes the use of a variety of strategies and tactics, including pest-resistant varieties and biological, cultural and chemical controls, in a way that reduces costs, conserves natural resources and minimizes health and environmental risks. Decision-making is a key component of IPM programs (Binns and Nyrop, 1992). So far, monitoring programs forecasting systems for diseases (*Botrytis* leaf-blight, downy-mildew and purple-blotch) and pests (onion maggot, onion thrips, leek moth, cutworms, beet armyworm, aster leafhopper, aphids and mites) have been set up. IPM will continue to be the preferred strategy as it takes a whole-system approach as environmental problems take on greater importance. Since chives compete poorly with weeds, the use of herbicides is widespread and the economic advantages of their use have been demonstrated (Menges, 1987; Rubin, 1990). Scientific studies



are starting to appear on the effects of organic production methods of weed control (Bond *et al.*, 1998).

### 9.3.3 Post-harvest and uses in food processing

Because chives are used as a vegetable or for seasoning, it is important to preserve the fresh green appearance as well as the unique aroma. After harvest, remove withered and damaged leaves. Immediately store in temperatures as low as 0°C (32°F), but not lower in order to prevent freezing. At 0°C, with humidity of 95–100 %, chives can be kept fresh for one to two weeks (Snowdon, 1991). The respiration rate of chives increases with temperature. At 0°C, mg CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> is 22, which increases to 110 at 10°C, and 540 at 20°C (Peiris *et al.*, 1997). In fact, when the temperature rises above 10°C, chives will wilt quickly (Cantwell and Reid, 1993). When transporting, chives are usually packed into 1–3 kg packets, also in bunches of 10–50 g and kept moist in wax cartons at around 2–6°C. Pre-cooling is recommended (Aharoni *et al.*, 1989). In an experiment, green tops were bunched, 25–30 g per bunch, packed in perforated or non-polythene bags (20 × 25 cm) and stored at 2, 5, 10, 15 or 20°C by Umiecka (1973). The control was kept unpacked. The tops stored better in non-perforated than in perforated bags and the longest satisfactory storage of 14–21 days was in non-perforated bags at 2°C, but deteriorated rapidly at the higher temperatures (Thompson, 2003). Studies have been conducted on freshly harvested chives under simulated conditions of air transport from Israel to Europe, and also with an actual shipment, during which temperatures fluctuated between 4 and 15°C (Aharoni *et al.*, 1989). Packaging in sealed polyethylene-lined cartons resulted in a marked retardation of both yellowing and decay. However, sealed film packaging was applicable only if the temperature during transit and storage was well controlled, otherwise perforated polyethylene was better.

Drying is a common technique to process chives. The usual methods are heat drying at 50°C, or freeze drying. Freeze drying is more costly, but preserves the flavour well. After drying/freeze drying, there are losses of 24–34 % vitamin C, 19–21 % chlorophyll, 11–18 % beta-carotene, and 47–82 % volatile sulphur (Lisiewska *et al.*, 1998). Chive leaves for freezing contained 13.9 g dry matter, 133 mg vitamin C 4.7, beta-carotene, 121 mg, chlorophylls (a + b), 40.4 mg nitrates, and 0.19 mg nitrites in 100 g of edible part. Blanching of the raw material before freezing reduced the level of dry by 22 %, vitamin C 29 %, beta carotene 20 %, chlorophylls 21 %, and nitrates 26 %, while the nitrites increased three times. A further enhancement of losses was observed with a storage temperature at –20°C. After 12 months storage of frozen chive, the preserved content of vitamin C ranged from 11 to 66 %, beta carotene 37 to 65 %, chlorophylls 65 to 75 % and the nitrates 58 to 81 % (Kmiciek and Lisiewska, 1999). The development of the catering business and industrial preparation of ready-to-cook food, most frequently pizza and au gratin dishes, has increased the demand for chives throughout the year. This demand can be met by preserving the vegetable as a dried or frozen product.

Chives can be used as seasoning for many dishes, or as garnish. Chives especially enhance the flavour of fish. There is a very delicious Chinese dish known simply as fish with chives. Chives can be included in many food items such as pancakes, buns, dumplings, and cookies. It can also be used in many dairy and meat products.

## 9.4 Varieties

There are many differences between the *Allium* species. The volatile sulphur content of different species ranges from 15 to 155 mg/10 g<sup>-1</sup> fresh weight (Ermakov and Arasimovich, 1961). It is easy to test the pyruvate of *Allium*'s sulphur precursors, to determine the difference between the species. The difference ranges from 1–22 µmolg<sup>-1</sup> fresh weight, while difficult to select through genetic breeding, are perhaps the key to improving flavours (Randle and Lancaster, 2002). The volatile sulphur content in *Alliums* is closely related to the soil and usable sulphur in the soil. Experiments have shown that in peat soil, where sulphur content is as high as 470 mg/10 g<sup>-1</sup>, the volatile sulphur content in the chives reaches 157 mg/10 g<sup>-1</sup>, while in clay soils with a sulphur content of 58 mg/10 g<sup>-1</sup>, the volatile sulphur content in the chives is only 42.8 mg/10 g<sup>-1</sup>. At the temperature range of 10–30 °C, volatile sulphur content increases from 42.8 mg/10 g<sup>-1</sup> to 130.9 mg/10 g<sup>-1</sup> (Ermakov and Arasinovich 1961). Also, improving cultivar, fertilization and cultivation techniques, shows that chive is feasible for commercial production. In summary, the flavour of chives is closely tied to its genetic traits, growing environment and as cultivation techniques. Chives are mildly flavoured. Chives have the highest beta-carotene and vitamin C content among all *Allium* species, and contain many antioxidants. These characteristics make Chives a superior and well appreciated vegetable and seasoning. However, there has not been much research done on chives. The understanding and improvement of chives has a lot of potential.

The formal name of Chives is *A. schoenoprasum* L. Syn. *A. sibiricum* (Kamenetsky and Fritsch, 2002), there are some other related species, including:

1. *A. schoenoprasum* L. var. *schoenoprasum*, also known as *A. raddeanum* Regel; *A. sibiricum* L. Leaves, leaf sheaths, and scape smooth. Fl. and fr. Jul–Sep. leaves 1 or 2, shorter than scape. Widely distributed in meadows, valleys, damp slopes; 2000–3000 m, in Xinjiang in China, India, Japan, Kazakhstan, Korea, Mongolia, Pakistan, Russia, SW Asia, Europe, and North America (Xu and Kamelin, 2000). Fl. May–June, second bloom in late summer possible (Kamenetsky and Fritsch, 2002).
2. *A. schoenoprasum* L. var. *scaberrimum* Regel, (Trudy Imp. S.–Peterburgsk. Bot. Sada 3(2): 80. 1875) Leaves, leaf sheaths, and scap scabrous-denticulate along angles, Fl. Aug. distributed in meadows, along streams; 2000–2500 m Xinjiang in China, Russia, Kazakhstan, and Mongolia.
3. *A. schoenoprasum* L. var. *foliosum* Mutel. Originated in the Mediterranean region, with two to five smooth leaves (Mutel, 1834).
4. *A. schoenoprasum* L. var. *orientale* Regel. Bulb usually solitary or paired, rarely clustered (Xu and Kamelin, 2000).

*A. schoenoprasum* section contains several closely related species, in part of polyploid nature, with partly unclear species status. It is not certain whether geographical varieties exist, probably need more work in near future to clear.

Nowadays there are a few plants commonly called 'chives' or 'green onions', but some of them are not *A. schoenoprasum*. For example, *A. cepiform*, a 'chive' (Rubatzky and Yamaguchi, 1997) from China with tender leaves named xi-xiang-cong, is possibly a cross of *A. cepa* x *A. fistulosum*. Many people also call *A. fistulosum*

*L. var. aespitosum* Makino 'chive'. These varieties are different in flavour from chives, and usually have only white flowers, or no flowers.

## 9.5 References and further reading

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## Cinnamon

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**Abstract:** The chapter focuses mainly on the chemical and flavouring properties of the spice cinnamon, *Cinnamomum zeylanicum*. The difference in spice properties and constituents between cinnamon and cassia, *Cinnamomom cassia*, the nutritional constituents of cinnamon, chemical variations of cinnamon bark oil, root oil and leaf oil and cassia oils are described. Harvesting of cinnamon bark, preparation of quills, grades and standard specifications are detailed in the chapter. Uses of cinnamon as a spice in the food industry, as a flavourant, as a colourant and as a medicinal are indicated.

**Key words:** cinnamon, cassia, quills, nutritional content, flavouring compounds, cinnamaldehyde.

### 10.1 Introduction

Cinnamon is an age-old spice. It is often mentioned in the Old Testament of the Bible and there are indications of its use in Egypt as early as 3000 BC. There are several possible derivations for the name cinnamon. One school of thought is that it comes from Phoenician through the Greek word *kinnámōmon*, meaning sweet wood. The taxonomical nomenclature for the spice *Cinnamomum zeylanicum* is derived from Sri Lanka's former name, Ceylon, which indicates the plant's centre of origin. The genus *Cinnamomum* belongs to the family lauraceae and has about 250 species spread over South East Asia, China and Australia, many of which are aromatic and flavouring. The cinnamon of commerce is the dried inner bark of the tree *Cinnamomum verum* (syn. *C. zeylanicum* Blume) native to Sri Lanka and the Malabar Coast of India. It is pale tan in colour and mildly sweet in flavour. Cassia is a similar spice to cinnamon but of an inferior quality. It is a native of Myanmar (Burma). The sources of cassia cinnamon are Chinese cassia (*C. cassia* syn. *C. aromaticum*) from China and Vietnam, Indonesian (*Cinnamomum burmanii*), from Sumatra and the Java region, and Indian cassia (*Cinnamomum tamala*) from the north-eastern region of India and Myanmar (Baruah and Nath, 2004).

Cinnamon is endemic to Sri Lanka, which is the major producer and exporter of bark oil and leaf oil with 24000 ha under cultivation. The Seychelles, Madagascar and India also produce true cinnamon bark of superior quality, though in small



quantities. In India, true cinnamon is cultivated in Kerala, coastal areas of Karnataka and various parts of Tamil Nadu (Krishnamoorthy *et al.*, 1996). Islands like the Andaman Islands, Nicobar Islands, Lakshadweep and the Maldives are also famous for cinnamon. According to the *International Herald Tribune*, in 2006 Sri Lanka produced 90 % of the world's cinnamon, followed by China, India and Vietnam. According to the FAO, Indonesia produces 40 % of the world's *Cassia* genus of cinnamon. The major importers are Mexico, West Germany, the USA, the UK, Saudi Arabia, Taiwan, Singapore, Hong Kong and France.

The cinnamon spice grown in Sri Lanka has unique advantages due to its organoleptic properties. There are significant compositional variations even within the plantations, and the presence of different cultivars has been recorded in the island (Wijesekera, 1978). These cultivars are recognized by sensory evaluation and denoted by such nomenclature as 'sweet', 'honey', 'camphoraceous' and also 'mucilaginous', wild and 'bloom'. The sweet and honey cultivars are extensively cultivated (Senanayake, 1977).

Bark oil is produced from the distillation of imported cinnamon and cassia in Western Europe and North America. Sri Lanka also supplies a major portion of the cinnamon bark oil. France is the biggest importer followed by the USA. Leaf oil is distilled in Sri Lanka and the Seychelles. The USA and Western Europe are the largest market for leaf oil. China is the major producer of cassia oil in the global market. Small quantities of cassia oils are produced in Indonesia, Vietnam, India and Nepal, but these are obtained from species of cinnamon other than *C. cassia* and are much less widely traded than the Chinese oil.

## 10.2 Description of cinnamon

The cinnamon tree is a tropical evergreen that can grow up to 7 m in its natural state. However, under cultivation, the plant is more often grown as a bush, limiting the plant height to less than 3 m, since the stems are continuously cut back to produce new shoots for bark/quills extraction. It has thick scabrous bark and strong branches. Cinnamon bark is widely used as a spice. The bark has a pleasing fragrance and warm, sweet, aromatic taste in its natural or cooked state. The leaves are ovate-oblong in shape and 7–18 cm long. The leaves are slightly hot and bitter in taste and have a mouth-watering fragrance when cooked or fried. The flowers are arranged in panicles, greenish in colour with distinct odour. The fruit is a purple, 1 cm long berry containing a single seed.

The tree thrives best in a hot, wet tropical climate at low altitude and is usually ready to harvest after three years. The soil should not be waterlogged as this produces a bitter-tasting bark. Eight or ten side branches grow on the bush and these are harvested after about three years to obtain quality cinnamon bark. Cinnamon spice is mainly obtained by drying the central part of the bark, which is marketed as quills or powder. Dried leaves, at times, form a constituent of spice mixtures also. The essential oil extracted from cinnamon bark is also used as concentrated source of cinnamon flavour, especially in the canned food and confectionery industries. Cinnamon gets its distinctive smell and aroma from volatile oils present in the bark or leaves.



A lot of confusion exists between cinnamon and cassia. While cinnamon and cassia are not precisely the same, they are closely related and the bark of the two is not all that different. It may be a surprise to many to know that what is sold in American stores as cinnamon is mostly cassia. Cassia is thick, hard and has a flavour that is extremely bitter and burning with somewhat of a bite in the aftertaste. Cassia has a double curl when it dries, meaning that this is a spiral of dried bark, a small bit of relatively straight bark, then the other long edge spirals in the opposite direction. Ground cassia has very reddish brown colour. True cinnamon has but a single spiral curl and is almost papery, brittle, easily crushed or powdered. Its flavour is more subdued, less bitter and has a decidedly sweet finish in the aftertaste. Its smell is sweet and aromatic. The bark of cinnamon is pale yellowish brown

### 10.2.1 Chemical structure

Cinnamon bark contains: moisture 9.9 %, carbohydrates 22.6 %, protein 4.65 %, fibre 20.3 %, total ash 3.55 %, calcium 1.6 %, phosphorous 0.05 %, iron 0.04 %, sodium 0.01 %, potassium 0.4 %, vitamin B<sub>1</sub> (0.14 mg/100 g), vitamin B<sub>2</sub> (0.21 mg/100 g), vitamin C (39.8 mg/100 g), niacin (1.9 mg/100 g). The nutritional composition of cinnamon is given in Table 10.1.

The geographical origin of the spice and the conditions under which it is processed influence the chemical composition. The primary chemical constituents of the spice crop are cinnamaldehyde, gum, tannin, mannitol, coumarins and essential oils.

#### *Volatiles*

Cinnamon bark contains volatile oil from 0.4–2.8 % (Angmor *et al.*, 1972; Wijesekera, 1978; Krishnamoorthy *et al.*, 1996), tannins constituting polymeric 5,7,3',4'-tetrahydroxy flavan-3-4-diol units, catechins and proanthocyanidins, resins,

**Table 10.1** Nutritional composition of cinnamon per 100 g

| Composition             | USDA   | ASTA   |
|-------------------------|--------|--------|
| Water (g)               | 9.52   | 10.00  |
| Food energy (Kcal)      | 261.00 | 355.00 |
| Proteins (g)            | 3.89   | 4.50   |
| Fat (g)                 | 3.18   | 2.20   |
| Carbohydrates (g)       | 79.85  | 79.80  |
| Ash (g)                 | 3.55   | 3.50   |
| Calcium(g)              | 1.23   | 1.60   |
| Phosphorous (mg)        | 61.00  | 50.00  |
| Sodium (mg)             | 26.00  | 10.00  |
| Pottasium (mg)          | 500.00 | 400.00 |
| Iron (mg)               | 38.07  | 41.00  |
| Thiamine (mg)           | 0.077  | 0.14   |
| Riboflavin (mg)         | 0.14   | 0.21   |
| Niacin (mg)             | 1.30   | 1.90   |
| Ascorbic acid (mg)      | 28.46  | 40.00  |
| Vitamin A activity (RE) | 26.00  | 26.00  |

*Source:* Tainter and Grenis (1993).

mucilage, gum, sugars, calcium oxalate, two insecticidal compounds (cinnezalin and cinnzeylanol), coumarins and others. The sweet taste of cinnamon is due to the presence of cinnamaldehyde.

The bark oil consists of cinnamaldehyde (80–90 %), eugenol, eugenol acetate, cinnamyl acetate, cinnamyl alcohol, methyl eugenol, benzaldehyde, cinnamaldehyde, benzyl benzoate, linalool, monoterpene, hydrocarbon, caryophyllene, safrole and others such as pinene, phyllandrene, cymene and cineol. Bark oil is a pale yellow to dark yellow liquid with a strong, warm, sweet, spicy, tenacious odour and a sweet, pungent but not bitter taste.

The root bark oil consists of camphor at 60 %. It is colourless to pale yellowish brown, similar in odour to stem bark oil but weaker, lacking in fragrance and camphoraceous odour. Cinnamon leaf oil, obtained by steam distillation, has a warm, spicy, but rather harsh odour, lacking the rich body of the bark oil. The leaf oil yield varies between 0.5 % and 1.8 % (Senanayake and Wijesekera, 1989). More than 47 compounds have been identified from the leaf oil and, among these, eugenol is the most significant, which constituting 65–92 % (Senanayake *et al.*, 1978). Mallavarapu *et al.* (1995) identified 53 constituents along with the major component eugenol (81–84.5 %) in cinnamon leaf oil. Leaf oil is cheaper than bark oil and is used as a source of eugenol for the preparation of synthetic vanillin. Cinnamon oleoresin is a dark brown highly concentrated and viscous liquid obtained by solvent extraction, containing 50 % or more volatile oil.

Cassia bark yields 1–2 % volatile oils, resembling that of cinnamon. Its value depends on the percentage of cinnamaldehyde. The oil also contains cinnamyl acetate, cinnamic acid, phenyl propyl acetate, orthocumaric aldehyde, tannic acid and starch.

Besides the essential oil constituents, several diterpenes have been isolated from the genus. These include cincassols A, B, C<sub>1</sub> and their glucosides, cincassols C<sub>2</sub> and C<sub>3</sub>, cincassols D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub> and their glucosides, cincassiol E, cinnzeylanol, cinnzeylanin, anhydrocinnzeylanol and anhydrocinnzeylanin. Jayaprakasha *et al.* (2006) isolated and identified non-volatile constituents from cinnamon fruits and reported its antioxidant properties. The polyphenolic polymers present in cinnamon may function as antioxidants, potentiate insulin action, and may be beneficial in the control of glucose tolerance and diabetes (Anderson *et al.*, 2004).

## 10.3 Harvesting and production of cinnamon

### 10.3.1 Harvest

Harvesting for bark is made after the second or third year of planting, and the subsequent harvest is made between 12 and 18 months after the previous harvest. The branches harvested this way are processed by scraping off the outer bark, then beating the branch evenly with a hammer to loosen the inner bark. The outer bark of the tree is thick and brownish. The inner bark is more useful than the outer one. The cutting of the tree is normally done in the wet season from central portions of shoots. The finest quality of bark is obtained from shoots with uniform brown colour, thin bark 1.0–1.25 m length and 1.25 cm diameter. The ideal time for cutting the stem is when the red flush of the young leaves turns to green and is the indication of the free

flow of sap between the bark and the wood. The shoots ready for peeling are removed from the stems and the terminal ends of shoots are also removed. Harvesting is done mainly in two seasons, the first in May and second in November, although harvesting on a limited scale continues throughout the year. The ideal time for cutting the shoots for peeling is determined based on the sap circulation between the root and the corky layer. Skilled peelers can judge this by making a test cut on the stem.

### 10.3.2 Processing

The bark must be processed immediately after harvesting, while still wet. Azam Ali (2007) describes bark processing as follows:

Processing accounts for about 60 % of the cost of production of cinnamon. This is because the peeling of bark from the stems is labour intensive and is usually done by hand, by skilled peelers. The quality of cinnamon depends on how well the bark is removed from the stems ... Drying is an important stage in the processing of cinnamon. It contributes to the quality of the final product ... The compound quills are placed on coir rope racks and dried in the shade to prevent warping. After 4 or 5 days of drying, the quills are rolled on a board to tighten the filling and then placed in subdued sunlight for further drying. In humid climates or during the rainy season it will be necessary to use a mechanical dryer to complete the drying process. There are a range of dryers available to suit different situations (electrical, gas fired, biomass fuelled).

The first harvest yields quills of 60–125 kg ha<sup>-1</sup>. Plants with an age of 10–12 years will give about 225–300 kg quills per hectare. The larger pieces or quills can be sold at a premium compared to the smaller broken pieces. It should be added that once removed from the stems, the bark will dry completely over a period of 4–6 hours, provided it is kept in a well-ventilated and relatively dry environment. Otherwise pests may proliferate in the bark, which then needs to be treated by fumigation. Fumigated bark is not considered as premium quality.

#### *Production of quills*

The commercial products of cinnamon are quills, quillings, featherings, chips, cinnamon bark oil and cinnamon leaf oil. There are a number of stages involved in the production of quills:

- **Peeling:** The harvested shoot needs to be peeled for the extraction of quills. A special knife is used to scrap off the rough outer bark. Subsequently, the scraped portion is polished with a brass rod to facilitate easy peeling. A longitudinal slit is made from one end to other and the bark is peeled off. The Agricultural Engineering University of Ruhuna in Sri Lanka has developed a small machine for removing the bark from cinnamon stems.
- **Rolling:** The barks are packed together, placed one above the other and pressed well. The bark slips are reduced to 20 cm length and are piled up in small enclosures made by sticks. Then they are covered with dry leaves or mat to preserve the moisture for the next day's operation and also to enhance the slight fermentation. The retention of moisture is important for the next operation, piping.
- **Piping:** Rolled slips are taken to the piping yard for piping operations. The outer skin is scraped off with a small curved knife. The scraped slips are sorted into different grades according to thickness. The graded slips are trimmed; the ends

are cut and pressed over pipes. Slips are rolled into pipes and, soon after, they are allowed to dry. During drying, smaller quills are inserted into bigger ones, forming smooth and pale brown compound quills, which are known as pipes. The quills are arranged in parallel lines in the shade for drying as direct exposure to the sun at this stage would result in wrapping. The dried quills, thus obtained, consist of a mixture of coarse and fine types and are yellowish brown in colour. The quills are bleached, if necessary, by sulphur treatment for about 8 hours.

The process of producing quills has several by-products, which are used in further processing:

- **Quillings:** These are broken pieces of quills used mainly for grinding but also for distillation of oil. The pieces vary considerably in size, being about 5–15 or 20 cm in length and about 10–25 mm in diameter.
- **Featherings:** These are feather-like pieces consisting of shavings and small pieces of leftovers from the processing of the inner bark into quills. Collectively, featherings present as a shade darker in colour than the quills and a shade lighter than the chips. The product is marketed as medium-quality cinnamon.
- **Chips:** These are small pieces of bark, greyish brown on the outside and a lighter brown on the inside. They are deficient in both aroma and taste and are not to be compared to the quills for flavour. There are two grades in chips: grade 1 contains small featherings obtained by scraping very small twigs together with a small amount of other bark material; grade 2 contains inner and outer bark and pieces of wood.

#### *Production of ground cinnamon*

Grinding can be a method of adding value to a product and cinnamon is sometimes ground to a powder prior to sale. The heat of grinding is very destructive to the volatile oil content of cinnamon. Cryogenic grinding, however, helps to retain more volatiles and is ideal in the case of cinnamon (Tainter and Grenis, 1993). Grinding also reduces the shelf-life of cinnamon: it renders the spice more vulnerable to spoilage because the flavours and aroma compounds are not stable and will quickly disappear. Other considerations are that it is very difficult for the consumer to judge the quality of a ground spice and also very easy for unscrupulous processors to contaminate the ground spice by adding other material.

#### *Production of oils and oleoresins*

Hydrodistillation or steam distillation methods are employed for the production of bark oil. Cinnamon chips, featherings or quillings are invariably used for extraction. Cinnamon bark produces two oils, namely a superior type derived from inner bark and a poorer quality from broken quills, chips and bark. The barks to be distilled for oil should not be placed in wet bundles or become damp as this may facilitate mould or fermentation which directly affects the oil profile. The leaves of cinnamon can be used as a raw material for the extraction of leaf oil. It is reported that about 1 tonne of leaf is obtained from 1 hectare which, on steam distillation, yields 2.5–3 kg of oil rich in eugenol (Thomas and Duethi, 2001).

Cinnamon and cassia oils are both normally rectified to produce oil with a more uniform composition. Cinnamon oleoresin is also produced from Indonesian cassia

for flavouring purposes. Oleoresin is produced by extracting barks with organic solvents; the yield using ethanol is 10–12 % and that using benzene is 2.5–4.3 %. The solvent 1,1,2- trichloro-1,2,2-trifluoroethane is widely used in recent years for oleoresin extraction.

### 10.3.3 Packaging

Packaging of cinnamon, especially if ground, requires polypropylene. Polythene cannot be used as the flavour components diffuse through it. Cinnamon quills are cut into pieces up to 10 cm in length and packed into moisture-proof polypropylene bags for sale. The bags should be sealed to prevent moisture entering. According to Azam Ali (2007),

The label needs to contain all relevant product and legal information – the name of the product, brand name (if appropriate), details of the manufacturer (name and address), date of manufacture, expiry date, weight of the contents, added ingredients (if relevant) plus any other information that the country of origin and of import may require (a barcode, producer code and packer code and all extra information that is required in some countries to help trace the product back to its origin).

For wholesale export, the quills are packed in compact cylindrical bales of 50 kg and wrapped in jute cloth. A new cinnamon-based active paper package was developed incorporating cinnamon essential oil (6 %w/w) to solid wax paraffin. The essential oil in the active coating formulation completely inhibits the growth of *Rhizopus stolonifer* (Rodriguez *et al.*, 2008).

### 10.3.4 Storage

According to Azam Ali (2007),

Dried cinnamon quills must be stored in moisture-proof containers away from direct sunlight. The stored cinnamon quills should be inspected regularly for signs of spoilage or moisture. If they have absorbed moisture, they should be re-dried to a moisture content of 10 %. The storage room should be clean, dry, cool and free from pests. Mosquito netting should be fitted on the windows to prevent pests and insects from entering the room. The ground powder should be packaged in moisture proof packaging (polypropylene bags) to retain the flavour.

## 10.4 Quality issues

The quality of the product is dependent on the thickness of the bark, the appearance and the aroma and flavour. The quality of bark is greatly influenced by the soil and ecological factors. The bark obtained from the central branches is superior to that from the outer shoots and that from either the base or the top. The bark of thick branches is coarse and that of young shoots is thin and straw-coloured with very little flavour. Plants grown under shade produce inferior quality quills.

The quality of cinnamon is assessed on the basis of its appearance and on the content and aroma/flavour characteristics of the volatile oil. Good-quality cinnamon

should not be thicker than a thick paper. It should be light brown with wavy lines and produce a sound of fracture when broken. When chewed it should become soft, melt in the mouth and sweeten the breath. Freshly ground cinnamon bark of good quality contains 0.9–2.3 % essential oil depending on the variety.

#### 10.4.1 Grading of quills

This is essentially done on the basis of physical appearance and there is no close relation with the volatile oil content. Compound quills measuring 42 inches long (just over 1 m) are sorted into grades according to the thickness of the bark. According to Parry (1969), three main qualities are exported, which are as follows:

- **Fine/ continental grades:** Quills are fine and are designed by a series of zeroes, C-00000 being the thinnest and best, while C-0 is the thickest (range from 10 mm in diameter or less in C-00000 and 19 mm in C-0 grade).
- **Hamburg grades:** H-1 to H-3 wherein H-1 grade is thicker and darker than C-0 grade. H-3 is very coarse, ranging from about 23 to 32 mm.
- **Mexican grades:** M-00000 and M-0000 are intermediate in quality between fine and Hamburg grades: M-00000 is equivalent to C-000 in thickness and M-0000 is equivalent to C-0.

The Sri Lankan grading system is also described elsewhere as dividing cinnamon quills into four main groups according to diameter:

- **Alba:** Less than 6 mm in diameter.
- **Continental:** Less than 16 mm in diameter.
- **Mexican:** Less than 19 mm in diameter.
- **Hamburg:** Less than 32 mm in diameter.

For cassia quills, the grade designations are:

- **Quality A:** Quills 1 m long taken from the main trunk.
- **Quality B:** From side branches.
- **Quality C:** Broken pieces.

#### 10.4.2 Quality specifications

The British Pharmacopoeia defines cinnamon as the dried bark of the *Cinnamomum zeylanicum* tree. It should contain less than 2 % foreign matter by weight, not less than 1 % volatile oil.

Whole and ground cinnamon quality is defined in ISO 6539 (ISO 1997b). According to this standard, Sri Lankan cinnamon should have:

- moisture: 12 % max
- total ash: 5 % max
- acid insoluble ash: 1 %
- volatile oils: whole – 1 % and ground – 0.7 %.

The American Spice Trade Association (ASTA) has suggested moisture levels should be at 14 % for all *Cinnamomum* spices. Most good-quality cinnamon should

have ash and acid insoluble ash levels of less than 5 % and 1 %, respectively. Insect fragment levels mandated by the Food and Drug Administration (FDA) must be less than 400 per 5 g in the ground spice and the minimum volatile oil content of the fortified ground cinnamon/cassia shall be 2.0 ml per 100 g. The FDA has set defect action levels of contaminants for cassia/cinnamon as:

- an average of 5 % mouldy pieces by weight;
- an average of 5 % insect infested pieces by weight;
- an average of 1 mg of excreta per pound of cinnamon.

The British Pharmacopoeia, 1973, lays out the following specifications for bark of Ceylon cinnamon:

- acid insoluble ash: 2.0 % max.
- foreign organic matter: 2.0 % max.
- volatile oil: 1.0 % v/w min.

In India, the sanitary and phytosanitary (SPS) standards for agricultural commodities are prescribed through the AGMARK specification. The AGMARK standards are basically used for grading the commodities and do not specify safety parameters. The safety standards are as per the Prevention of Food Adulteration (PFA) Act & Rules of India 1954 and amended in October 1984, whereby Cinnamon (*Dalchini*) Whole and Powder were placed under the codes A.05.06 and A.05.06.01, respectively. Whole refers to the dried pieces of the inner bark of *Cinnamomum zeylanicum* (Blume). It shall not contain any other foreign vegetable or colouring matter. It shall contain not less than 0.5 % (v/w) of volatile oil. The amount of insect-damaged matter shall not exceed 5 % by weight. Cinnamon Powder (A.05.06.01) is obtained by grinding the dried inner bark of *Cinnamomum zeylanicum* (Blume). It shall be free from added colouring matter. The stipulated standards of the powder are as follows:

- moisture: 12.0 % by weight max.
- total ash: 8.0 % by weight max.
- ash insoluble in dilute HCl: 2.0 % by weight max.
- non-volatile ether extract: 0.5 % (v/w) min.

The dried bark of *Cinnamomum cassia* Blume, Syn. *Cinnamomum aromaticum* Nees (Chinese Cinnamon, or Cassia) is placed under Cassia (Taj), Whole with PFA code A.05.06.02. The Whole shall not contain any other foreign vegetable or colouring matter.

There is no international standard for cinnamon bark oil although batches containing cinnamaldehyde at the higher end of the range fetch higher prices. In the USA, the Essential Oil Association (ESA) standard specifies an aldehyde content of 55–78 %. However International (ISO) standards exist for cinnamon leaf (ISO, 2003) and cassia oils (ISO, 1997a). Sri Lanka now accounts for almost all of the leaf oils in the international market and specifies 75–85 % eugenol content and a maximum of 5 % cinnamaldehyde. In the USA, an FMA monograph, which replaces the old EOA standards, specifies the eugenol content of leaf oil in terms of solubility in KOH (80–88 %). For cassia oil, cinnamaldehyde is the major constituent and a minimum 80 % is specified in the ISO standard.



### 10.4.3 Adulteration

Cinnamon is frequently adulterated with a rougher, thicker and less aromatic bark from cassia and *C. tamala*. Bark oil is usually adulterated with leaf oil. Artificial cinnamon was prepared by Schmal in 1940, mixing 3.4 % of a mixture of 96 % cinnamaldehyde and 4 % eugenol with a carrier such as powdered hazelnut or almond shells and colouring the mixture with yellow brown dye (Pruthi 1998). Bark oil is often distilled from a mixture of bark and leaves. Bark powder is adulterated with powdered beechnut husks, aromatized with cinnamaldehyde. It may often also be adulterated with sugar, ground walnut shells, galangal rhizome, etc. The addition of cassia oil to cinnamon bark oil represents another form of adulteration. The oil sometimes contains resin, petroleum or oil of cloves.

The cinnamon can be detected by thin-layer chromatography (TLC) by European/American distillery criteria where the bark oil eugenol content should not exceed 14 %. The cinnamaldehyde should fall between 60 and 75 %. Ceylon cinnamon, if tested with one or two drops of tincture of iodine to a fluid ounce of a decoction of the powder, is but little affected, while with cassia a deep blue colour is produced. The cheaper kinds of cassia can be distinguished by the greater quality of mucilage, which can be extracted by cold water.

## 10.5 Main uses in the food industry

A large proportion of the total usage of cinnamon is for culinary purposes. It can be bought as whole sticks, used to flavour rice and meat dishes and hot apple cider, but recipes can also call for ground cinnamon. Cinnamon, being more delicate than cassia, is mostly used in dessert dishes, such as apple pie and cinnamon buns. It is used to spice mulled wines, creams and syrups in Europe and is a common ingredient in chai tea. In the USA, cinnamon and sugar are often used to flavour cereals, bread-based dishes and fruits, especially apples. Cinnamon-sugar mixtures are sold separately for such purposes. In Mexico, the largest importer of Sri Lankan cinnamon, it is drunk with coffee and chocolate or brewed as a tea. Although in Western cuisine, it is mainly used in sweet dishes, its primary use is within savoury dishes in the East. In Indian cuisine, it is used in curries and *pilau*s, and it is an important ingredient in *garam masala*. Cinnamon sticks are used in beverages, boiled beef, pickles, chutneys and ketchup. It is common in many Middle Eastern, North African dishes in flavouring lamb tagines, chicken dishes or stuffed aubergines. Cinnamon powder has long been an important spice in Persian cuisine, used in a variety of thick soups, drinks and sweets. It is often mixed with rosewater or other spices to make a cinnamon-based curry powder for stews or just sprinkled on sweet treats. It is often overlooked that cinnamon does more than add flavour to cakes, cookies, ice creams and other high-fat desserts. The bark oil is antifungal and antibacterial, slowing meat spoilage, so its use as a spice for meat dishes in warmer climates is sensible. It has potential more generally as a natural food preservative (Shan *et al.*, 2007).

The stronger flavour of cassia is preferred in chocolate manufacture by Germans and Italians and is used less frequently in the kitchen. Cassia oil is used mainly for flavouring cola-type drinks, with smaller amounts used in bakery products, sauces,

confectionery and liqueurs. Dried unripe fruit, or Chinese cassia buds, have the odour and taste of the bark, and are rather like small cloves in appearance. They are employed in confectionery and in making pot-pourri.

### **10.5.1 Cinnamon as a flavourant**

In India, South East Asia, the USA and European countries, cinnamon is used for flavouring foods. It is commonly used for de-odouring/masking in the food industry in the USA. Bark oil is employed mainly in the flavouring industry where it is used in meat and fast food seasonings, sauces and pickles, baked goods, confectionery, cola-type drinks, tobacco flavours and in dental and pharmaceutical preparations. Perfumery applications are far less numerous because the oil has some skin-sensitizing properties, limiting its use.

Cinnamon oleoresin is used in flavouring, cake and similar mixes, pickles, prepared meats, convenience foods and related products. Leaf oil is used as a flavouring agent for seasonings and savoury snacks to a small extent. The iso-eugenol, derived from ex-eugenol cinnamon leaf oil, is another flavouring agent in confectionery and liqueurs. Cinnamon buds are as good for flavouring and spicing as the bark itself (Pruthi, 1997).

It is reported that when combined with sweet food, the sweet sensation of the food is enhanced because of the synergetic effect between the sweet taste of sugar and the sweet aroma of cinnamon.

### **10.5.2 Cinnamon as a colourant**

Natural colourants are generally derived from plants, fruits or vegetables which possess sufficiently deep colour to stain any mixture. Various colours are employed in natural soaps, including shredded carrots to make yellow – orange and cinnamon for making a tan or brown colour. Cinnamon is widely used for colouring in baking and in the soap industry. For certain purposes, it is desirable to give high cinnamon colour for baked product and relatively mild flavouring. In such instances, the consumer would look for red-coloured cinnamon (cassia) with moderate oil content, or even a cinnamon blend. The blending of different varieties of cinnamon or grades to form a tailor-made cinnamon for various types of baked items has become a standard practice.

## **10.6 Functional properties and toxicology**

### **10.6.1 Medicinal uses**

Cinnamon has been used medicinally for thousands of years to fight toothache, clear up urinary tract infections and soothe stomach irritation. It has a broad range of historical uses in different cultures, including the treatment of diarrhoea, arthritis and various menstrual disorders. It is employed as an adjuvant in stomachic and carminative medicines and is also administered in cases of anorexia, inflammation, vomiting and tubercular ulcers (Warrier *et al.*, 1994). It is very effective for rheumatism and inflammation (Wagner *et al.*, 1986), and its application in small quantities

to the forehead gives relief in neuralgic headaches. It is used also externally as a rubefacient and is employed to counteract the stings of poisonous insects. The large number of medicinal applications for cinnamon indicates the widespread appreciation of folk herbalists for its healing properties.

In Ayurveda, cinnamon is considered a medicine as well as a spice. Some of the Ayurvedic medicines prepared from cinnamon bark include *Astanga lavana churna*, *caturjata churna*, *sitopaladi churna*, *sudarsana churna*, *talisadya churna*, *chandraprabha vati*, *khadirarishta*, *pippayadyasava*, *lavanbhaskara churna* (Dey, 1980) and *vyaghri haritaki* (Asolkar *et al.*, 1994). In the Indian System of Ayurvedic medicine, it is used against a wide spectrum of diseases like bronchitis, colds, congestion, diarrhoea, dysentery, oedema, flu, gas, metabolic and heart strengthening, hiccups, indigestion, liver problems, menorrhagia, melancholy, muscle tension, nausea and vomiting. It assists uterine contractions during labour and menstrual pain from low metabolic function. For external applications, it is used against headaches and pain.

In Unani medicine, it is used as a cephalic tonic and cardiac stimulant and for the treatment of coughs. Flowers are used in the European tradition as a blood purifier. Cinnamon may find its way to a diabetic's daily diet. It contains a chemical called methoxy hydroxy chalcone polymer, which can reduce the blood glucose level. Cinnamon is used for religious purposes also. It is believed, by some, that burning cinnamon incense will promote high spirituality and aid in healing. Some people believe that it can stimulate the passions of the male (Pittman, 2000). It is now becoming more widely used as a herbal remedy in Europe and the USA. The generally recommended medicinal dosage for cinnamon powder is 0.5–1 g as tea, 0.5–1 ml as fluid extract in 1:1 in 70 % alcohol and 0.05–0.2 ml bark oil (Blumenthal *et al.*, 1998).

### 10.6.2 Antimicrobial research

Cinnamon oil and extracts possess various microbial properties against several microorganisms. *In vitro* studies showed that the bark extracts were active against 27 strains of *Vibrio cholerae* and also against *Shigella* (Islam *et al.*, 1990). Cinnamon bark oil has fungicidal and insecticidal properties and also prevents aflatoxin production. Cinnamon bark oil inhibits gram-positive bacteria such as *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*; the gram-negative bacteria *Alcaligenes faecalis*, *Enterobacter cloacae*, *Escherichia coli* and *Pseudomonas aeruginosa*; the fungi *Aspergillus niger* and *Rhizopus oligosporous*; and the yeast *Candida albicans* (Chao *et al.*, 2000). Bara and Vanetti (1995) reported the bacteriostatic effect of ground cinnamon spice against *Yersinia enterocolitica*. Ground cinnamon also inhibits the growth of *Aspergillus parasiticus* and aflatoxin production. Leaf oil of cinnamon is antimicrobial and is sensitive to yeast and fungi such as dermatophytes and moulds (Mangiarotti *et al.*, 1990). The oil inhibits the growth and production of *Alternaria alternata* and can be used as a preservative in processed tomato products (Hasan, 1995). The essential oil obtained from cinnamon seed shows antimicrobial activities (Chaurasia and Jain, 1978). The use of seed oil in industrial application of seed oil is to be explored further for commercialization.

Cinnamaldehyde is the major constituent of cinnamon and cassia barks, responsible for antibacterial and antifungal activities. It has shown strong antifungal activities against the fungus *Malassezia furfur* and also strong nematocidal activity against the root-knot nematode, *Meloidogyne javanica*. Cinnamaldehyde, cinnamic acid, cinnamyl alcohol and eugenol possesses antibacterial, astringent, carminative and stomachic properties (Lee and Ahn, 1998). Cinnamon incorporating creams are also found to be beneficial in fighting 'acne' producing bacteria, *Propionibacterium acnes*. A commercial product, Sepicontrol A5, consisting of cinnamon bark powder and a lipo-amino acid (lypoglycine) in a cream formulation is reported to be very effective in improving the state of oily acne-prone skin situations. In short, cinnamon shows selective action against a wide range of human pathogenic bacteria, sparing some of the beneficial ones. The bactericidal and bacteriostatic effects of cinnamon have been studied widely and it is broad spectrum antifungal substance.

### 10.6.3 Diabetes and other conditions

Various studies have indicated a potential role for cinnamon and cassia in the prevention and management of diabetes. Not all investigations have produced results that have been positive or statistically significant, though, and the generally small number of studies in this area limits the conclusions that can be drawn at this stage. Studies undertaken by the United States Department of Agriculture also suggested that components with biological activity relevant for insulin control may be present in the water soluble, rather than the fat soluble fractions.

Gruenwald *et al.* (2010) provide a review of the health benefits of cinnamon and Dugoua *et al.* (2007) provide a review of the efficacy and safety of both cinnamon and cassia.

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# 11

## Cloves

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**Abstract:** The clove, *Syzygium aromaticum* (L.) Merrill et. Perry, belongs to the family Myrtaceae. The clove is harvested primarily for the unopened flower buds borne in clusters, which are dried to produce whole clove buds, i.e. the familiar spice of commerce. The other products of clove are ground clove, volatile oils produced from clove buds, stem or leaf and oleoresins. Clove contains volatile oil; eugenol (up to 95 %), acetyl eugenol,  $\beta$ -caryophyllene, methyl salicylate, pinene, vanillin. The majority of cloves are used by kretek cigarette manufacturers in Indonesia and only about 10 % for other purposes such as folk medicine, food flavouring, food preservatives, fragrance and pharmaceuticals. Since some studies have indicated that clove and clove oil can be used as an antibacterial, antifungal and antiseptic agent, the use of clove and clove oil is expected to increase in line with the 'back to nature' trend which encourage a preference for natural products.

**Key words:** clove, eugenol, antibacterial, antifungal, preservative, antiseptic, medicinal.

### 11.1 Introduction

The clove, *Syzygium aromaticum* (L.) Merrill et. Perry, belongs to the family Myrtaceae. The species is indigenous to certain volcanic islands of the North Moluccas (formerly known as the spice islands), in the eastern part of Indonesia where cultivated and wild forms are found. The islands include Ternate, Tidore, Motir, Makian, Bacan and Halmahera. A considerable wild population of cloves has also been found in Ambon and the western part of Papua. The common synonyms are *Caryophyllus aromatica* L., *Eugenia aromatica* Kuntze, *E. caryophyllata* Thunb. and *E. caryophyllus* (Sprengel) Bullock & Harrison.

The tree is of medium size, is evergreen and reaches up to 20 m in height. Its canopy shape varies from cylindrical to pyramidal, depending on the variety. The tree can live for up to 100 years, and there are individual records of trees over 350 years old in Ternate. The trunk diameter can reach 50 cm in mature plants. The leaves are opposite, single, very aromatic, pointed oval, oval-lanceolate or lanceolate in shape, depending on the variety. The newly formed leaves, which are pink turn to dark glossy green when mature. The flowers are arranged in inflorescences, grow at terminal branches and range in number from 15–50, depending on variety and cultural practices. The flower is a hermaphrodite with a fleshy hypanthium that



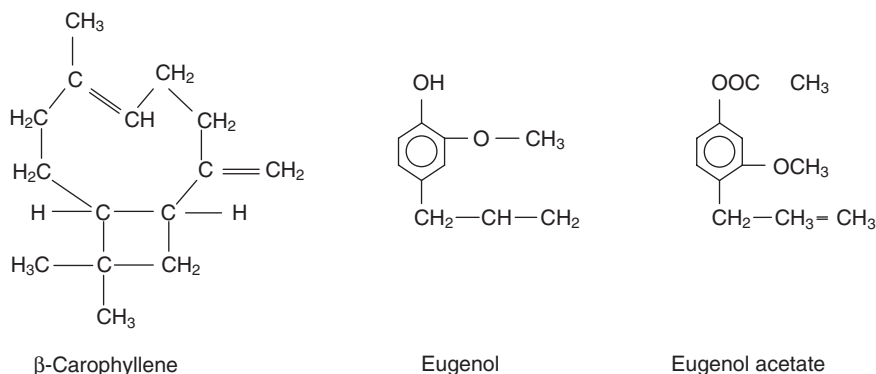


**Fig. 11.1** Chemical structures of eugenol, eugenol acetate and  $\beta$ -carophyllene (Guenther, 1950).

is surmounted by the sepals. The colour of unopened buds at the young stage is usually green, turning to flushed pink when they reach their full size, at which time they are ready for harvest. At that stage, the stamens are still inside and covered by the petals which form the head of the dried cloves. The flowers have four petals, yellow roses and several pistils. The fruits (small and elongated dark red berries) sprout from the flowers.

The clove is harvested primarily for the unopened flower buds borne in clusters, which are dried to produce whole clove buds, i.e. the familiar spice of commerce. The other products of clove are ground clove and volatile oils produced from the clove buds, stem or leaf and oleoresins. Clove contains volatile oil; eugenol (up to 95 %), acetyl eugenol,  $\beta$ -caryophyllene, methyl salicylate, pinene, vanillin. The chemical structures of eugenol, eugenol acetate and  $\beta$ -caryophyllene are illustrated in Fig. 11.1.

The majority of cloves produced are used for kretek (a type of clove cigarette) manufacture in Indonesia and only about 10 % for other purposes such as folk medicine, food flavouring, food preservation, fragrance and pharmaceuticals. The use of clove and clove oil is expected to increase in line with the 'back to nature' trend which encourages a preference for natural products.

## 11.2 Production and post-harvest processing

Clove is native to Indonesia and is grown in almost all provinces. From Indonesia, clove was brought to Mauritius in the eighteenth century by the French administrator (Poivre), then to Zanzibar by an Arab trader. Zanzibar and Pemba became clove producers (Purseglove *et al.*, 1981). Clove was then later spread to Madagascar, Sri Lanka, India, Malaysia and recently to parts of the Mediterranean region such as Turkey. According to the WHO, Indonesia leads the world in clove area and production. As mentioned above, the main use of clove in Indonesia is for kretek cigarette manufacture. Today, the total area under clove production in Indonesia is still increasing despite an ongoing campaign against cigarette smoking. The total clove area in Indonesia in 2008 was 456 471 ha producing a total of

70535 t of dried cloves. The total area reached 470045 ha in 2010 and clove bud production reached 110807 t (Directorate General of Tree Crop Estate, 2011). The other world clove producers were Madagascar and Zanzibar (Tanzania) with total production of approximately 20000–27000 t/year. Comoros, Sri Lanka, Malaysia, China, Grenada, Kenya and Togo supplied around 5000–7000 t/year (Indonesian Agency for Agricultural Research and Development, 2005). Tropical countries which are also production centres for the clove are Brazil and the Caribbean island of Jamaica. Global demand for cloves is reported to have increased to 5500 t, a trend which is expected to continue as the world population increases.

### 11.2.1 Dried clove buds

Dried clove buds are harvested at the time when the buds have reached their full size before the corolla open, and the hypanthium have turned reddish. The harvested buds are separated from the stems by hand or thresher machine. A thresher machine with 1 HP electric power can separate 76 kg fresh clove buds per hour. After separation, the buds are dried by the sun or an artificial dryer. The colour and oil content of sun and artificially dried clove buds are not significantly different (Hidayat and Nurdjannah, 1992). Harvesting clove buds should be undertaken at the right stage. Early picking, fallen and over-ripe buds will produce buds of a lower quality (Table 11.1). The essential oil content and the ratio between eugenol and eugenol acetate influences the aroma. Higher content of essential oil is preferred. Higher content of eugenol will produce a harsher aroma, on the other hand, higher content of eugenol acetate produces a smoother aroma. Indonesia has three standard grades for clove quality, their characteristics are shown in Table 11.2. According to Alma *et al.* (2007), clove from Turkey contains eugenol 87 %, eugenyl acetate 8 % and caryophyllene 3.56 %.

**Table 11.1** The characteristics of clove bud at several stages of maturity

| Characteristic                           | Bud maturity stage |                 |                     |               |
|------------------------------------------|--------------------|-----------------|---------------------|---------------|
|                                          | Fallen flower      | Undeveloped bud | Fully developed bud | Over-ripe bud |
| <b>Clove bud</b>                         |                    |                 |                     |               |
| Water content (% v/b)                    | 12.3               | 5.0             | 12.8                | 6.5           |
| Oil content (% v/b)                      | 13.9               | 14.9            | 16.4                | 16.1          |
| Ash content (%)                          | 4.7                | 3.8             | 4.7                 | 6.11          |
| Fibre content (%)                        | 11.8               | 10.8            | 8.5                 | 13.3          |
| Si content (%)                           | 0.15               | 0.11            | 0.11                | 0.10          |
| <b>Clove bud oil</b>                     |                    |                 |                     |               |
| Total eugenol content % <sup>1</sup>     | 91.0               | 90.0            | 94.0                | 93.0          |
| Eugenol content (%) <sup>2</sup>         | 54.2               | 55.6            | 68.5                | 72.2          |
| Eugenol acetate content (%) <sup>2</sup> | 34.4               | 36.5            | 22.0                | 9.4           |

<sup>1</sup> Cassia method.

<sup>2</sup> By gas chromatography.

Source: Nurdjannah *et al.* (1977).

**Table 11.2** Indonesian Clove Quality Standard (SNI 01-3392-1998)

| Standard parameters                        | Grade quality  |                |                | Analysis method                   |
|--------------------------------------------|----------------|----------------|----------------|-----------------------------------|
|                                            | I              | II             | III            |                                   |
| Size                                       | Even           | Even           | Uneven         | Organoleptic                      |
| Colour                                     | Brownish black | Brown          | Brown          | Organoleptic                      |
| Aroma                                      | Specific clove | Specific clove | Specific clove | Organoleptic                      |
| Foreign matter, <sup>1</sup> % (w/w), max. | 0.5            | 1.0            | 1.0            | SP-SMP-32-1975 (ISO/R927-1969(E)) |
| Clove stem, % (w/w), max.                  | 1.0            | 3.0            | 5.0            | SP-SMP-32-1975 (ISO/R927-1969(E)) |
| Inferior clove, <sup>2</sup> % (w/w), max. | 2.0            | 2.0            | 5.0            | SP-SMP-32-1975 (ISO/R927-1969(E)) |
| Moulded clove <sup>3</sup>                 | negative       | negative       | negative       | SP-SMP-32-1975 (ISO/R927-1969(E)) |
| Moisture content, % (w/w), max.            | 14.0           | 14.0           | 14.0           | SP-SMP-32-1975 (ISO/R927-1969(E)) |
| Essential oil content, % (v/w)             | 20.0           | 18.0           | 16.0           | SP-SMP-37-1975                    |

<sup>1</sup> Foreign matter = all material not from clove bud.

<sup>2</sup> Inferior clove = shrunk, broken and fertilised flowers.

<sup>3</sup> Moulded clove = clove which is already moulded.

Source: BSN, (1998).

### 11.2.2 Ground clove

Ground clove is produced by milling and/or grinding of dried clove buds. The process is usually conducted at room temperature (25–35 °C) to prevent the loss of much of the valuable volatile components during processing. Various techniques such as pre-chilling, water cooling or refrigeration of the grinding chambers have been developed to minimize the heat formed during processing. Ground clove is produced with several degree of fineness, depending on the nature of the spice, the ultimate application and consumer preferences. For extraction and distillation, coarsely ground clove is accepted, while for direct used in food seasoning, a finer product is required. To produce a very fine powder, a two-step procedure is usually conducted, the buds are reduced first to a very coarse powder by passage through a slow-speed breaker or cutter mill, before being ground to the desired fineness. US consumers prefer a finer powder compared to consumers in the UK. Another method of producing ground clove is by cryogenic grinding, a method of powdering herbs at sub-zero temperature ranging from 0 to –70 °C. By this method, the loss of volatile components and heat-sensitive constituents in herbs can be prevented or reduced. Cryogenic grinding at temperatures below 50 °C resulted in 29.5 % more volatile components compared to ambient grinding (Sing and Gswami, 2000).

### 11.2.3 Clove oil

Clove oil is a mixture of different compounds, with the three main active ingredients being eugenol, eugenyl acetate and caryophyllene. Jirovetz *et al.* (2006) found 23

constituents, with eugenol (76.8 %), followed by  $\beta$ -caryophyllene (17.4 %),  $\alpha$ -humulene (2.1 %), and eugenyl acetate (1.2 %) as the main components. Santin *et al.* (2010) reported eugenol (89.6 %),  $\beta$ -caryophyllene (8.6 %) and eugenol acetate (1.7 %) while Alma *et al.* (2007) reported eugenol (87 %), eugenyl acetate (8 %) and caryophyllene (3.56 %). Variation in components and composition depends on variety, agro-ecological condition, pre-treatments, processing and methods of extraction.

Clove oil is colourless or pale yellow with the characteristic aroma and taste of cloves. According to the FAO, Indonesia produced 70 % of world clove oil in 2005, followed by Madagascar with around 12 %. Tanzania and the Comoros followed with shares of 10 % and 2.4 %, respectively. Indonesia also contributed 75 % of the world's clove oil derivatives, such as eugenol, iso-eugenol, methyl eugenol, etc. (Tekriwal, 2009). Indonesia exports close to 2500 MT of the world demand of 3500 MT/year of crude clove oil and its derivatives. The global market for clove oil is expanding in line with its multiple uses and an increase in demand for nutraceuticals, foods, cosmetics, and body care products derived from natural products. The market has an annual growth rate of approximately 5 %.

Clove oil can be obtained from distillation of buds, leaf or stem, each resulting in an oil having different characteristics of oil (Table 11.3). The yield and quality of the oil are influenced by origin, variety, post-harvest processing, pre-treatment before distillation, distillation method and post-distillation treatment. Clove buds and stem are comminuted before distillation to break the oil cells and widen the surface so that the oil can be released more easily from the cells. Clove leaf does not need pre-treatment as it is already thin. The materials are distilled using water and steam or steam distillation for between 8 and 24 hours. The highest yield derived from high-quality clove bud is 20 %. In the UK, the finest oil containing 85–89 % eugenol, is obtained by water distillation (Purseglove *et al.*, 1981).

Clove bud oil, a colourless or yellow liquid, is obtained from distillation of buds. Clove buds contain 15 to 20 % of oil by weight. The main oil constituents are eugenol (70–95 %), eugenol acetate (up to 20 %) and  $\beta$ -caryophyllene (12–17 %). According to Gildemeister and Hoffimer, cited by Guenther (1950), the distillation of whole

**Table 11.3** Characteristics of clove oil derived from buds, stem and leaf of Indonesian origin

| Compound               | Bud (%) | Range     | Stem (%) | Range     | Leaf (%) | Range     |
|------------------------|---------|-----------|----------|-----------|----------|-----------|
| Essential oil          | 18      | 15–20     | 6        | 5–10      | 3–4      | 1–4       |
| Specific gravity       | 10425   | 1004–1057 | 10495    | 1048–1056 | 1030     | 1030–1060 |
| Refractive index       | 15296   | 1528–1538 | 15320    | 1534–1538 | 15295    | 1520–1540 |
| Optical rotation       | –1.1    | 0–(–1.58) | –1.05    | 0–(–1.50) | –1.58    | –1.58     |
| Total eugenol content  | 80      | 85–93     | 85       | 89–85     | 82       | 78–93     |
| Solubility in ethanol  | 1:2     | 1:2       | 1:2      | 1:2       | 1:2      | 1:2       |
| True eugenol           | 61.62   |           | 80       |           | 77.1     |           |
| Eugenol acetate        | 18.72   |           | 2.1      |           | trace    |           |
| $\beta$ -Caryophyllene | 15.27   |           | 12.70    |           | 17.02    |           |

Source: Nurdjannah *et al.*, (2001).

clove bud produces clove oil with high eugenol content and specific gravity above 1.06. Comminuted clove buds produce clove oil with a slight lower eugenol content and specific gravity lower than 1.06, because evaporation of the oil occurred during comminution. To prevent evaporation, distillation of comminuted material should be done immediately. Belcher (1965) stated that the eugenol content of the oil is dependent on the time taken to distill the charge. Rapid distillation produces oil with eugenol content far higher than that normally found in commercial practice. Supercritical fluid extraction (SFE) using CO<sub>2</sub> as solvent can be used to extract clove bud oil at sub-critical condition using extraction conditions of 50–80 bar pressure and temperature 0–10°C. This method is used as an alternative to steam distillation. The oil product has better characteristics, i.e. no solvent residue, no off-notes, more top notes, more back notes, better solubility and greater concentration of aromatic components (Moyler, 1977). The technique results in more optimum extraction yield, with oil yield, eugenol and eugenol acetate contents higher than those obtained by steam distillation.

Clove stem oil, pale yellow, obtained after distillation is used in the mass-market products. It is less expensive than the clove bud oil. Clove stem oil comprises 5–7 % of oil by weight, the major component being eugenol (90–95 %), while eugenyl acetate and  $\beta$ -caryophyllene and others are present in smaller amounts. Clove stem oil of Indonesian origin, obtained from water and steam distillation, yields 5–6 % oil with eugenol content 90–98 %. Variation in yield and eugenol content depend on the length of distillation time (Nurdjannah *et al.*, 1990).

Clove leaf oil dark brown liquid obtained after distillation of the dry leaves, is the main traded clove oil, because it is less expensive than the formers. It is used as a main source for the production of eugenol, which is used as an analgesic and as a raw material to produce synthetic vanillin. Clove leaf oil contains 3–4 % of oil by weight, the main component being eugenol (80–88 %) with low eugenyl acetate and high content of caryophyllene (Nurdjannah *et al.*, 1993; Hariono, 2009). Distillation of leaf in a 100 l still capacity for 8 hours yielded 3.5 % oil with total eugenol content 76.8 % (water content 7–12 %) (Nurdjannah *et al.*, 1993). The main producers of clove leaf oil are Indonesia and Madagascar. Indonesian clove leaf oil capacity is about 2500 t/year, whereas the potential production based on clove plantation is 11500 t/year (Hariono, 2009). The centres for production of clove leaf and stem oil in Indonesia are Java, Sumatra, Bali and Sulawesi region.

#### 11.2.4 Oleoresin

Clove oleoresin is extracted from the stems or the buds. Its flavour and odour are similar to the spice, for which it is sometimes substituted. Clove oleoresin prepared by solvent extraction of clove bud yielded about 18–22 % oleoresin (90–92 % volatile components) using benzene and 22–31 % using alcohol (Weiss, 1997). It can be produced from ground clove which is extracted by suitable solvent(s) then evaporated or distilled to obtain oleoresin. According to Somaatmadja (1981) ethanol is a very safe solvent because it is non-toxic. Oleoresin is an extremely concentrated product, containing all the flavouring ingredients soluble in the particular solvent used, so that it is much closer to the original clove odour and flavour (Heat, 1973). Oleoresin can also be produced by supercritical CO<sub>2</sub> extraction,

which is conducted at 200–300 bar pressure at 50–80°C. *In situ* fractionation is possible at 80–100 bar and temperature 0–50°C. This could extract all the soluble components of oleoresin in a similar way to organic solvent extraction. The product is free of solvent residue(s), and can be further fractionated to produce oil. Solvent(s) extraction is, however, more cost-effective than supercritical extraction (Moyler, 1977).

### 11.2.5 Eugenol and iso-eugenol

Eugenol (4-allyl-2-ethoxyphenol) is the major compound of clove oil, comprising 83–95 % of the oil. It is slightly water-soluble and easily soluble in organic solvent, colourless or yellowish in colour. Eugenol can be isolated from clove bud, stem and leaf oil. Isolation from clove leaf oil is cost-effective and can be done in small distilleries. The common method to isolate eugenol is by adding NaOH at concentration 4–7 %, the higher yield (74.5 %) with high purity (98 %) is obtained at 4 % NaOH using hexan as washing solution (Nurdjannah *et al.*, 1997).

Iso-eugenol can be produced by isomerization of eugenol derived from clove leaf oil. The chemical structure of iso-eugenol ( $C_{10}H_{12}O_2$ ) is 1-hydroxy-2-methoxy-4-propenylbenzene, 4-hydroxy-3-methoxy-1-propenylbenzene, 2-methoxy-4-propenylphenol, 4-propenylguaiacol. Iso-eugenol was known as the precursor used in commercial production of synthetic vanillin ( $C_8H_8O_3$ ): 3-methoxy-4-hydroxybenzaldehyde. Iso-eugenol is mainly used for cigarettes, non-alcoholic beverages, ice cream and chewing gum. In Indonesia, iso-eugenol is mainly produced by small distilleries around Java, Sumatera, Sulawesi and Bali (Hariono, 2009).

### 11.2.6 Storage

Storage is one of the important factors in maintaining a good quality of clove. Dried whole clove bud is usually packed in gunny bags and stored in a clean, dry room with good ventilation. This manner of storing should not cause any significant changes except loss of sheen. Storage usually causes loss of oil by evaporation, the rate depending on the physical condition of the spice, mainly the moisture content of the products, temperature and relative humidity during storage (Purseglove *et al.*, 1981). The essential oil and eugenol acetate content of whole clove decreased slightly after storing for six months, while eugenol content increased (Table 11.4). Loss of volatile oil during storage from whole clove is relatively slow compared to ground clove. Ground clove is more sensitive to high ambient temperature and moisture content which can change its stability and flavour profile. Moisture content and temperature storage of ground clove should fall within 8–10 %, and 10–15°C, respectively, with relative humidity 55–65 %. Poor storage conditions could cause more loss of volatile oil, mould growth and development of musty flavour and odour (Reineccius, 1994).

Storage can also change the composition of CO<sub>2</sub>-extracted clove bud. Reduction in caryophyllene content is 11 % at  $0 \pm 1^\circ\text{C}$ , while at ambient temperature reduction of CO<sub>2</sub>-extracted oil is higher (up to 18 %) compared to commercially distilled oil (only up to 13 %) (Gopalakhrisnan, 1994). Moreover, eugenol content increased after storage, whereas eugenol acetate remained fairly constant (Table 11.5).

**Table 11.4** The changes of quality of dried clove bud during storage

| Source    | Storage duration (months) | Oil content of clove bud (%) | Clove oil*          |                             |
|-----------|---------------------------|------------------------------|---------------------|-----------------------------|
|           |                           |                              | Eugenol content (%) | Eugenol acetate content (%) |
| Samalayu  | 0                         | 17.22                        | 78.30               | 7.97                        |
|           | 6                         | 16.48                        | 80.25               | 6.8                         |
| Cigombong | 0                         | 17.04                        | 77.56               | 11.20                       |
|           | 6                         | 14.10                        | 78.10               | 8.78                        |

\* By gas chromatography.

Source: RISC M (1988).

**Table 11.5** Changes in composition of CO<sub>2</sub> extracted clove bud oil during storage

| Component          | Day of storage                   |      |      |      |      |                              |      |      |
|--------------------|----------------------------------|------|------|------|------|------------------------------|------|------|
|                    | Carbon dioxide extracted oil (%) |      |      |      |      | Commercial distilled oil (%) |      |      |
|                    | 0                                | 45   | 90   | 45   | 90   | 0                            | 45   | 90   |
| $\alpha$ -Cububene | 1.5                              | 1.1  | 1.3  | 1.2  | 1.2  | 0.7                          | 0.7  | 0.6  |
| $\alpha$ -Copaene  | 1.7                              | 1.4  | 1.5  | 1.4  | 1.4  | 1.0                          | 1.0  | 1.0  |
| Caryophyllene      | 16.6                             | 15.0 | 14.7 | 15.1 | 13.6 | 10.8                         | 10.5 | 9.4  |
| Eugenol            | 62.2                             | 61.4 | 64.8 | 61.3 | 65.7 | 71.0                         | 70.1 | 72.6 |
| Isoeugenol         | 0.9                              | 0.9  | 0.8  | 0.8  | 0.6  | 0.6                          | 0.6  | 0.5  |
| Nerolidol          | 1.2                              | 1.2  | 1.2  | 1.2  | 1.2  | 0.4                          | 1.2  | 1.1  |
| Eugenol acetate    | 14.3                             | 13.8 | 13.6 | 14.6 | 13.6 | 12.1                         | 13.4 | 12.3 |
| Farnesol           | 0.1                              | 0.3  | 0.3  | 0.4  | 0.3  | 0.2                          | 0.3  | 0.4  |
| MVMC               | 0.2                              | 0.1  | 0.3  | 0.1  | 0.2  | 0.1                          | –    | 0.1  |
| LVMC               | 1.1                              | 2.7  | 1.4  | 3.6  | 2.2  | 2.2                          | 2.3  | 1.8  |

MVMC = more volatile minor components; LVMC = less volatile minor components.

Source: Gopalakrishnan (1994).

### 11.3 Main uses in food processing

The main use of clove in whole or ground form is for domestic culinary purposes and as a flavouring agent in the food industry. Whole cloves are rarely used in food processing as they are not a ready source of flavour. In some cases, whole clove is inserted into ham and baked apples, and it is used for pickles. Usually, only small amounts, perhaps as many as five whole cloves, are used for pickling sauce blend as for meat such as corned beef and stews.

In the food industry, cloves are often used in the form of ground, extracted essential oils or oleoresin in a small amount because of their intense flavour. The advantages of using ground cloves are that they retain a considerable degree of their original stability during storage and are better able to withstand high-temperature processing (e.g. baking) than many of the extracted processed products. Oleoresin is preferred over other clove products, because it contains both volatile essential oil



and non-volatile resinous material, which accounts for the flavour mimicking the original ground spice. Oleoresin also has low risk of bacterial contamination. Food products which use clove are mainly curry powder, sauces and baked foods. According to Farrell (1990), curry powder uses 2 % (mild) to 3 % (sweet) by weight of ground clove buds, meat sauces 0.37 % ground clove or 0.111 % clove oil, food seasonings such as Bologna seasoning A, B and C use 0.39 % ground clove, 0.07 % clove oil and 0.45 % clove oil, respectively. Chilli sauce uses 0.025 % oil, mustard 0.111 % and 0.222 % ground clove in Dijon and Dusseldorf, respectively. Tomato ketchup uses 0.139 % clove oil, whereas sausages (Sweet Italian) use 0.111 % ground clove (Farrell, 1990). The highest average maximum use level reported for cloves is 0.236 % in condiments and relishes, and 0.06 % clove stem oil and 0.078 % clove bud oleoresin in alcoholic beverages (Leung, 1980).

Clove oil is one of the most important essential oils used for flavouring all kinds of food products, such as sausage, baked goods, confectioneries, etc. Clove bud oil is used in perfumery and pharmaceuticals and in the food industry for meat products, sauces and pickles, confectionery and bakery products. Clove leaf oil is not suitable for food flavouring because of its harsher note, and does not reproduce the genuine clove flavour. It is mainly used for production of eugenol and caryophyllene (Weiss, 1997).

## 11.4 Functional properties of cloves

Currently, concerns over safety of food additives, method of preservation and food preservatives are increasing (Oiyee and Muroki, 2002). Much research has been conducted with a view to find safe food additives and preservatives. Natural antimicrobial agents derived from natural sources such as plant-derived oils have been recognized and used for centuries. Clove and clove bud oil can be used as an antibacterial, antifungal and antiseptic agent, this means that they can be used as a food preservative, hence improving shelf-life of foods (Sulieman *et al.*, 2007). Clove has been used as a preservative to disguise/cover up spoilage in meat, syrups, sauces and sweetmeats (Tajkarimi *et al.*, 2010). Despite its potential use as a food preservative, clove still remains primarily a food condiment.

### 11.4.1 Antibacterial properties

Many essential oils or their fractions of plant origin showed antimicrobial activity, and some are classified as generally recognized as safe (GRAS) and therefore could be used to prevent post-harvest growth of native and contaminant bacteria (Moreira *et al.*, 2004). Nychas (1995) cited by Moreira (2004) reported that essential oils from clove possess antimicrobial activity against both bacteria and moulds.

Clove oil possesses inhibitory activity against food pathogens, *Campylobacter jejuni* (Palmer *et al.*, 1998), *Salmonella enteritidis* (Palmer *et al.*, 1998, 2001; Hoque *et al.*, 2007), *Salmonella typhimurium* (Suleiman *et al.*, 2007), *Escherichia coli* (Palmer *et al.*, 1998; Moreira *et al.*, 2004; Hoque *et al.*, 2007; Sulieman *et al.*, 2007), *Staphylococcus aureus* (Palmer *et al.*, 1998; Hoque *et al.*, 2007; Sulieman *et al.*, 2007), *Listeria monocytogenes* (Palmer *et al.*, 1998; Singh *et al.*, 2003; Hoque *et al.*, 2007; Du *et al.*,

2009), *Vibrio parahaemolyticus* (Hoque *et al.*, 2007) and *Bacillus cereus* (Hoque *et al.*, 2007), and *Bacillus subtili* (Blank *et al.*, 1987; Sulieman *et al.*, 2007), together with four food spoilage bacteria: *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Alcaligenes faecalis* and *Aeromonas hydrophila* (Hoque *et al.*, 2007). Palmer *et al.* (1998) found that oil of clove is amongst the most inhibitory essential oils, having a bacteriostatic concentration of 0.075 % or less against five important foodborne pathogens (*C. jejuni*, *S. enteritidis*, *E. coli*, *S. aureus* and *L. monocytogenes*). Hoque *et al.* (2007) found that the ethanol extracts aqueous extracts and essential oils of cloves (*Syzygium aomaticum*), showed antimicrobial activity against 21 food pathogens: *L. monocytogenes* (five strains), *S. aureus* (four strains), *E. coli* O157:H7 (six strains), *S. enteritidis* (four strains), *Vibrio parahaemolyticus* and *B. cereus*—and five food spoilage bacteria. *P. aeruginosa*, *P. putida*, *Alcaligenes faecalis* and *Aeromonas hydrophila* (two strains). The essential oil of clove showed antimicrobial activity even after treatment at 100°C for 30 minute. It appears that the high temperature does not affect the activity of these essential oils.

Concerns over *L. monocytogenes* contamination in the food industry have received a lot of attention, especially for food preserved at low temperature (refrigerator), because this bacteria remains able to multiply at low temperature (Junttila *et al.*, 1988). Singh *et al.* (2003) found that clove was highly effective against *L. monocytogenes* in peptone water (1 g/l) and reduced the bacterial population below detection limits at concentration of 1 ml/l. The essential oil of cloves (5 %) reduced *L. monocytogenes* cells to an undetectable level in ground chicken meat within one day of exposure, therefore, it can be used to control *L. monocytogenes* in ground chicken meat (Hoque *et al.*, 2007). Moreover, clove oil reduced the total *L. monocytogenes* population at 1 ml/l in hotdogs with different level of fat content (zero-fat, low-fat and full-fat). Increasing the concentration to 10 ml/l had no significant effect on the full-fat hotdogs, indicating that the antimicrobial action was highly dependent on the fat content (Singh *et al.*, 2003). Menon and Garg (2001) reported that clove oil at 1 % concentration could inhibit the growth of *L. monocytogenes* at 30°C or 7°C. A most promising approach would be to combine essential oil with other preservation methods such as low-temperature, low-O<sub>2</sub>, high-pressure techniques to develop a synergistic alternative for preventing food contamination (Singh *et al.*, 2003). Clove bud oil is also effective against many foodborne pathogens such as *E. coli*, *S. aureus*, *B. subtili* and *S. typhimurium*, so that it can be used to prevent foodborne diseases (Sulieman *et al.*, 2007).

Microbial spoilage limits the shelf-life of salt-cured fish, mainly due to red halophilic bacteria. Treatment with clove oil at 0.02 and 0.5 % concentration caused a steep decrease in the counts of red halophiles and slime-producing halophilic bacteria and complete elimination of *Halomonas* spp. Exposure to brine, even at a clove oil concentration of 0.02 %, overnight resulted in complete elimination of red halophiles and slime-producing halophilic bacteria. It is clear that clove oil can be used to control the growth of halophiles in cured fish (Prasad and Seenayya, 2000).

#### 11.4.2 Antifungi properties

Many reports have indicated that clove oil exhibited antifungal properties. From 20 essential oils tested, clove oil was one of the oils displaying strongest inhibition

to the growth of *Penicillium* spp. The inhibition against *P. digitalis* and *P. italicum* mycelia occurred upon direct contact with the oil or its vapour. The strong antifungal activity of clove oil is due to its high phenolic content which vaporizes easily (Plaza *et al.*, 2004). Aflatoxin is a toxic secondary metabolite produced by foodborne fungi *Aspergillus flavus* and *A. parasiticus*. Clove oil at 0.6 mg/ml showed inhibitory activity against aflatoxin production by *A. parasiticus* (Farag *et al.* 1989, cited by Kalemba and Kunicka, 2003). Clove oil exhibited some antifungal activity against all isolates of *Eurotium* spp., *Aspergillus* spp. and *Penicillium* spp. which often contaminate bakery products. These findings strengthen the possibility of using plant essential oils as an alternative to chemicals to preserve bakery products (Guynot *et al.*, 2005). Sulieman *et al.*, (2007) found that clove oil has a potent antimicrobial activity against *Candida albicans* (yeast), *A. niger* and *Rhizopus nigricans* (moulds), but complete inhibition (100 %) occurred against *A. niger*. These studies indicated that clove and clove bud oil can be used as a food preservative, hence improving shelf-life of foods. However, the application of this oil in the food industry will require safety and toxicity issues to be addressed.

Some experiments proved that phenol has stronger antifungal activity compared to other components in essential oil such as alcohol, aldehyde, ketone and ester (Faid *et al.*, 1996, cited by Plaza *et al.*, 2004). According to Chami *et al.* (2004, 2005), eugenol is the component in clove oil which has antifungal properties. This statement is based on their experiment which proved that Madagascar clove leaf oil (90 % eugenol content) has stronger antifungal activity compared to *Indian clove bud oil* (72.0 % eugenol content).

#### 11.4.3 Antioxidant properties

Oxidation of lipids is one of the basic processes causing rancidity in food products. The search for natural antioxidants has been intensive. Clove has been shown to possess strong antioxidant properties. Antioxidant content varies depending on the type of clove product. Ground clove contains 1.8 %, while in soluble fraction 1.4 % (petroleum ether-soluble fraction) and 1.7 % (alcohol-soluble fraction). Antioxidant activity of clove and clove oil are higher than the standard butylated hydroxytoluene (BHT). Gallic acid and eugenol (Kramer, 1985) and eugenyl acetate (Lee and Shibamoto, 2001a) have been identified as the major antioxidants. The antioxidant activity of eugenol and eugenyl acetate was found to be comparable to that of the natural antioxidant,  $\alpha$ -tocopherol (vitamin E) (Lee and Shibamoto, 2001b). *Iso*-eugenol, more rarely found in nature, exhibited higher antioxidative efficiency than eugenol during methyl oleate (Davcheva *et al.* 1992) and sunflower oil oxidation (Ivanov and Davcheva 1992). Eugenol and *iso*-eugenol also have an inhibiting effect on the peroxidation of lecithin induced by the  $\text{Fe}_{2+}$ - $\text{H}_2\text{O}_2$  system (Toda *et al.* 1994). In a study comparing the effectiveness of antioxidants having several different plant origins in meat lipids, the antioxidant activity of ground clove was found to be higher than that of ginger, oregano, sage and thyme. Ginger and thyme exerted the weakest effect (Shahidi *et al.*, 1995). The antioxidative activities of clove buds are due in part to the contributions of aroma chemicals such as eugenol and eugenyl acetate. The presence of various aroma chemicals may explain the improvement of food stability.

#### 11.4.4 Medicinal properties

Since very ancient times, cultures in South East Asia have been using cloves for the preparation of many herbal remedies. The tradition of using cloves in remedies goes back thousand of years. Clove oil especially has been used by traditional folk healers as well as by modern pharmacists and dentists in alleviating the symptoms associated with toothache and dental decay. Clove herbal tea prepared by boiling the dried clove buds in water has been used to cure nausea, aid digestion, cure stomach disorders and as pain relief (Rumphuis, 1741; Rosengarten, 1969). Some of these therapeutic properties have been associated with the role of eugenol. Clove oil has been used successfully for inflamed oral and pharyngeal mucus and for topical anaesthesia in dentistry. Clove balm containing the oil as the active ingredient has also been used for soothing pain caused by rheumatism (Nurdjannah *et al.*, 1997).

The antioxidative activities of clove buds are also due in part to the contributions of aroma chemicals such as eugenol and eugenyl acetate. Ingestion of these compounds may help to prevent *in vivo* oxidative damage, such as lipid peroxidation, which is associated with many diseases, including cancer, arteriosclerosis, diabetes and immune deficiency (Lee and Shibamoto, 2001b).

Clove oil showed activity against murine vaginitis in model animals. Topical administration of the liposimized clove oil was found to be most effective against treatment of vaginal candidiasis (Ahmad *et al.*, 2005). Nunez *et al.* (2001) found that clove oil dispersing in sugar solution possessed antifungal activity against *C. albicans* equivalent to the chemical antiseptics *povidone-iodine* and *chloroxylonol*. It has also been suggested that clove has a potential chemopreventive effect through apoptogenic and antiproliferative properties (Banerjee *et al.*, 2006). Clove oil derived from buds, stem or leaf showed antibacterial activity against *Streptococcus viridans* and *S. mutans*. Mouth rinse or gargles from clove bud oil, stem and leaf can inhibit *S. viridans* (Nurdjannah *et al.*, 2001). Other research has a show that clove oils inhibit the growth of *supragingival plaque*, *S. sanguis* and *Porphyromonas gingivalis* (Kalemba and Kunicka, 2003). Activity of mouth gargles made of clove stem oil is twice as strong activity as from clove bud and leaf oil, probably due to eugenol. Eugenol content as stem oil is higher (79.59 %) compared to bud (61.62 %) and leaf (77.11 %) (Nurdjannah *et al.*, 2001). An *in vitro* study (Ail *et al.*, 2005) indicated that eugenol could significantly reduce the viability of *Helicobacter pylori*, a bacterium indigenous in the human stomach, suggesting the potential of eugenol as an anti-*H.pylori* agent. However, additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential use of clove oil or eugenol in medication.

#### 11.4.5 Biopesticides

Increasing concern over the safety of chemical pesticides has led to research on alternative approaches to controlling pathogens and pests using natural plant products. Essential oil and some of the components have shown bactericidal, virucidal, fungicidal, antiparasitical, insecticidal activities and offer potential applications in pest and pathogens control. Clove oil and its components such as eugenol, methyl eugenol and iso-eugenol showed potent antibacterial and antifungal activities.

Clove oil with its major compound eugenol inhibited vanilla stem rot disease caused by *Fusarium oxysporum* sp. *vanillae* (Tombe *et al.* 1992, 1993). Clove oil also showed fungicidal activity against several plant pathogenic fungi such as *Fusarium solani* (Asman *et al.*, 1995), *Phytophthora capsici*, *Sclerotium rolfsii*, *Rigidoporus lignosus*, *Colletotrichum* (Manohara *et al.*, 1994), *Pseudomonas solanacearum* (Hartati *et al.*, 1994), and nematodes (Mustika and Rahmat 1994).

In combination with other essential oils, formulae containing clove and citronellal oils (5 %) could also kill dry-wood termite (*Cryptotermes cynocephalus*) almost completely (score 9.8 out of 10) (Atmaja *et al.*, 2009). A mixture of clove and cinnamon oils tested against the anthracnose and crown rot pathogens in banana showed that the oils were fungistatic and fungicidal against the test pathogens at concentration 0.03–0.11 % (v/v). Eugenol also showed toxicity against several insects and termites comparable to the other botanical pesticides (Isman, 2000).

Other clove components such as methyl eugenol have been used in insect traps and lure products to attract certain fruit flies such as the Tephritidae flies of the order Diptera, including Oriental fruit fly, Mediterranean fruit fly and solenaceous fly. According to the US Environmental Protection Agency (US EPA), methyl eugenol showed few adverse effects indicating it is 'slightly toxic' for acute oral and dermal routes of exposure, but not toxic for acute inhalation toxicity, primary eye irritation and primary skin irritation studies. Although clove oil and its major compound eugenol showed bactericidal and fungicidal properties, the oil has been reported to be safer than the active ingredients of several other botanical insecticides (Stroh *et al.*, 1998). Clove oil containing 90 % eugenol is approximately 1500 times less toxic than the botanical insecticide pyrethrum, and 15000 times less toxic than the organophosphate insecticide azinphosmethyl, four times less toxic than thyme oil (90 %) and 15 times less toxic than Neem (3 % azadirachtin) (Stroh *et al.*, 1998).

## 11.5 Toxicology of cloves

Despite very promising reports on the antioxidative activity of clove oil and various uses of clove and clove oil as a flavouring agent in food, condiments, pharmaceuticals and cigarettes, as an attractant in insecticides and as a fragrance in perfumes and soaps, some reports have raised concern that eugenol, the major component of clove oil, could be a carcinogen. The carcinogenic activity has been associated with methyl eugenol (ME), due to the similarity between its chemical structure and those of other flavourants that are known to be carcinogenic (e.g. safrole and estragole) (Craigmill, 2000). Methyl eugenol can be found naturally in many kinds of spices such as clove oil, nutmeg, etc. Many reports have shown that methyl eugenol is not among the major compounds of clove oil, its presence in clove oil is very small or even only in trace amounts (<1 %) (Gopalakrishnan, 1994; Alma *et al.*, 2007). The major components are eugenol, eugenol acetate and caryophyllene. Moreover, the use of clove oil in food is only in a very small amount or even a few drops, so that the concentration of methyl eugenol would be very small. The FDA in its Code of Federal Regulations (CFR) 21 has affirmed clove oil as generally recognised as safe (GRAS) as a substance added directly to human food (21 CFR 184.1257) and

**Table 11.6** Nutritional composition of clove, per 100 g

| Composition               | USDA (ground) | ASTA |
|---------------------------|---------------|------|
| Water                     | 6.86          | 5    |
| Food energy (kcal)        | 323           | 430  |
| Protein (g)               | 5.98          | 6.0  |
| Fat (g)                   | 20.06         | 14.5 |
| Carbohydrate (g)          | 61.22         | 68.8 |
| Ash (g)                   | 5.88          | 5.0  |
| Ca (g)                    | 0.646         | 0.7  |
| P (mg)                    | 105           | 110  |
| Na (mg)                   | 243           | 250  |
| K (mg)                    | 1102          | 1200 |
| Fe (mg)                   | 8.68          | 9.5  |
| Thiamin (mg)              | 0.115         | 0.11 |
| Riboflavin (mg)           | 0.267         | ND   |
| Niacin (mg)               | 1.458         | 1.5  |
| Ascorbic acid (mg)        | 80.81         | 81   |
| Vitamin A activities (RE) | 53            | 53   |

Source: Tainter and Grenis (1993).

clove oil and its derivatives as a food additive (21 CFR 172.515). The FDA has approved it as a food additive in both natural and synthetic forms.

Clove oil has also been added into various consumer products which are applied topically or as fragrance. Concern on hypersensitive reactions against human skin has led to a number of tests on the application of eugenol itself or various products containing eugenol and/or clove leaf oil. The results indicated that at a very low concentration or at the concentration present in consumer products from various companies, eugenol alone or as part of clove leaf oil has a very low potential to induce hypersensitivity ('induced' reaction) (Rothenstein *et al.*, 1983). Therefore, eugenol or clove oil used either for food or other various consumer products may be safe, and its carcinogenic potential or other adverse effects need to be established.

Clove oil and eugenol are GRAS, but according to Prashar *et al.* (2006), an *in vitro* study demonstrated cytotoxic properties of both the oil and eugenol towards human fibroblast and endothelial cells. Clove oil was found to be highly cytotoxic at concentration as low as 0.03 % (v/v) with up to 73 % of this effect attributable to eugenol.

Besides being a source of natural flavour, cloves also contain nutrients, such as proteins, vitamins, minerals, etc. The composition of nutrients in 100 g is illustrated in Table 11.6. It appears that clove contains fat and carbohydrate in high concentration and has relatively high food energy.

## 11.6 Quality and regulatory issues

The quality required for clove products varies between countries and depends on whether they are intended for domestic consumption or for export and on the



nature of the product. The ASTA (American Spice Trade Association) and FDA (Food and Drug Administration) recommendations for whole spice are described in Table 11.7. The specific requirement for ground clove is minimum quercitannic content 12 %, maximum clove stem content 5 %, and minimum volatile ether extract 15 % (Reineccius, 1994). For clove oil, specifications are illustrated in Table 11.8. For oleoresin, Salzer (1975) recommended determination of volatile oil content and subsequent assay of eugenol content as the measure of quality. There are specifications on the maximum residues limit in the foodstuff as a result of the use as extraction solvents in preparing food flavourings from natural flavouring materials, and there should be applied to solvent-extracted clove oleoresin (Table 11.9).

**Table 11.7** Whole clove chemical and physical specification

| Organisation                                                                                                      | Suggested limit               |              |
|-------------------------------------------------------------------------------------------------------------------|-------------------------------|--------------|
|                                                                                                                   | Whole clove                   | Ground clove |
| <b>ASTA cleanliness specification</b>                                                                             |                               |              |
| Whole dead insect by count                                                                                        | 4                             | —            |
| Mammalian excreta, by mg/lb                                                                                       | 5                             | —            |
| Other excreta, by mg/lb                                                                                           | 8.0                           | —            |
| Mould, % by weight                                                                                                | 1.00                          | —            |
| Insect defiled/infested, % by weight                                                                              | 1.00                          | —            |
| Extraneous, % by weight                                                                                           | 1.00                          | —            |
| A 5 % allowance for unattached clove stems over and above the tolerance for other extraneous matters is permitted |                               |              |
| <b>FDA DALs</b>                                                                                                   |                               |              |
| Adulteration with stem by weight                                                                                  | Ave. of 5 %                   |              |
| Volatile oil                                                                                                      | 16.0 % min.                   | 14.0 % min.  |
| Moisture                                                                                                          | 8.0 % max.                    | 8.0 %        |
| Ash                                                                                                               | 5.0 % max.                    | 5.0 %        |
| Acid insoluble ash                                                                                                | 0.5 % max.                    | 0.5 %        |
| Military specification (EE-S-635J)                                                                                | 5 % or more by weight of stem | —            |
| Average bulk index (mg/100 g)                                                                                     | 240                           | —            |

DALs = deficed action levels.

Source: Tainter and Grenis (1993).

**Table 11.8** Specifications of oil of cloves

| Characteristics                | Sources of oil            |                      |                             |
|--------------------------------|---------------------------|----------------------|-----------------------------|
|                                | Flower bud                | Flower stem          | Leaf                        |
| Colour                         | Colourless to pale yellow | Yellow to dark brown | Straw coloured or very pale |
| Specific gravity (25°/25°C)    | 1.038–1.060               | 1.048–1.056          | 1.036–1.046                 |
| Optical rotation (20°C)        | –1° 30–0                  | –1.5° to 0           | 0 to –2°                    |
| Refractive index (20°C)        | 1.527–1.535               | 1.534–1.538          | 1.531–1.535                 |
| Solubility (70 % ethanol)      | 2 vols                    | 2 vols               | 2 vols                      |
| Total phenols (min. by volume) | 85 %                      | 89–95 %              | 84–88 %                     |

Source: Reineccius (1994).



**Table 11.9** The maximum solvent residues limit in foodstuff

| Solvent name   | Max. residue limits (mg/kg) | Solvent name         | Max. residue limits (mg/kg) |
|----------------|-----------------------------|----------------------|-----------------------------|
| Diethyl ether  | 2                           | Ethyl methyl ketone* | 1                           |
| Hexane*        | 1                           | Dichloromethane      | 0.02                        |
| Methyl acetate | 1                           | Methyl-propan-1-ol   | 1                           |
| Butan-1-ol     | 1                           | Propan-1-ol          | 1                           |
| Butan-2-ol     | 1                           | Cyclohexane          | 1                           |

\* Combined used is forbidden.

Source: Ziegler and Ziegler (1998).

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## Coriander

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**Abstract:** Coriander is an annual herb. It is a member of the carrot family (Umbelliferae) and often used in flavoring substances. The root, stem, leaves and fruits all have an aromatic odor that most considered pleasant. In addition to India, coriander is cultivated commercially in Morocco, Romania, France, Spain, Italy, the Netherlands, Myanmar, Pakistan, Turkey, Mexico, Argentina, South and Western Australia and to some extent in the UK and USA. Coriander seeds are also known for their medicinal properties. As such, coriander is a popular ingredient in the preparation of Ayurvedic medicines. The new value-added products, volatile oil and oleoresins, obtained from coriander seeds are also in high demand on the international markets.

**Key words:** *coriandrum*, herb, medicinal uses, volatile oil, linalool.

### 12.1 Introduction

Coriander is a member of the carrot family (Umbelliferae), and is considered an annual herb and a spice, since both its leaves and seeds are used as a condiment. Fresh coriander leaves are more commonly known as cilantro and bear a strong resemblance to Italian flat leaf parsley (*Eryngium foetidum*). The root, stem, leaves and fruits all have a pleasant aromatic odor, and are widely used in flavorings. The entire young plant is used in preparing chutneys and sauces. The leaves are used for flavoring continental curries and soups. The fruits are extensively employed as a condiment in the preparation of curry powder, pickling spices, sausages and seasonings, and are also used to flavor pastry, biscuits, buns, cakes and liquors, particularly gin. Coriander seeds are also known for their medicinal properties and are considered carminative, diuretic, antibilious, refrigerant and aphrodisiac. Coriander is consequently a frequent ingredient in the preparation of Ayurvedic medicines and is a traditional home therapy for a variety of ailments. New research studies have found that coriander helps in controlling blood sugar, cholesterol and free radical production. Volatile oil and oleoresins are new value-added products obtained from coriander seeds and are in high demand in international markets. The volatile oil is mainly used in flavoring liquors and for disguising unpleasant odors in medicines. The oleoresin is employed for seasoning beverages, pickles, sweets and numerous other products.

### 12.1.1 Origin and distribution

Coriander is native to the Mediterranean and Middle East, and found wild in Egypt and Sudan. It was one of the first spices used by mankind and has been cultivated since antiquity (Small, 1997). The earliest record of coriander dates back to 5000 BC, and its cultivation is mentioned in the Bible, where the color of 'manna' is compared to that of coriander. It was used as a spice in both Greek and Roman cultures; the latter used it to preserve meats and flavor breads. The Romans also introduced coriander to Britain, and it was widely used in English cookery until the Renaissance, when it was largely replaced by the new exotic spices that had become available. Early physicians, including Hippocrates, used coriander for its medicinal properties, including as an aromatic stimulant. Coriander leaves also feature in the culinary traditions of Latin American, Indian and Chinese cuisine. It is believed that the plant has been known in India since the Vedic Period, but at that time it was mostly used for its fresh leaves. The seeds were not used as a spice until the Muslims arrived in India: this may explain why the bulk of coriander seeds are consumed in Mughlai preparations, which are unquestionably of Muslim origin.

Two species of coriander are found; however, only *Coriandrum sativum* L. is cultivated widely, mainly in the tropics. The other species, which is wild, is known as *C. tordylium* (Ifenzl) Bomm. India is the world's leading producer of coriander: it is cultivated over an approximate area of  $4.0$  to  $6.1 \times 10^5$  ha with an annual production of  $3.0$ – $4.0 \times 10^5$  tonnes. The main coriander-growing states of India are Andhra Pradesh, Rajasthan, Madhya Pradesh, Karnataka, Tamil Nadu and Uttar Pradesh. In addition to India, coriander is also commercially cultivated in a number of countries, including Morocco, Romania, Bulgaria, France, Spain, Italy, the Netherlands, Myanmar, Pakistan, Turkey, Mexico, Canada, Argentina and Australia and, to some extent, in the UK and USA. India is also the world's leading exporter of coriander seeds, accounting for 35.9 % of total global exports in 2008. In the same year, Bulgaria was next, with 27.9 %, followed by Morocco (6.8 %) and Canada (4.7 %). Thus, these four countries accounted for over 75 % of global trade quantities; since many of the other exporters are 're-exporters' of coriander that has been imported from one of the four main producing countries, the percentages of total trade quantity for these producer countries are effectively higher (Table 12.1).

### 12.1.2 Botanical description

*Coriandrum sativum* L. ( $2n = 22$ ) has the following botanical classification:

- Division: Angiospermae
- Series: Calyciflorae
- Class: Dicotyledonae
- Sub-class: Polypetalae
- Order: Umbellales (Apiales)
- Family: Umbelliferae (Apiaceae)
- Genus: *Coriandrum*
- Species: *Coriandrum sativum*

**Table 12.1** Top 10 exporters of coriander fruit

| Rank | Reporter                   | Trade value (USD) | Trade quantity (kg) | % of total trade quantity |
|------|----------------------------|-------------------|---------------------|---------------------------|
| 1    | India                      | 46 309 220        | 35 872 012          | 35.86                     |
| 2    | Bulgaria                   | 32 491 063        | 27 920 019          | 27.91                     |
| 3    | Morocco                    | 10 484 463        | 6 843 476           | 06.84                     |
| 4    | Canada                     | 6 789 323         | 4 689 118           | 04.69                     |
| 5    | China                      | 3 835 034         | 3 175 743           | 03.17                     |
| 6    | Romania                    | 3 267 990         | 3 156 303           | 03.16                     |
| 7    | Italy                      | 5 976 060         | 2 990 498           | 02.99                     |
| 8    | Netherlands                | 4 625 568         | 2 308 354           | 02.31                     |
| 9    | USA                        | 2 029 376         | 2 022 074           | 02.02                     |
| 10   | Singapore                  | 2 755 261         | 1 661 644           | 01.66                     |
| 11   | <i>All other exporters</i> | 15 812 694        | 9 394 413           | 09.39                     |
|      | <b>Total Exports</b>       | 134 376 052       | 100 033 654         | 100.00 %                  |

Source: United Nations COMTRADE Database (year 2008).

Below are some different names for the herb:

- botanical name: *Coriandrum sativum* L.
- common name (English): Coriander
- Hindi name: dhaniya
- Ayurvedic name: dhanyaka
- Siddha name: kottumalli vitai
- Unani name: kishneez

The pharmacopoeial names are *Coriandri fructus* for the coriander fruit and *Coriandri aetheroleum* for the coriander fruit oil.

Purseglove *et al.* (1981) provide a detailed botanical description of the plant. There are two distinct morphological plant types: one erect and tall with a comparatively stronger main shoot and shorter branches, the other bushy with a relatively weaker main shoot and longer, spreading branches. Coriander is an annual, erect herb with a tap root system. The plants attain heights from 20–120 cm and flower 45–60 days after sowing; the flowers mature in 65–120 days, depending upon the variety and whether irrigated or rain fed. The upper leaves are wispy and finely divided; the lower ones broad, undivided and trilobite. Some varieties form a rosette of leaves at the base. Each branch as well as the main shoot terminates in a compound umbel bearing 3–10 umbels, each umbel containing 10–50 pentamerous flowers. Hermaphrodite and staminate flowers may occur in each umbel. The flowers are small, protoandrous and difficult to manipulate for controlled pollination. Like other Umbelliferae plants, coriander is also a cross-pollinated crop. The degree of cross-pollination has been reported to range from 50 % (Ramanujam *et al.* 1964) to 60 % (Dimri *et al.*, 1977). Hore (1979) considered a poor seed set as a major factor that restricts yield. Pillai and Nambiar (1982) considered coriander to be andromonoecious, while Singh and Ramanujam (1973) reported significant varietal differences in distribution of male and perfect flowers in the umbels. Hermaphrodite flowers opened earlier than males, and the selection of a higher proportion of hermaphrodite flowers was considered an effective criterion for obtaining a higher seed set.



Coriander fruit consists of the dry cremocarp of *C. sativum* L. and is nearly globular with a 1.5–5.0 mm diameter. The fruit consists of two single-seeded mericarps and is tan to yellow–brown in color when ripe. Coriander may also be divided into two types according to the size of the fruit: macrocarpum types, with a fruit diameter of 3.0–5.0 mm, are usually grown in tropical and sub-tropical regions, while microcarpum types, with a fruit diameter of 1.5–3.0 mm, tend to be grown in temperate regions (Smallfield *et al.*, 2001).

Coriander oil is the essential oil obtained by steam distillation, while coriander oleoresin, a brown–yellow to green liquid, is obtained by the solvent extraction of the dried fruits of *C. sativum* L. Ph Eur-grade fruit (dried drug) must contain a minimum of 3 ml/kg of essential oil (Anon., 2009).

## 12.2 Chemical composition

Coriander contains variable amounts of proteins, fats, carbohydrates, fibers, minerals and vitamins, like other seed spices. However, owing to the very small quantity used in foods, their contribution to nutrient requirements is not significant. The essential/volatile oil is most important in determining the quality of spices.

The green leaves of coriander contain 87.9 % moisture, 3.3 % protein, 0.6 % fat, 6.5 % carbohydrates and 1.7 % mineral matter. Dried ripe coriander seeds have 6.3–8.0 % moisture and contain essential oil (0.3–2.06 %), fatty oil (13–18 %), crude protein (11.5–21.3 %), fat (17.8–19.15 %), crude fiber (28.4–29.1 %) and ash (4.9–6.0 %) (Coskuner and Karababa, 2006). The composition of coriander seed is given in Table 12.2.

**Table 12.2** Composition of coriander seed

| Composition        | USDA Handbook 8.2 <sup>1</sup> | ASTA <sup>2</sup> |
|--------------------|--------------------------------|-------------------|
| Water (g)          | 8.86                           | 6.0               |
| Protein (g)        | 12.37                          | 12.0              |
| Fats (g)           | 17.77                          | 19.6              |
| Carbohydrate (g)   | 54.99                          | 56.5              |
| Ash (g)            | 6.02                           | 5.3               |
| Vitamin A (IU)     | Trace                          | ND                |
| Niacin (mg)        | 2.13                           | 3.2               |
| Riboflavin (mg)    | 0.290                          | 0.230             |
| Thiamin (mg)       | 0.239                          | 0.26              |
| Calcium (g)        | 0.709                          | 0.8               |
| Iron (mg)          | 16.32                          | 5.9               |
| Phosphorus (mg)    | 409                            | 440               |
| Potassium (mg)     | 1267                           | 1200              |
| Sodium (mg)        | 35                             | 20                |
| Energy (Kcal)      | 298                            | 450               |
| Ascorbic acid (mg) | –                              | ND                |

<sup>1</sup> Composition of Foods, Spices and Herbs, USDA Agricultural Handbook, 8–2, January 1977.

<sup>2</sup> The Nutritional Composition of Spices, ASTA Research Committee, February 1977.

ND = not detected.

**Table 12.3** Volatile oil contents of coriander and its source

| Country  | Per cent volatile oil |
|----------|-----------------------|
| Russia   | 0.8–1.8               |
| Norway   | 1.4–1.7               |
| Poland   | 0.6–1.15              |
| Germany  | 0.6–1.0               |
| Bulgaria | 0.3–1.5               |
| Romania  | 0.3–0.8               |
| Italy    | 0.2–0.6               |
| Morocco  | 0.2–0.6               |
| India    | 0.1–0.5               |

Source: Purselove *et al.* (1981).

Glycolipids (GL) were detected in coriander seed, including acylated steryl glucoside (ASG), steryl glucoside (SG) and glucocerebroside (Ramadan and Morsel, 2003). Coriander seeds were also shown to have a selenium content of 23.53 ppm, higher than any other spice (Ozcan *et al.*, 2008). Ray fluorescent analysis revealed the presence of other minerals, namely magnesium, aluminum, silicon, phosphorus, sulfur, potassium, calcium, tin, manganese, iron, copper and zinc (Al Bataina *et al.*, 2003).

The aromatic odor and taste of coriander seeds is largely due to its volatile oil, which is a clear, colorless to light yellow liquid. The flavor of the oil is warm, spicy–aromatic, sweet and fruity. Telci *et al.* (2006) reported that in the ripe fruits, the content of essential oil is low (typically, less than 1 %) compared to that of immature fruits. The oil content of the seeds is reported to vary widely with geographical origin, as shown in Table 12.3, with the microcarpum varieties (mainly the Russian and northern European types) having a higher oil content than the tropical / subtropical microcarpum varieties. The highest volatile oil content is found in Norwegian coriander, which has an average volatile oil content of 1.4–1.7 %, and even in some cases up to 2.7 %, while Bulgarian coriander has 0.3–1.5 % (Purselove *et al.*, 1981). Indian seeds are poor in volatile oil content, with only 0.1–0.4 % (Agrawal and Sharma, 1990). However, exceptions to this are not uncommon: the bold-seeded Indian varieties CS-4 and CS-6 have a higher oil content than the small-seeded Indian variety RCr.41. The smaller-seeded types, with their high oil content, are preferred for distillation purposes, while bold-seeded types have a better appearance and are more suitable for use as a spice. The small-seeded, high-oil-yielding types of coriander are generally late in flowering and maturity (Kumar *et al.*, 1977).

Coriander oil has the following physical properties:

- specific gravity at 25 °C: 0.863–0.875
- refractive index at 20 °: 1.463–1.472
- optical rotation: +8 to +15 °

The essential oil is made up of monoterpene hydrocarbons and oxygenated monoterpenes (Anitescu *et al.*, 1997; Bandoni *et al.*, 1998) with the former accounting for about 20 % of the essential oil. The principal oxygenated compound present is *d*-linalool, also known as coriandrol: coriander essential oil can contain between

19.8 and 82 % of this compound. Other major components of the oil are  $\alpha$ -pinene (10.5 %),  $\gamma$ -terpinene (9.0 %), geranyl acetate (4.0 %), camphor (3.0 %) and geraniol (1.9 %) (Diederichsen, 1996). Minor components in the oil are  $\beta$ -pinene, camphene, myrcene, limonene, *p*-cymol, dipentene,  $\alpha$ -terpinene, *n*-decylaldehyde, borneol and acetic acid esters. Different studies have identified various compounds present to different extents in coriander essential oil:

Rastogi and Mehrotra (1993), using thin-layer chromatography (TLC), reported the presence of  $\alpha$ -pinene, limonene,  $\beta$ -phellandrene, eucalyptol, linalool, borneol,  $\beta$ -caryophyllene, citronellol, geraniol, thymol, linalyl acetate, geranyl acetate, caryophyllene oxide, elemol and methyl heptenol.

Telci *et al.* (2006) found that the oil consisted mainly of linalool (50–60 %) and about 20 % terpenes (pinenes,  $\gamma$ -terpinene, myrcene, camphene, phellandrenes,  $\alpha$ -terpinene, limonene and cymene).

Nazrul *et al.* (2009) identified 53 compounds in seed essential oil (Table 12.4) of which the major compounds were linalool (37.65 %), geranyl acetate (17.57 %) and  $\gamma$ -terpinene (14.42 %). Other minor compounds reported in the same study are  $\beta$ -pinene (1.82 %), *m*-cymene (1.27 %), citronellal (1.96 %), citronellol (1.31 %), citral (1.36 %), geraniol (1.87 %), citronellyl acetate (1.36 %),  $\alpha$ -cedrene (3.87 %),  $\alpha$ -farnesene (1.22 %) and  $\beta$ -sesquiphellandrene (1.56 %).

Other studies on the composition of coriander seed oil include those of Coleman and Lawrence (1992), Leung and Foster (1996), Tashinen and Nykanen (1975) and Pino *et al.* (1996).

The variation in the oil composition is down to the relative proportions of the constituents and not to the presence/absence of a particular component. Location, fertilization, weeds, cultivar and seeding date were all shown to affect the chemical profile of coriander oil (Gil *et al.*, 2002; Zheljazkov *et al.*, 2008). The chemical composition and relative proportions of the components in the essential oil of coriander seeds are also dependent on the stage of maturity (Msaada *et al.*, 2007).

Essential oils extracted from coriander seeds from different geographical locations have also been found to differ in their composition (Coleman and Lawrence, 1992; Tashinen and Nykanen, 1975; Leung and Foster, 1996; Pino *et al.*, 1996). For example, Indian coriander oil differs from European oil in that it has a lower linalool content and a higher ester (linalyl acetate) content (Rao *et al.*, 1925). Asolkar *et al.* (1992) reported a type from Mysore with a high geranyl acetate content. Nazrul *et al.* (2009) reported that these variations observed between cultivars are not due to geographic divergence and ecological conditions but are instead the result of different chemotypes.

Hirvi *et al.* (1986) also observed differences in the composition of volatile oil extracted through steam distillation, CO<sub>2</sub> and three other commercial extraction methods. A summary of the results is shown in Table 12.5. Boelens *et al.* (1989) reported that volatile oil extracted by hydrodistillation had a higher linalool content (70.4 %) and fewer monoterpene hydrocarbons (23.6 %) than that extracted by hydrodiffusion, which had a linalool content of 66.2 % and a monoterpene hydrocarbon content of 27.3 %.

The odor and flavor of fresh herbage is completely different from that of the mature seed. While aliphatic aldehydes (mainly C10–C16 aldehydes) with a

**Table 12.4** Composition of essential oil of *C. sativum* L. seed

| SL. No. | Name of chemical compounds                    | %     |
|---------|-----------------------------------------------|-------|
| 1       | $\gamma$ -Terpinene                           | 14.42 |
| 2       | Camphene                                      | 0.14  |
| 3       | E-verbenol                                    | 0.27  |
| 4       | Sabinene                                      | 0.23  |
| 5       | $\beta$ -Pinene                               | 1.82  |
| 6       | 2-Oxabicyclo[2.2.2]Octan-6-ol,1,2,3-trimethyl | 0.02  |
| 7       | $\beta$ -Myrcene                              | 0.55  |
| 8       | Cyclooctanol                                  | 0.02  |
| 9       | $\alpha$ -Thujene                             | 0.04  |
| 10      | <i>m</i> -Cymene                              | 1.27  |
| 11      | Limonene                                      | 0.40  |
| 12      | E-ocimene                                     | 0.05  |
| 13      | Z-ocimene                                     | 0.04  |
| 14      | Lilac alcohol                                 | 0.11  |
| 15      | $\alpha$ -Terpinene                           | 0.04  |
| 16      | Z-verbenol                                    | 0.11  |
| 17      | Linalool                                      | 37.65 |
| 18      | Isothujol                                     | 0.04  |
| 19      | $\alpha$ -Campholenal                         | 0.22  |
| 20      | Citronellal                                   | 1.96  |
| 21      | Umbellulone                                   | 0.11  |
| 22      | Borneol                                       | 0.32  |
| 23      | 4-Terpineol                                   | 0.06  |
| 24      | Terpinyl acetate                              | 0.31  |
| 25      | Decanal                                       | 0.14  |
| 26      | Z-verbenone                                   | 0.10  |
| 27      | Citronellol                                   | 1.31  |
| 28      | Citral                                        | 1.36  |
| 29      | Geraniol                                      | 1.87  |
| 30      | Eugenol                                       | 0.90  |
| 31      | Carveol                                       | 0.15  |
| 32      | Undecanal                                     | 0.58  |
| 33      | Methyl geranate                               | 0.17  |
| 34      | Myrtenyl acetate                              | 0.43  |
| 35      | Citronellyl acetate                           | 1.36  |
| 36      | Geranyl acetate                               | 17.57 |
| 37      | Z-myrtanyl acetate                            | 0.10  |
| 38      | $\beta$ -Elemene                              | 0.06  |
| 39      | Dodecenal                                     | 0.15  |
| 40      | Caryophyllene                                 | 0.33  |
| 41      | $\beta$ -Farnesene                            | 0.07  |
| 42      | 2-Dodecenal                                   | 0.18  |
| 43      | Curcumene                                     | 0.98  |
| 44      | $\alpha$ -Cubebene                            | 0.13  |
| 45      | $\alpha$ -Cedrene                             | 3.87  |
| 46      | $\alpha$ -Farnesene                           | 1.22  |
| 47      | $\beta$ -Bisabolene                           | 0.80  |
| 48      | $\beta$ -Sesquiphellandrene                   | 1.56  |
| 49      | E-nerolidol                                   | 0.13  |
| 50      | Artumerone                                    | 0.04  |
| 51      | 8-Hexadecenal, 14-methyl-, (Z)                | 0.22  |
| 52      | $\alpha$ -Bisabolol                           | 0.15  |
| 53      | <i>n</i> -Hexadecanoic acid                   | 0.08  |

**Table 12.5** Chemical composition of oils and extracts of *C. sativum* L.

| Compound            | Steam-distilled oil | CO <sub>2</sub> extract | Soxhlet extract | Commercial extract |      |      |
|---------------------|---------------------|-------------------------|-----------------|--------------------|------|------|
|                     |                     |                         |                 | 1                  | 2    | 3    |
| $\alpha$ -Pinene    | 0.6                 | 0.1                     | 0.6             | 0.2                | 0.8  | 0.2  |
| $\beta$ -Pinene     | 1.4                 | 0.6                     | 1.2             | 0.7                | 0.7  | 0.9  |
| Limonene            | 2.6                 | 1.2                     | 3.8             | 1.6                | 3.4  | 7.7  |
| $\gamma$ -Terpinene | 4.7                 | 2.3                     | 6.8             | 10.5               | 4.6  | 3.7  |
| <i>p</i> -Cymene    | 0.7                 | 0.6                     | 1.6             | 1.6                | 1.6  | 2.6  |
| Camphor             | 4.3                 | 2.9                     | 4.7             | 2.4                | 4.0  | 4.5  |
| Linalool            | 71.9                | 83.2                    | 70.4            | 70.3               | 74.8 | 72.5 |
| Terpinen-4-ol       | 0.2                 | 0.1                     | 0.2             | 0.2                | 0.2  | 0.2  |
| $\alpha$ -Terpineol | 0.5                 | 0.4                     | 0.5             | 0.3                | 0.6  | 0.3  |
| Geranyl acetate     | 7.5                 | 5.7                     | 7.2             | 5.7                | 6.1  | 4.7  |
| Geraniol            | 4.0                 | 2.4                     | 2.8             | 3.4                | 1.7  | 1.8  |

Source: Hirvi et al. (1986).

**Table 12.6** Physicochemical properties of leaf and seed essential oil of *C. sativum* L.

| Parameters               | Leaf oil | Seeds oil       |
|--------------------------|----------|-----------------|
| Essential oil (%)        | 0.1      | 0.42            |
| Specific gravity         | 0.815    | 0.893           |
| Optical rotation at 32°C | –        | +9 <sup>0</sup> |
| Refractive index at 32°C | 1.428    | 1.459           |

fetid-like aroma are predominant in the fresh herb oil (Potter, 1996); on ripening, the seeds acquire a more pleasant and sweet odor, mainly because of an increase in linalool content. Ghani reported (2003) that linalool, pinene, cymene, phellandrene, geraniol and borneol were present in both leaf and seed oil; however, the composition of the leaf oil is on the whole markedly different from that of the seed oil (Guenther, 1950).

The physicochemical properties and components of the essential oil from both the leaves and seeds of *C. sativum* were analyzed by gas chromatography–mass spectroscopy (GC–MS) and compared with those of the seed oil (Table 12.6). The leaf oil contains 44 compounds mostly of aromatic acids, principally 2-decenoic acid (30.82 %), E-11-tetradecenoic acid (13.4 %), capric acid (12.7 %), undecyl alcohol (6.4 %) and tridecanoic acid (5.5 %). Other constituents in the leaf oil are undecanoic acid (2.13 %), 2-dodecanal (1.32 %), 2-undecenal (3.87 %), cyclododecane (2.45 %), decamethylene glycol (1.15 %), decanal (1.35 %) and dodecanoic acid (2.63 %) (Table 12.7) (Nazrul *et al.*, 2009).

Besides the essential oil, the seeds have a fatty oil content that can vary between 9.9 and 27.7 % (the usual range is 19–21 %) (Diederichsen, 1996). The fatty oil has a dark, brownish green color and an odor quite similar to that of the essential oil. The main components of the fatty acids found in coriander seeds are petroselinic acid (C18: 1) 68.8 %, linoleic acid (C18: 2) 16.6 % oleic acid (C18: 1) 7.5 % and palmitic acid (C16:0) 3.8 %. Minor components are stearic acid, vaccenic acid and

**Table 12.7** Chemical composition of essential oil of *C. sativum* L. leaf

| SL. | Name of chemical            | %  | SL. | Name of chemical         | %  |
|-----|-----------------------------|----|-----|--------------------------|----|
| 1   | <i>g</i> -Thionodecalactone | 0  | 23  | E-11-tetradecenoic acid  | 13 |
| 2   | 1,2-Decanediol              | 0  | 24  | Ethylidenecyclooctane    | 0  |
| 3   | <i>n</i> -Cetyl alcohol     | 0  | 25  | Eucalyptol               | 0  |
| 4   | 2,4-Dimethylheptane         | 0  | 26  | 1-Pentadecene            | 0  |
| 5   | 2-Decenoic acid             | 30 | 27  | $\alpha$ -Caryophyllene  | 0  |
| 6   | 2-Dodecanal                 | 1  | 28  | $\alpha$ -Pinene         | 0  |
| 7   | 2-Tridecenal, (E)           | 0  | 29  | <i>m</i> -maminioaniline | 0  |
| 8   | 2-Undecenal                 | 3  | 30  | Capric acid              | 12 |
| 9   | 4-Allylphenyl acetate       | 0  | 31  | Nonanoic acid            | 1  |
| 10  | 5-Nonanol, 5-methyl         | 0  | 32  | Nonanol                  | 0  |
| 11  | Anisole, P-allyl            | 0  | 33  | Octanoic acid            | 0  |
| 12  | Benzene                     | 0  | 34  | o-Cymene                 | 0  |
| 13  | $\beta$ -Cinene             | 0  | 35  | Oleic acid               | 0  |
| 14  | Z-nonene                    | 0  | 36  | Oxirane tetradecyl       | 0  |
| 15  | Cyclododecane               | 2  | 37  | Oxirane, octyl           | 0  |
| 16  | Cyclooctanol                | 0  | 38  | E-2-ethyl-3-methyl       | 0  |
| 17  | Decahydroazulene            | 0  | 39  | E-undecanoic acid        | 4  |
| 18  | Decamethylene glycol        | 1  | 40  | Tridecanoic acid         | 5  |
| 19  | Decanal                     | 1  | 41  | Undecanal                | 0  |
| 20  | <i>d</i> -Limonene          | 0  | 42  | Undecanoic acid          | 2  |
| 21  | Dodecanal                   | 1  | 43  | Undecyl alcohol          | 6  |
| 22  | Dodecanoic acid             | 2  | 44  | Undecylenic acid         | 0  |

myristic acid. The oil solidifies if allowed to stand for a short period. Good-quality oleoresin can also be extracted from coriander seeds (IENICA report, 2000).

There were no photoactive compound furocumarins present in coriander, but two unknown slightly photoactive compounds were identified (Ceska *et al.*, 1987). Antinutritive compounds such as glucosinolates (27.5  $\mu\text{mol/g}$ , sinapne 4 mg/g), condensed tannins 1.1 mg/g and inositol phosphate 17.4 mg/g were found in the seeds (Matthaus and Angeline, 2005).

## 12.3 Cultivation of coriander

### 12.3.1 Climate and soils

Coriander is a tropical crop; it requires a cool and comparatively dry frost-free climate, particularly at flowering and seed formation stages, for good-quality and high seed yields. Frost following the flowering stage reduces production drastically. High temperature and high wind velocity during anthesis and seed formation lead to increased sterility and reduce yield. Cloudy weather at the time of flowering increases the number of aphids and diseases. Coriander can be grown in a wide range of soils, provided that they are rich in organic matter and well drained. It is grown as an irrigated crop on loamy to moderately heavy soils and as a rain-fed crop with conserved moisture on black cotton or heavy soil with high moisture retention capacity. Saline, alkaline and sandy soils are not suitable for coriander cultivation.

### 12.3.2 Field preparation and sowing

#### *Field preparation*

The coriander field is brought to a fine tilth by two or three plowings, the first of which should preferably be carried out with a soil-turning plow. If the soil moisture level is low, the soil may be lightly irrigated before plowing. For a rain-fed crop, field preparation should be carried out when the moisture level in the soil falls to an optimum level following the preceding rains, and the soil should be thoroughly planked to check the moisture losses until sowing.

#### *Sowing time*

The optimum temperature for germination and early growth of coriander is 20–25°C. In northern India, the main crop is sown from the last week of October to the second week of November. Seed germination and early growth are adversely affected by high temperature if the crop is sown earlier. In southern India, when the sowing is done after the winter season, a second crop is also taken during the summer, which is sown between 15 May and 15 June. Delay in sowing reduces the plant growth and increases the incidence of diseases and pests.

#### *Seed rate*

To achieve optimum plant density in irrigated conditions, a seed rate of 12–15 kg/ha, depending upon the seed size, with a slightly higher seed rate for bold-seeded type, is sufficient. For rain-fed conditions, a seed rate of 25–30 kg/ha is recommended. Seeds are crushed or divided into two halves and treated with 1.0 g carbendazim per kg of seed or any other mercurial fungicide at the rate of 2.0 g per kg of seeds.

#### *Sowing method*

Sowing of the treated and crushed seed (halves) is carried out by scattering/broadcasting or by sowing in lines 30 cm apart in shallow rows behind the plow. Line sowing facilitates intercultural operations in the standing crop. In heavy soils or under high soil fertility conditions, row spacing of 40 cm is recommended. An optimum distance between plants in rows is 10 cm. Care should be taken in both methods of sowing to ensure that seeds are uniformly covered with soil not deeper than 4.0 cm.

### 12.3.3 Plant management

#### *Manure and fertilizers*

During field preparation, about 10–20 tonnes/ha of farmyard manure (FYM) or compost should generally be applied. In addition to the FYM/compost, 20–30 kg nitrogen, 30 kg phosphate and 20 kg potash per hectare should be applied in the form of fertilizers at the time of sowing. In irrigated conditions, an additional dose of 40 kg nitrogen/ha should be applied with irrigation in two equal portions, the first at 30 days and the second at 75 days after sowing. Doses of fertilizers can be manipulated in accordance with the available soil nutrients, which can be detected through soil testing.



*Hoeing and weeding*

Coriander initially grows at a slow rate. Hoeing and weeding should first be carried out 30 days after sowing, at which point thinning to remove excessive plants may also be done. The second hoeing and weeding may be carried out between 50 and 60 days after sowing, depending upon the appearance or re-growth of weeds. The use of chemicals reduces the market value of the produce; however, if manual control of weeds is not possible, effective chemical control of weeds can be achieved by applying the herbicide fluchloralin before planting at the rate of 0.75 kg/ha, or through a pre-emergent application either of oxyfluorfen at the rate of 0.15 kg/ha or of pendimethalin at the rate of 1.0 kg/ha dissolved in 400–500 l of water.

*Irrigation*

Depending upon the climatic conditions, the moisture-retaining capacity of the soil and the variety used, four or five irrigations are required after germination. The first irrigation should be given at 30–35 days after sowing, the second at 60–70, the third at 80–90, the fourth at 100–105 and the fifth at 110–150 days. In addition to these scheduled irrigations, a light irrigation may sometimes be required between five and eight days after sowing to facilitate proper germination.

**12.3.4 Harvesting and threshing**

The ideal harvesting time for coriander is difficult to establish, as flowering and fruit maturity cannot be accurately predicted. It is extremely important to harvest the fruit at the correct stage of maturity, as this determines the quality of the spice and the essential oil. Although the volatile oil content is higher in immature fruits, their aroma is generally considered to be disagreeable by consumers. The sweet and spicy aroma that is typical of coriander does not develop until the fruit has reached maturity and has begun to dehydrate on the plant. If the harvesting is delayed in order to allow secondary umbels to reach maturity, there is a risk that the fruits on the primary umbels may shatter. In windy conditions, seed shatter is more likely, and loss of a complete class of umbel can even occur. As the fruit ripens progressively rather than simultaneously, the optimum time for harvesting must be judged very carefully. Normally, in order to obtain a good luster of seed with maximum yield, the harvesting should be done when 50 % of the seeds have changed color from green to gray-green or yellow. To minimize breakage, the plants should be cut either early in the morning or late in the evening. When harvested, the fruits may have a moisture content of above 20 %: this must be reduced to 9 % or less during drying (Smallfield *et al.*, 2001). If the harvest is carried out using a harvester-thresher, this should be done when the seed moisture content is less than 15 %, early in the morning hours, because after the dew dries the fruits fall off easily.

The harvested material should be dried in the shade to ensure that seed color and quality are maintained; if this is not possible, then the material should be kept in bundles upside down to avoid direct sunlight on the seeds, which adversely affects the color of the produce. Coriander is artificially dried in some countries, including Russia at temperatures of 80–90°C. Temperatures of over 100°C result in the loss of volatile oils.

Once the harvested material is dried, the seeds are separated by light beating with sticks and winnowing. The dried bundles should be threshed on a clean floor or on tarpaulin. Once the seeds are dried and separated, they are packaged in jute fabric bags.

To obtain extra income, 50 % of the leaves may be plucked at 75 days after sowing, without causing a reduction in seed yield. If good management practices are adopted, and high-yielding varieties used, an average yield of 1200–1500 kg/ha under irrigated conditions and 700–800 kg/ha under rain-fed conditions can be easily obtained.

## 12.4 Post-harvest management and processing

The size, volatile oil content and aroma/flavor character of dried, mature fruit are principally governed by the intrinsic properties of the cultivar grown, and the stage of harvest is also important in determining the quality of the produce. However, there are also some post-harvest factors that can affect the quality of the final product.

The cleanliness of the product is important for marketing the spice. Fruits should be thoroughly cleaned to remove extraneous matter such as stalks, plant debris and soil. Proper care should be exercised during post-harvest operations to ensure that the produce retains the required quality level. The produce should be properly cleaned with vacuum gravity separators or a destoner spiral gravity separator and graded. The graded material should be packed in lint-free bags and stored in a damp-free aerated storehouse to ensure insect-free conditions. The stowage factor for bagged dried coriander is 3.54–3.68 m<sup>3</sup>/t (jute bags, 30 kg). The main requirements for the stowage space are that it should be cool and dry with good ventilation. Rats, mice, beetles, moths and mites may infest coriander seeds during storage and transport; care must therefore be taken to avoid this.

Coriander, like other seed spices, can be used in value-added products such as volatile oil, oleoresin, ground spices, curry powder and consumer-packed spices. There are certain disadvantages associated with spices in raw form: whole or ground spices do not impart their total flavor readily; they are bulky, making storage more difficult, and are often unhygienic owing to bacterial contamination; and they experience wide fluctuations in price. Some of these defects can be reduced by extracting oils through steam distillation and by preparing oleoresins using organic solvents.

The value-added form of spices has recently shown tremendous growth potential. The global market is increasingly shifting away from the commodity form towards the value-added form of consumer-packed branded spices, overcoming the disadvantages of raw spices.

### 12.4.1 Volatile oil

The volatile oil is aromatic and is primarily recovered from the dried ripe seeds. To produce the oil, the dried seeds are placed in stainless steel distillation vessels equipped with a steam inlet, vapor outlet, condenser and separator assembly. Live steam is introduced below the charge; the steam rising through the plant charge carries the volatile oil. The volatile oil is condensed and separated from the water. The advantages of using essential oil are that it has uniform flavor quality, is free from enzymes and tannins and does not impart color to the end-product.

### 12.4.2 Oleoresin

Oleoresin represents the complete flavor and non-volatile resinous fraction present in the spices. The resinous fraction comprises heat components, fixative, natural antioxidant and pigments. Hence, oleoresin is the true essence of the coriander. Oleoresin is a free-flowing, dark brown to yellow liquid at ambient temperatures and is soluble in alcohol (95 %). It can be obtained by solvent extraction of the ground seed, when it has a fruity, aromatic, slightly balsamic flavor, or from the roasted seeds, which gives a more rounded flavor with a caramel taste.

The volatile oil content in the oleoresin ranges from 2 to 12 ml per 100 g. In coriander, the volatile oil is found only in very small quantities; therefore, the volatile oil content and oleoresin are less significant value-added products in comparison to others.

### 12.4.3 Ground spices

These are the whole spices milled to a certain degree of fineness required by the food processor. The grinding technique should be studied in more detail in order to develop efficient methods to prevent undesirable changes with respect to flavor and pungency. Ground spices can be incorporated into food dishes more uniformly than can whole spices. However, they also have disadvantages: they have a limited shelf-life and are subject to oxidation, flavor loss and degradation owing to microbial contamination if stored for long periods.

### 12.4.4 Consumer-packed spices

Exported spices are consumed in three main sectors, namely industrial, institutional and retail. Different packaging media are used according to the consumer's preference. Packaging has gained considerable importance as it increases the shelf-life of spices. The development of new and improved plastic films, aluminum foil, laminations, and high-speed film-sealing machines has created new opportunities for packaging the spices as instant spices, spice pastes and spice powder. Consumer-packed spices can command a higher unit price for the same quantity. The prices of these retail spice packs are between 50 and 100 % higher than those of bulk spices. The weights of retail packs generally range between 30 g and 500 g. However, institutional packs range between 500 g and 1 kg in weight. It is important to note that, with the stiff competition that India is facing in the spice market, building brand image is essential, particularly for packed spices.

## 12.5 Main uses of coriander

### 12.5.1 Uses in the food industry

Coriander is commonly used as a flavoring substance. The two primary products that are used for flavoring purposes are the fresh green herb and the spice; the latter is the dried form of the whole mature seed capsule (fruit) and frequently termed 'coriander seed' in commerce. The odor and flavor of these two products are markedly different. The herb is used for culinary flavoring purposes in Asia, the

Middle East and Central and South America. The entire plant, when young, is used in preparing chutneys and the leaves are used for flavoring curries, sauces and soups. The fresh leaves may also be used as a garnish for curries and other dishes, and are used as a green vegetable by some ethnic groups.

The seeds are used as an important ingredient in some curry powders, a type of seasoning made from five to 20 different spices and usually consisting of turmeric, garlic, chillies, coriander, cumin, fennel, fenugreek and black pepper. Coriander seeds are also used as a pickling spice, in seasonings and sausages and also in pastries, buns, cakes and other confectionery and tobacco products. Coriander oil is also used as a flavoring for alcoholic beverages, candies, meat, sauces and tobacco. The oleoresin is employed for seasoning beverages, pickles, sweets and numerous other delicacies. Lastly, coriander flowers produce a lot of nectar and attract many different insects for pollination. Sieved coriander honey is known for its taste as well as for its characteristic aroma. A 1 ha coriander field allows honey bees to collect about 500 kg honey (Lukjanav and Reznikov, 1976).

### 12.5.2 Traditional medicine

The seeds, leaves and oil of coriander have a variety of uses in traditional and folk medicine. Coriander is a valuable herb in promoting digestion and treating gastro-intestinal disorders such as dyspepsia, flatulence, loss of appetite, griping pain and vomiting (Jabeen *et al.*, 2009). One or two teaspoons of coriander juice, added to fresh buttermilk, is considered highly beneficial in treating indigestion, nausea, dysentery, hepatitis and ulcerative colitis. It is also helpful in the treatment of typhoid fever. Dry coriander treats diarrhea and chronic dysentery, as well as being useful in preventing acidity. Chutney made from dry coriander, green chillies, grated coconut, ginger and black grapes without seeds is a remedy for abdominal pain caused by indigestion. Coriander is also used as a diuretic, particularly in Morocco (Aissaoui *et al.*, 2008). Regular drinking of coriander water, water helps lower blood cholesterol by stimulating the kidneys. The water is prepared by boiling dry seeds of coriander and straining the decoction after cooling.

Coriander is used as both an anti-inflammatory and an analgesic, and also has antimicrobial properties. An extract of the seeds, combined with castor oil, is used as a cure for rheumatism and joint pain (Anon., 1950; Chopra *et al.*, 1956; Asolkar *et al.*, 1992; Yusuf *et al.*, 1994; Ghani, 2003). As well as the effects on diarrhea and chronic dysentery mentioned above, a decoction prepared from freshly dried coriander is an excellent eyewash for the treatment of conjunctivitis. It relieves burning and reduces pain and swelling. A teaspoon of coriander juice, mixed with a pinch of turmeric powder, is said to be an effective remedy for pimples, blackheads and dry skin. The mixture should be applied to the face, after washing, before going to bed. The seeds in particular are recommended as a cure for anxiety and insomnia, especially in Iran (Emamghoreishi *et al.*, 2005). They can also be used to control excessive menstrual flow. Six grams of the seeds should be boiled in half a liter of water, until only half the water remains. Sugar should be added, and the resulting liquid taken while still warm. After three or four days of taking this preparation, the patient should experience relief.

Coriander leaves are considered to be an aphrodisiac, and can also be used in the treatment of stomatitis and bad breath, and to prevent pyorrhea. They also help in the removal of catarrh and phlegm from the bronchial tubes, thereby counteracting any spasmodic disorders. Externally, the juice of the leaf can be applied on the forehead as a treatment for migraine and other types of headaches. In some parts of Europe coriander is also considered to have antidiabetic properties. The various uses of coriander in different systems of medicine are summarized below:

- **European Community:** Coriander fruit is regulated as a herbal medicinal product (HMP) for oral use (prepared as a herbal tea or fluid extract or as the essential oil) for treating loss of appetite, digestive disturbances with mild spasms of the gastrointestinal tract, distension and flatulence.
- **Indian systems of medicine (Ayurvedic, Siddha and Unani medicine):**
  - *Ayurveda:* Coriander is tridoshar (equilibrium of *vat*, *cough* & *pitta*), trishnanigrah (thirst control), rochan (laxative), deepan (appetizer), pachan (digestive), krimidhan (parasite killer), coughdhan (cough reducer), mutrajanan and mutravaraniva (diuretic), sukhardhatu, jawarhan (antipyretic), sheet parasaman (cold remover) and masteekbalya (brain tonic). Sansthanik upyog (local application) – sodhar (anti-inflammatory) and sulhar (pain killer). It comes under category Deepanadii Varg (work on digestive system) (Sharma, 1995) and it is used therapeutically for treatment of Jvara (fever), Trsna (thirst), Chardi (emesis), Daha (burning sensation), Ajirna (dyspepsia) and Atisara (diarrhea).
  - *Siddha:* Coriander is used therapeutically for treatment of Carayaveri (alcoholic delirium), Kulirkaiccal (fever with rigor), Navaratci (dryness of mouth), Pittamantam (indigestion), Pun (ulcers), Takam (thirst), Vanti (emesis) and Veppam (fever).
  - *Unani:* Coriander is used therapeutically for treatment of Suda (headache), Zof-e-Qalb (cardiac weakness), Zof-e-Meda (weakness of stomach), Nafkh-e-Shikam (flatulence) and Zof-e-Dimagh (weakness of brain).

### 12.5.3 Other uses

Many of the healing properties of coriander discussed above can be attributed to its exceptional phytonutrient content. The seeds and volatile oil are rich in beneficial phytonutrients including carvone, geraniol, limonene, borneol, camphor, elemol and linalool. The flavonoids present in coriander include quercetin, kaempferol, rhamnetin and epigenin. Coriander also contains active phenolic acid compounds including caffeic and chlorogenic acid. Coriander is a very good source of dietary fiber and a good source of iron, magnesium and manganese. Coriander leaves also constitute one of the richest sources of vitamin C (250 mg/100 g) and vitamin A (5200 IU/100 g). Coriander juice is thus highly beneficial in treating deficiencies of vitamins A, B<sub>1</sub>, B<sub>2</sub> and C and iron.

Coriander is a valuable ingredient in perfumes, and is also used in cosmetics, soaps, detergents and other toiletries (Smith *et al.*, 1997). Its soft, pleasant, slightly spicy note blends into scents of oriental character. The leaf juice can also be used as a cosmetic agent, and the essential oil can be used in aromatherapy, although it

is not as popular as other seed oils in this respect. Coriander is also used in surfactants and emulsifiers (Smith *et al.*, 1997). The IENICA report (2000) also provides a detailed discussion of one significant recent use of coriander, in the oleo-chemical industry. Coriander is of interest because of its high level of petroselinic acid, an isomer of oleic acid, which is used as a plastics lubricant, in the manufacture of nylons and for cosmetics. Higher oil percentage yields would be required for coriander to be a viable industrial crop. One possibility, therefore, would be to transfer the genes responsible for petroselinic acid production into an established oilseed crop. One further use of coriander is as a feedstuff for ruminants. The residues left after the extraction of the essential oil are used for this purpose, since their composition is nearly the same as that of the whole fruit; they still contain digestible fat and protein. Because of the high crude fiber content, coriander oil cake can only be fed to ruminants, but the value of the feed is limited (Diederichsen, 1996).

## 12.6 Modern research into the medicinal properties of coriander

### 12.6.1 Antioxidant activity

The ethanol extract of *C. sativum* leaves is an excellent antioxidant, which is stable at high temperature and can serve as a substitute for synthetic antioxidants (Shyamala *et al.*, 2005). Further studies carried out by Melo *et al.* (2005) indicated that the four coriander extract fractions obtained from the crude extract using chromatography in silica gel possessed similar antioxidant activities, which can be measured by the  $\beta$ -carotene/linoleic acid system. The antioxidant activity was due to several phenolic acids and caffeic acid, which were present in all four fractions (Melo *et al.*, 2005). Extracts of coriander seeds obtained with supercritical CO<sub>2</sub> in semi-continuous lab-scale equipment with low-density (0.60 g/mL) CO<sub>2</sub> and high-density (0.73–0.83 g/mL) CO<sub>2</sub> (pressures from 116–280 bar and temperatures from 311–31 K for the latter) exhibited significant activity in removing free radicals present in a methanol solution of DPPH in a manner which was comparable to those of commercial antioxidants (Yepez *et al.*, 2002). The aqueous extract of coriander seed inhibited peroxidized lipid-induced lysis (induced by FeSO<sub>4</sub>-ascorbate 10:100  $\mu$ mol/system) by 72 % in human erythrocyte membranes (Sujatha and Srinivas, 1995).

An evaluation of the antioxidant power of polyphenolic compounds in *C. sativum* against hydrogen peroxide-induced oxidative stress in human lymphocytes showed that the polyphenolic fractions (50  $\mu$ g/mL) increased the activity of antioxidant enzymes and the glutathione content and reduced the levels of thiobarbituric acid-reacting substances significantly. Peroxidative damage was decreased, as there was a reduction in the level of lipid peroxides (Hashim *et al.*, 2005). The principal component in coriander antioxidant action was  $\beta$ -carotene as it represented 61.14 % of the carotenoids detected in the ether extracts (Guerra *et al.*, 2005). The antioxidant capacity of phenolic compounds in coriander leaves was higher than that of the seeds in three different bioassays, namely scavenging of free radicals by DPPH, inhibition of 15-lipoxygenase (15-LO) and inhibition of Fe<sup>2+</sup> induced phospholipids peroxidation in the brain. The seed lipid content, which was extracted with dichloromethane, gave low activities in radical scavenging and inhibition of lipid peroxidation. The ethyl acetate extract of the leaves exhibited the most potent activity (IC<sub>50</sub>



value  $147 \pm 3 \mu\text{g/mL}$ ) (Wangensteen *et al.*, 2006). The greater antioxidant effect of a crude extract of coriander compared to its component fractions suggested a synergistic action between the carotenoids. Assessment of the total antioxidant activity of methanol and water extracts coriander leaves and stems using an iron-induced linoleic acid oxidation model system showed that the methanol-derived leaf extracts exhibited significantly greater radical-scavenging activity towards both lipid- and water-soluble radicals, which was attributed to the total phenolic content (Wong and Kitts, 2006). Pre-treatment with *C. sativum* protected Wistar albino rats against gastric mucosal damage induced by ethanol. The protective effect might be related to the free-radical scavenging property of the different antioxidant constituents present in *C. sativum* (Al-Moflelh *et al.*, 2006).

### 12.6.2 Antimicrobial properties

#### *Antibacterial properties*

Coriander has strong antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* (Al-Jedah *et al.*, 2000). In 2002, a study carried out by Delaquis *et al.* reported that coriander oil strongly inhibited gram-positive bacteria (*Listeria monocytogenes* and *S. aureus*), but had little effect against gram-negative bacteria (*Pseudomonas fragi*, *E. coli*, *S. typhi*) (Delaquis *et al.*, 2002). Coriander contains an antibacterial compound, dodecenal, which is twice as effective as the commonly used antibiotic drug gentamicin in killing Salmonella (Kubo *et al.*, 2004). In addition to dodecenal, eight other antibiotic compounds were isolated from fresh coriander, which has led food scientists to suggest that dodecenal might be developed as a tasteless food additive to prevent foodborne illness. Methanol and water extracts of coriander leaves and stems were tested for antimicrobial activity against *Bacillus subtilis* and *E. coli* by determining cell damage. The greater bacterial cell damage caused by the methanol stem extracts resulted in a greater growth inhibition of the bacteria, which corresponded to the ferrous sequestering activity of the methanol-derived stem extracts (Wong and Kitts, 2006). However, some studies have found that the decoction of coriander does not have antibacterial potential against gram-positive or gram-negative bacteria and urinary pathogens (Chaudry and Tariq 2006; Saeed and Tariq, 2007).

#### *Antifungal activity*

Coriander oil did not affect mycelia growth (*Aspergillus parasiticus*) and did not affect the aflatoxin content of the fungus (Atanda *et al.*, 2007). Delaquis *et al.* (2000) reported that coriander oil had an inhibitory effect on *Saccharomyces cerevisiae*; but Chaudry and Tariq, (2006) and Saeed and Tariq (2007) reported that a decoction of coriander had no antifungal effect against *Candida albicans*.

#### *Anthelmintic activity*

Crude aqueous and hydro-alcoholic extracts of the seeds of *C. sativum* completely inhibited the hatching of nematode eggs at concentrations lower than 0.5 mg/mL with no statistically significant difference between the extracts. However, the hydro-alcoholic extract showed better *in vitro* activity against adult parasites than the aqueous extract (Egualle *et al.*, 2007).



The efficacy of the anthelmintic activity *in vivo* was tested by faecal egg count reduction (FECR) and total worm count reduction (TWCR) in sheep artificially infected with *Haemonchus contortus*. Significant FECR was detected on the second day after treatment with 0.9 g/kg of crude aqueous extract of *C. sativum*. On days 7 and 14 FECR was also detected at 0.45 g/kg dose of crude aqueous extract. A significant TWCR was only detected with a 0.9 g/kg dose of crude aqueous extract (Egualle *et al.*, 2007).

### 12.6.3 Antidiabetic activity

*C. sativum* showed significant hypoglycemic action in rats fed with a high-cholesterol diet. The activity of glycogen synthase was increased, which also revealed an increase in the concentration of hepatic glycogen, while the activity of glycogen phosphorylase and gluconeogenic enzymes revealed a decrease in the rate of glycogenolysis and gluconeogenesis. There were also increased activities of glucose-6-phosphate dehydrogenase and glycolytic enzymes, suggesting that the glucose is used by the pentose phosphate pathway and glycolysis, respectively (Chitra and Leelamma, 1999b). In an *in vitro* study to assess the possible effects of aqueous coriander plant extract (50 g plant extract/l) on glucose diffusion across the gastrointestinal tract, it was found that the extract significantly decreased glucose diffusion compared to control with mean external glucose concentration of  $6.4 \pm 0.2$  mmol/l at 26 hours. Part of the antihyperglycemic action of *C. sativum* may be due to decreased glucose absorption *in vivo* (Gallagher *et al.*, 2003). Other recent research studies have confirmed that coriander helps to stimulate the secretion of insulin and cause lowering of the blood sugar (Gray and Flatt, 1999), while Srinivasan (2005) has also discussed the hypoglycemic properties of coriander.

### 12.6.4 Anticholesterol activity

In a study conducted on triton-induced hyperlipidemic rats, *C. sativum* at 1.0 g/kg body weight reduced the level of cholesterol and triglycerides. Coriander decreases the uptake and enhances the breakdown of lipids, and may therefore provide preventative and curative effects against hypolipidemia (Chithra and Leelamma, 1997; Lal *et al.*, 2004). Coriander has been found to reduce the amount of damaged fats (lipid peroxides) in cell membranes and to lower the levels of total and LDL (the 'bad' cholesterol), while actually increasing levels of HDL (the 'good' cholesterol) (Chitra and Leelamma, 1999a).

### 12.6.5 Antimutagenicity

Coriander played a protective role against the deleterious effects in lipid metabolism in experimental colon cancer induced by 1, 2-dimethyl hydrazine in rats (Chitra and Leelamma, 2000). The antimutagenicity of coriander juice against the mutagenic activity of 4-nitro-o-phenylenediamine *m*-phenylenediamine and 2-aminofluorene was investigated using the Ames reversion mutagenesis assay (his- to his+) with the *S. typhi* TA98 strain as the indicator organism. It was found that aqueous crude coriander juice significantly decreased the mutagenicity of metabolized amines

(Cortes-Eslava *et al.*, 2004). The capacity of coriander essential oil to induce nuclear DNA damage-responsive genes was tested using suitable Lac-Z fusion strains for *RNR3* and *RAD51*, which are genes involved in DNA metabolism and repair, respectively. At equitoxic dose, the essential oil demonstrated significant gene induction, approximately the same as that caused by hydrogen peroxide, but much lower than that caused by methyl methane sulfonate (MMS). It affected the mitochondrial structure and function and was shown to be able to stimulate the transcriptional expression of DNA damage-responsive genes. It appeared that the induction of microbial damage was closely linked to the overall cellular cytotoxicity by the essential oil, which also appeared to mask the occurrence of nuclear genetic events (Bakkali *et al.*, 2005).

#### **12.6.6 Immunomodulatory activity**

The aqueous crude extracts of *C. sativum* stimulated the proliferation of human peripheral blood mononuclear cells (PBMC) and the secretion of IFN- $\gamma$  at concentrations between 50 and 200  $\mu\text{g/mL}$ . Further, studies on several bioactive compounds known to be of the extract showed that the flavonoid quercetin stimulated the proliferation of human PBMC and the secretion of IFN- $\gamma$ . However, the flavonoid rutin and coumarins bergapten and xanthotoxin were shown to modulate the secretion of IFN- $\gamma$  but did not enhance the proliferation of human PBMC, while the coumarin isopimpinellin promoted the proliferation of PBMC but did not modulate the secretion of IFN- $\gamma$  (Cherng *et al.*, 2008).

#### **12.6.7 Other effects**

##### *Anxiolytic and sedative effects*

The aqueous extract of *C. sativum* seed has an anxiolytic effect and may also act as a sedative and muscle relaxant. The aqueous extract (100 mg/kg i.p.) showed an anxiolytic effect in male albino mice using the elevated plus maze model: there was increasing time spent on the open arms of the maze and the percentage of open arm entries also increased. Furthermore, the aqueous extract (50, 100 and 500 mg/kg) significantly reduced spontaneous activity and neuromuscular coordination compared to the control group (Emamghoreishi *et al.*, 2005). The extract and essential oil of coriander seed possess (unknown) sedative–hypnotic activity. However, the major active component(s) responsible for hypnotic effect are mainly present in the aqueous extract (Emamghoreishi and Heidari-Hamedani, 2006).

##### *Diuretic effect*

The aqueous extract of coriander increased diuresis and the urinary excretion of sodium potassium chloride and the glomerular filtration rate at doses of 40 and 100 mg/kg administered by intravenous infusion (120 min) in anesthetized Wistar rats. The mechanism of diuretic action of coriander appeared to be similar to that of furosemide (Aissaoui *et al.*, 2008). However, furosemide was more potent as a diuretic and saluratic.

### *Antifertility activity*

The effect of the aqueous extract of fresh coriander seeds on female fertility in rats was studied. The extract (250 and 500 mg/kg orally) produced a dose-dependent significant anti-implantation effect, but failed to produce complete infertility (Ai-Said *et al.*, 1987).

### *Prevention of heavy metal deposition*

It has been suggested that *C. sativum* has a preventive effect on localized lead deposition in ICR mice. The administration of *C. sativum* significantly decreased lead deposition in the femur and reduced severe lead-induced injury in the kidney of ICR mice which were given lead (1000 ppm) as lead acetate trihydrate in drinking water for 32 days (Aga *et al.*, 2001).

### *Effects on bowel disorders*

A polyherbal ayurvedic formulation containing ripe coriander fruits as one of its major ingredients was tested on two different experimental animal models of inflammatory bowel disease (acetic acid-induced colitis in mice and indomethacin-induced enterocolitis in rats). The results obtained showed that the formulation was an effective remedy for inflammatory bowel disease (Jagtap *et al.*, 2004). A herbal medicine carmint containing a total extract of *C. sativum*, *Melissa officinalis* and *Menta spicata* was found to be effective and to have antispasmodic, carminative and sedative effects, observed in a pilot study in patients with irritable bowel syndrome (Vejdani *et al.*, 2006).

### *Effects on the lipid composition of meat*

Dietary supplementation by coriander seed greatly affected the lipid composition of meat by decreasing the saturated fatty acids (SFA) content (plasmic and stearic acids) and by increasing the non-saturated and polyunsaturated fatty acid (MUFA and PUFA) proportions in comparison to the control group ( $p < 0.01$ ). The highest dosage of coriander seed (4 % added to the ration) has systematically induced the most significant effect on the fatty acid composition. Consequently, dietary supplementation by coriander seed would improve the quality of lipids of quintals by lowering the SFA proportion and by enhancing the PUFA content, particularly of n3-PUFA (Ertas *et al.*, 2005).

## **12.7 Quality issues**

The quality of coriander relates to its characteristics in terms of size, shape, appearance, color, odor and aroma. These characteristics vary widely, depending upon the variety, the agro-climatic conditions in the production region and harvest and post-harvest operations. The moisture, volatile oil and oleoresin content of coriander, along with the major chemical constituents present, all determine its intrinsic quality. The produce must be safe and must not contain health-hazardous substances or other contaminants at levels above those stipulated in the relevant quality regulations or specifications.

### 12.7.1 Contaminants

#### *Physical contaminants*

Physical contaminants are known as macro-contaminants and determine the extrinsic quality (seed size, shape, appearance and color) of the produce. Major defects in this class include immature or shriveled seeds, products infested or damaged by insects, excreta of mammals (rodents, cattle, etc.), excreta of other animals such as insects and birds, extraneous foreign matter (other parts of the coriander plant, or parts of other plants) and filth. Filth can be classified as heavy filth, including sand and mud particles, and light filth, including parts of insects, birds or animals, which are considered to be unacceptable in any food material.

#### *Chemical contaminants*

With respect to chemical contaminants, the most significant defects are those due to the presence of added coloring material, preservatives, antioxidants, fumigants (SO<sub>2</sub>, ethyl oxide, methyl bromide), aflatoxin, trace metals (lead, arsenic, chromium, cadmium, copper, zinc, etc.) and pesticide residues.

#### *Microbial contaminants*

Microbial contaminants include pathogenic bacteria, such as *Salmonella*, *E. coli* and yeasts and molds. Some microbial contaminants pose a serious risk to human health.

### 12.7.2 Factors influencing seed quality

#### *Production practices*

The major focus of efforts to improve production practices for seed spices in the past was on higher production or productivity, while quality considerations were generally given little attention in developing countries, principally for the following reasons:

- increased yield is of immediate benefit to the farmers;
- no adequate scientific grading method was developed, mainly because seeds of different quality did not command different prices;
- lack of understanding of the quality characteristics and how to achieve them;
- lack of facilities to evaluate quality objectives.

The advent of modern chromatographic techniques has made it easier to assess the quality of seed spices; there is thus a greater awareness of quality issues and fierce competition for quality in the international market for export-oriented seed spices including coriander. Due attention is now being given to the development of high-quality varieties with appropriate post-harvest management techniques.

#### *Climatic conditions*

The role of climatic conditions on the biosynthesis pattern of volatile oil constituents in coriander is well known and the differences are discussed above in Section 12.2. Lytkin (1953) has observed that the cooler and drier climate of northern Europe produces more linalool in coriander than the tropical climate of India and Morocco. Hotin (1957) observed that when the fruit ripens at high humidity, the volatile oil content in the fruit may be high, but its organoleptic quality is poorer, owing to lower linalool contents and more aldehyde contents.

*Soil and soil fertility*

Coriander is grown under a wide range of conditions; however, the best yield and highest volatile oil content are obtained on a medium to heavy soil in a sunny location with good drainage and well-distributed moisture. Coriander is usually grown as a rain-fed crop, and judicious use of fertilizers has also been shown to have a beneficial effect on the volatile oil content as well as on the seed yield of coriander (Pillai and Bhoominathan, 1975; Prakash Rao *et al.*, 1983; Rahman *et al.*, 1990).

*Weeds*

Adulteration of the spices by weeds affects their quality. The presence of weed seeds in coriander seed will adversely affect the volatile oil content and test weight of coriander.

*Diseases*

Diseases such as wilt, powdery mildew and stem gall attack the crop, causing heavy loss of yield and leading to a deterioration in quality. These diseases can all be controlled by following good management practices and control measures (Sharma *et al.*, 1996).

**12.7.3 Quality assurance**

Quality assurance through an effective and efficient quality control system is pivotal to the achievement of increased sales of spices and its products, and grading and standardization are essential in ensuring product quality. Therefore, after processing, coriander to be exported should be graded according to International Organization for Standardization (ISO) standards, or according to the specific grades of the importing countries, most of which have their own systems. The importers prescribe grade specifications for various spices depending upon the end-use. For whole seed entering the grocery trade, the appearance of the seeds is the primary quality determinant. Appearance is less important for coriander intended for industrial extraction purposes, i.e. for essential oil and oleoresin. In these cases, the quantity and quality of volatile oil and its constituents are more important. There are food grade specifications in India for different grades of coriander fruit and powder contained in the Government of India AGMARK Spices Grading and Marking Rules. Tables 12.8 and 12.9 show the AGMARK 'special' and 'standard' grades for whole coriander fruits and powder.

Coriander for export must conform to the quality standards demanded by the importing countries (Sharma and Agrawal, 1998). In particular, spices exported to any country must conform to the cleanliness specifications stipulated by that country. These countries set limits for cleanliness specifications such as number of dead insects, amount of mammalian excreta and other excreta in the sample. If the exporting country does not fulfill these requirements, the consignment may be detained for reconditioning or be rejected. The major coriander importing countries, namely the UAE, Sri Lanka, Singapore, Malaysia, the UK, the USA and South Africa, are quality conscious and have strict quality standards. The USA, Japan, Canada, Australia and the European countries have their own stringent food laws and regulations to protect the health and the safety of their citizens. The exporting countries

**Table 12.8** Agmark Grade designations and quality of coriander (whole) – SCHEDULE XVI (see rules 3 and 4)

| Grade Designation                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                          | Quality                                           |                                    |                                                                                                                                                                                                 |                                |                                |                                          |                                         |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|---------------------------------------------------|------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|--------------------------------|------------------------------------------|-----------------------------------------|
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                          | Special Characteristics                           |                                    |                                                                                                                                                                                                 |                                |                                |                                          |                                         |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Organic<br>extraneous matter,<br>% (m/m) (Max.)          | Inorganic extraneous<br>matter, % (m/m)<br>(Max.) | Split fruits,<br>% (m/m)<br>(Max.) | Damaged, discolored,<br>shriveled, insect bored<br>fruits, % (m/m) (Max.)                                                                                                                       | Moisture,<br>% (m/m)<br>(Max.) | Total ash<br>% (m/m)<br>(Max.) | Acid insoluble<br>ash, % (m/m)<br>(Max.) | Volatile oil %<br>(ml/100 gm)<br>(Min.) |
| (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | (2)                                                      | (3)                                               | (4)                                | (5)                                                                                                                                                                                             | (6)                            | (7)                            | (8)                                      | (9)                                     |
| Special                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 0.8                                                      | 0.2                                               | 5.0                                | 2.0                                                                                                                                                                                             | 9.0                            | 7.0                            | 1.0                                      | 0.2                                     |
| Standard                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 3.5                                                      | 0.5                                               | 30.0                               | 5.0                                                                                                                                                                                             | 10.0                           | 7.0                            | 1.5                                      | 0.1                                     |
| General Characteristics                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                          |                                                   |                                    |                                                                                                                                                                                                 |                                |                                |                                          |                                         |
| (10)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                          |                                                   |                                    |                                                                                                                                                                                                 |                                |                                |                                          |                                         |
| <p>(1) Coriander (Whole) shall be dried mature fruit of <i>Coriandrum sativum</i> L.</p> <p>(2) It shall not contain any added coloring matter, or preservatives;</p> <p>(3) It shall be free from mold growth and living insects and practically free from dead insects, insect fragments and rodent contamination;</p> <p>(4) It shall comply with restrictions in regard to Aflatoxins, Metallic Contaminants, Insecticide or Pesticide residue, poisonous metals, naturally occurring Contaminants, Microbial load and the like as specified by the Codex Alimentarius Commission or as per buyers requirements for Export purposes and the Prevention of Food Adulteration Rules, 1955 for domestic trade.</p> |                                                          |                                                   |                                    |                                                                                                                                                                                                 |                                |                                |                                          |                                         |
| <b>Definitions:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                          |                                                   |                                    |                                                                                                                                                                                                 |                                |                                |                                          |                                         |
| (a)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 'Damaged, discolored, shriveled,<br>insect bored fruits' |                                                   |                                    | includes whole or split fruits that are damaged, discolored or shriveled and fruits showing signs of partial or whole bores as a result of their having been eaten by weevils or other insects; |                                |                                |                                          |                                         |
| (b)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 'Inorganic extraneous matter'                            |                                                   |                                    | includes stones particles of soil and sand;                                                                                                                                                     |                                |                                |                                          |                                         |
| (c)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 'Organic extraneous matter'                              |                                                   |                                    | All organic material other than Coriander seeds and includes all matter of vegetable origin;                                                                                                    |                                |                                |                                          |                                         |
| (d)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 'Split fruits'                                           |                                                   |                                    | Includes fruits which have been split longitudinally into two parts.                                                                                                                            |                                |                                |                                          |                                         |

**Table 12.9** Agmark Grade designations and quality of coriander (powder) – SCHEDULE XVI (see rules 3 and 4)

| Grade Designation                                                                                                                                                                                                                                                                                                                                                                   | Quality                  |                           |                                    |                             |                                           |                                   |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|---------------------------|------------------------------------|-----------------------------|-------------------------------------------|-----------------------------------|
| Special Characteristics                                                                                                                                                                                                                                                                                                                                                             |                          |                           |                                    |                             |                                           |                                   |
|                                                                                                                                                                                                                                                                                                                                                                                     | Moisture, % (m/m) (Max.) | Total ash, % (m/m) (Max.) | Acid insoluble ash, % (m/m) (Max.) | Crude fiber, % (m/m) (Max.) | Non-volatile ether extract % (m/m) (Min.) | Volatile oil % (ml/100 gm) (Min.) |
| (1)                                                                                                                                                                                                                                                                                                                                                                                 | (2)                      | (3)                       | (4)                                | (5)                         | (6)                                       | (7)                               |
| Special                                                                                                                                                                                                                                                                                                                                                                             | 9.0                      | 6.5                       | 1.0                                | 25.0                        | 15.0                                      | 0.10                              |
| Standard                                                                                                                                                                                                                                                                                                                                                                            | 10.0                     | 7.0                       | 1.5                                | 28.0                        | 12.0                                      | 0.05                              |
| General Characteristics                                                                                                                                                                                                                                                                                                                                                             |                          |                           |                                    |                             |                                           |                                   |
| (8)                                                                                                                                                                                                                                                                                                                                                                                 |                          |                           |                                    |                             |                                           |                                   |
| (1) Coriander powder shall be obtained by grinding clean, sound, dried and mature fruits of <i>Coriandrum sativum</i> L;                                                                                                                                                                                                                                                            |                          |                           |                                    |                             |                                           |                                   |
| (2) It shall be ground to such a fineness that it shall pass completely through a 500 micron sieve. However, for standard grade, 95% of it should pass through 1000 micron sieve;                                                                                                                                                                                                   |                          |                           |                                    |                             |                                           |                                   |
| (3) It shall have a typical aroma and flavor characteristic of the spice and shall be free from musty odor;                                                                                                                                                                                                                                                                         |                          |                           |                                    |                             |                                           |                                   |
| (4) It shall not contain any added coloring matter, preservatives or any foreign matter;                                                                                                                                                                                                                                                                                            |                          |                           |                                    |                             |                                           |                                   |
| (5) It shall be free from living insects and practically free from molds, dead insects, insect fragments and rodent contamination;                                                                                                                                                                                                                                                  |                          |                           |                                    |                             |                                           |                                   |
| (6) It shall comply with restrictions in regard to Aflatoxins, Metallic Contaminants, Insecticide or Pesticide residue, poisonous metals, naturally occurring Contaminants, Microbial load and the like as specified by the Codex Alimentarius Commission or as per buyers requirements for Export purposes and the Prevention of Food Adulteration Rules, 1955 for domestic trade. |                          |                           |                                    |                             |                                           |                                   |

must adhere to the practices for cultivation, post-harvest operations, packaging and storage, to maintain high-quality standards in order to compete in the international markets.

Prior to export, the quality standards in the destination market should be thoroughly understood. There are official quality standards monographs for coriander fruit published in the Ayurvedic Pharmacopoeia of India (API), European Pharmacopoeia (PhEur), Siddha Pharmacopoeia of India (SPI) and Unani Pharmacopoeia of India (UPI). Table 12.10 compares these official pharmacopoeial quality standards. The Spices Board India provides a useful discussion of the major quality specifications for spices, including coriander, as follows:

The most popular specification for spices and herbs the world over is the 'ASTA Cleanliness Specifications for Spices, Seeds and Herbs'. The unified ASTA, US FDA Cleanliness Specifications for Spices, Seeds and Herbs were made effective from 1 January 1990. Major producing countries have built up their facilities to meet the requirements as per ASTA Cleanliness Specification [Table 12.11]. Countries such as the UK, Germany and Netherlands have laid down cleanliness specification for spices [Table 12.12].



**Table 12.10** Comparison of coriander fruit pharmacopoeial quality standards

| Standard                   | API and UPI                                | PhEur                                                              | SPI                                                     |
|----------------------------|--------------------------------------------|--------------------------------------------------------------------|---------------------------------------------------------|
| Identification tests:      | Macroscopic<br>Microscopic<br>Organoleptic | Macroscopic<br>Microscopic<br>Thin-layer chromatography            | Macroscopic<br>Microscopic<br>Thin-layer chromatography |
| Foreign matter             | ≤2.0 %                                     | ≤2.0 % and none of the cremocarps show perforations due to animals | ≤2.0 %                                                  |
| Loss on drying             | No standard                                | ≤10.0 %                                                            | No standard                                             |
| Total ash                  | ≤6.0 %                                     | ≤8.0 %                                                             | ≤6.0 %                                                  |
| Acid-insoluble ash         | ≤1.5 %                                     | No standard                                                        | ≤1.5 %                                                  |
| Alcohol-soluble extractive | ≥10.0 %                                    | No standard                                                        | ≥10.0 %                                                 |
| Water-soluble extractive   | ≥19.0 %                                    | No standard                                                        | ≥19.0 %                                                 |
| Essential oil              | ≥0.3 % (v/v)                               | ≥3 ml/kg                                                           | ≥0.3 % (v/w)                                            |

Notes:

API I (2001): Ayurvedic Pharmacopoeia of India, Part I, Volume I, First Edition.

PhEur 6.0 (2009): European Pharmacopoeia, Sixth Edition.

SPI I (2008): Siddha Pharmacopoeia of India, Part I, Volume I.

UPI I (1999): Unani Pharmacopoeia of India, Part I, Volume I.

**Table 12.11** American Spice Trade Association (ASTA) cleanliness specifications for spices and herbs

| Cleanliness specifications | Whole insects, dead <sup>1</sup> | Excreta, mammalian | Excreta, mammalian | Mold    | Insect defiled/infested | Extraneous/foreign matter <sup>2</sup> |
|----------------------------|----------------------------------|--------------------|--------------------|---------|-------------------------|----------------------------------------|
| Name of spice              | By count                         | By Mg/Lb           | By Mg/Lb           | %/by Wt | %/by Wt                 | %/by Wt                                |
| Coriander                  | 4                                | 3                  | 10                 | 1       | 1                       | 0.5                                    |

<sup>1</sup> Whole insects, dead: cannot exceed the limits shown.

<sup>2</sup> Extraneous matter: includes other plant material. e.g. foreign leaves.

**Table 12.12** Cleanliness specifications for spices in major importing countries

| Name of country/spice | Extraneous matter %/wt | Moisture %/wt. | Total ash %/wt | Acid insoluble ash %/wt. |
|-----------------------|------------------------|----------------|----------------|--------------------------|
| Germany               |                        |                |                |                          |
| Coriander             | –                      | –              | 8.0            | 2.5                      |
| Netherlands           | 1.5                    | 10.0           | 8.0            | 2.5                      |
| UK                    | 2.0                    | 10.0           | 8.0            | 1.5                      |

Methodology used in setting standards:

Moistures ISO939

Total ash ISO928

Acid insoluble ash ISO930

Volatile oil ISO6571

Refer to the above methods when analysing the products.

Source: specification in Germany, Netherlands & UK (Importers' specifications).

The European Spice Association (ESA), comprising the members of the European Union, has come out with the 'quality minima for herbs and spices' [Table 12.13]. This serves as guideline specifications for member countries in the European Union. The European Union has yet to finalize the cleanliness specification for spices and spice products. The importing countries, where they do not have specifications for spices, used to request the exporting countries to supply spices as per the ASTA Specification.

In addition to the cleanliness specification, the importing countries insist on the specification for parameters such as pesticide residues, aflatoxin, trace metal contamination and microbial contamination. Individual member countries in the European Union have fixed maximum residue levels (MRLs) for pesticide residues. The European Union has not prescribed the limits for pesticide residues in spices and spice products. The USA and Japan have prescribed the MRLs in spices. Under the Codex,

**Table 12.13** European Spice Association (ESA) specifications of quality minima for

| Subject            | Specifications                                                                                                                                                                                                                                                                                                           |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Extraneous matter  | 1%                                                                                                                                                                                                                                                                                                                       |
| Sampling           | (For routine sampling) Square root of units/lots to a maximum of 10 samples. (For arbitration purposes) Square root of all containers, e.g. 1 lot of coriander may = 400 bags, therefore square root = 20 samples.                                                                                                       |
| Foreign Matter     | Maximum 2%                                                                                                                                                                                                                                                                                                               |
| Ash                | 7(ISO) % W/W max.                                                                                                                                                                                                                                                                                                        |
| Acid Insoluble Ash | 1.5 (ISO) % W/W max.                                                                                                                                                                                                                                                                                                     |
| H <sub>2</sub> O   | 12 (ISO) H <sub>2</sub> O % W/W max.                                                                                                                                                                                                                                                                                     |
| Packaging          | Should be agreed between buyer and seller. If made of jute and sisal, they should conform to the standards set by CAOBISCO Ref C502-51-sj of 20-02-95. However, these materials are not favored by the industry, as they are a source of product contamination, with loose fibers from the sacking entering the product. |
| Heavy metals       | Shall comply with national/Eu legislation.                                                                                                                                                                                                                                                                               |
| Pesticides         | Shall be utilized in accordance with manufacturers' recommendations and good agricultural practices and comply with existing national and/or EU legislation.                                                                                                                                                             |
| Treatments         | Use of any EC approved fumigants in accordance with manufacturers' instructions, to be indicated on accompanying documents. (Irradiation should not be used unless agreed between buyer and seller.)                                                                                                                     |
| Microbiology       | Salmonella absent in (at least) 25 g.<br>Yeast and Molds 105/g target, 106/g absolute maximum<br><i>E. coli</i> . 102/g target, 103/g absolute maximum<br>Other requirements to be agreed between buyer and seller.                                                                                                      |
| Off odors          | Shall be free from off odor or taste                                                                                                                                                                                                                                                                                     |
| Infestation        | Should be free in practical terms from live and/or dead insects fragments and rodent contamination visible to the naked eye (corrected in necessary for abnormal vision).                                                                                                                                                |
| Aflatoxins         | Should be grown, harvested, handled and stored in such a manner as to prevent the occurrence of aflatoxins or minimize the risk of occurrence. If found, levels should comply with existing national and/or EU legislation.                                                                                              |
| Volatile oil       | 0.3 (ESA) V/O % V/W min.                                                                                                                                                                                                                                                                                                 |
| Adulteration       | Shall be free from.                                                                                                                                                                                                                                                                                                      |
| Bulk density       | To be agreed between buyer and seller.                                                                                                                                                                                                                                                                                   |
| Species            | To be agreed between buyer and seller.                                                                                                                                                                                                                                                                                   |
| Documents          | Should provide details of any treatments the product has undergone; name of product; weight; country of origin; lot identification/batch number; year of harvest.                                                                                                                                                        |

**Table 12.14** Summary of legislation on aflatoxins in ESA member countries and other major importing countries

| Country        | Permitted levels                                | For which products                                                      | Comments                                                                                               |
|----------------|-------------------------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Austria        | B1 < 1 ppb                                      | All foodstuffs (except mechanically prepared cereals in the case of B1) |                                                                                                        |
| Belgium        | <5 ppb for peanuts. EU legislation is expected. |                                                                         | In Belgian law, aflatoxins (and toxins in general) may not present in foodstuffs, i.e. not detectable. |
| Germany        | B1 + B2 + G1 + G2 < 4 ppb                       | All foodstuffs                                                          |                                                                                                        |
| Denmark        | B1 < 2 ppb                                      |                                                                         |                                                                                                        |
| Netherlands    | B1 < 5 ppb                                      | All foodstuffs                                                          | No control on B2                                                                                       |
| Switzerland    | B1 < 1 ppb                                      | All foodstuffs (except maize)                                           |                                                                                                        |
|                | B2 + G1 + G2 < 5 ppb                            | All foodstuffs                                                          |                                                                                                        |
| UK             | <50 ppb                                         | Chilli                                                                  | Only aflatoxin Regulations on nuts/nut products                                                        |
|                | <10 ppb                                         | Peanuts                                                                 | Dried figs/dried fig products, which when sold to the consumer must contain <4 ppb total aflatoxin.    |
|                | <4 ppb                                          | Other nuts/dried figs, etc.                                             |                                                                                                        |
| Spain          | B1 < 5 ppb<br>B1 + B2 + G1 + G2 < 10 ppb        | All foodstuffs                                                          |                                                                                                        |
| Sweden         | B1 + B2 + G1 + G2 < 5 ppb                       | All foodstuffs                                                          |                                                                                                        |
| Finland        | B1 + B2 + G1 + G2 < 5 ppb                       | All foodstuffs                                                          |                                                                                                        |
| Italy + France | <10 ppb for B1                                  |                                                                         | No regulations                                                                                         |
| USA            | <20 ppb                                         | All foodstuffs                                                          | Guideline FDA                                                                                          |

Source: EU Draft Legislation.

MRLs for pesticide residues have not been prescribed. Some countries have prescribed pesticide residual limits for some specific spices. India has taken the initiative to fix the MRLs for spices at the Codex level. The European Union has prescribed limits for aflatoxin as 5 ppb for aflatoxin B1 and 10 ppb for aflatoxin total. Member countries in the European Union and others have fixed limits for aflatoxin varying from 1 ppb to 20 ppb [Table 12.14].

Importing countries are cautious about the microbial contamination in spices at the time of import. Almost all the importing countries have fixed the limits for Salmonella as absent in 25 g. Specifications have been prescribed by major importing countries for the microbial parameters such as total plate count (TPC), *E. coli*, yeast, mold, coliforms, etc. The limits for the above parameters vary from country to country. [Table 12.15].

**Table 12.15** General microbiological specification – Germany and the Netherlands

| Parameter                      | Standard value           | Danger value                                 |
|--------------------------------|--------------------------|----------------------------------------------|
| <b>Germany</b>                 |                          |                                              |
| Total aerobic bacteria         | $1 \times 10^5/\text{g}$ | $1 \times 10^6/\text{g}$                     |
| <i>E. coli</i>                 | Absent                   | Absent                                       |
| <i>Bacillus cereus</i>         | $1 \times 10^4/\text{g}$ | $1 \times 10^5/\text{g}$                     |
| <i>Staphylococcus aureus</i>   | $1 \times 10^2/\text{g}$ | $1 \times 10^3/\text{g}$                     |
| <i>Salmonella</i>              | Absent in 25 g           | Absent in 25 g                               |
| Sulfite-reducing clostridia    | $1 \times 10^4/\text{g}$ | $1 \times 10^5/\text{gm}$                    |
| <b>Netherlands</b>             |                          |                                              |
| <i>Bacillus cereus</i>         | Absent in 20 g           | The danger values similar to that of Germany |
| <i>E. coli</i>                 | " "                      |                                              |
| <i>Clostridium perfringens</i> | " "                      |                                              |
| <i>Staphylococcus aureus</i>   | " "                      |                                              |
| <i>Salmonella</i>              | " "                      |                                              |
| Total aerobic bacteria         | $1 \times 10^6/\text{g}$ |                                              |
| Yeast and mold                 | $1 \times 10^3/\text{g}$ |                                              |
| Coliform                       | $1 \times 10^2/\text{g}$ |                                              |

It is clear from the above that the utmost care in production practices and post-harvest technology is essential for any country interested in exporting coriander.

#### 12.7.4 Adulteration

Coriander seed is available both whole and in ground form. It is subject to adulteration by addition of exhausted or spent seed, from which oil or oleoresins have been extracted, excess stem and plant debris, chaff, earth and dust, etc. In addition, good-quality coriander seeds may be adulterated by addition of seeds of inferior grade. The oil is adulterated with herbage oil, which is poor in linalool content. These adulterations can be detected by gas chromatography or by TLC coupled with high-performance liquid chromatography. Adulteration at any level can be detected by using the specifications outlined above for the whole seed, powder, volatile oil and oleoresins. A trained perfumer or essential oil analyst might immediately spot quality problems with an oil sample before carrying out the sophisticated analysis tests. The trained nose can still be the single most important arbiter of quality despite the technology that is now available.

#### 12.7.5 Health hazards and toxicity

There is no toxicity reported to be associated with the use of products derived from coriander leaves and seeds, but the skin can become irritated by prolonged exposure to the concentrated liquid (oil and oleoresins) and vapors. These can also irritate and even damage the eyes and may be harmful if swallowed. The use of coriander

oil as a food ingredient is considered safe at the current levels and (Burdock and Carabin, 2008).

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## Appendix 1

Maximum pesticides residues limits in Netherlands & United Kingdom

| Active Substance       | Limiting Values in PPM |                |
|------------------------|------------------------|----------------|
|                        | Netherlands            | United Kingdom |
| HCH without Lindane    | 0.02                   | 0.02           |
| Lindane                | 0.02                   | –              |
| Hexachlorobenzene      | –                      | 0.01           |
| Aldrin and Dieldrin    | 0.03                   | 0.01           |
| Sum of DDT             | 0.15                   | 0.05           |
| Malathion              | 0.05                   | 8.00           |
| Dicofol                | 0.05                   | 0.50           |
| Chlorpyrifos           | 0.01                   | –              |
| Ethion                 | 0.01                   | –              |
| Chlordan               | 0.01                   | 0.02           |
| Parathion              | 0.10                   | 1.00           |
| Parathion methyl       | 0.10                   | 0.20           |
| Mevinphos              | 0.05                   | –              |
| Sum of Endosulfan      | 0.02                   | 0.10           |
| Phosalon               | 1.00                   | 0.10           |
| Vinclozolin            | –                      | 0.10           |
| Dimethoat              | 0.01                   | 0.05           |
| Quintozen              | –                      | 1.00           |
| Metacriphos            |                        |                |
| Heptachlor and epoxide | 0.21                   | 0.01           |
| Methidathion           |                        |                |
| Diazinon               | 0.05                   | 0.05           |
| Fenitrothion           | 0.05                   | 0.05           |
| Bromophos              |                        |                |
| Mecarbam               |                        |                |
| Methoxychlor           | 0.05                   |                |
| Omethoat               |                        | 0.20           |
| Dichlorvos             | 0.05                   |                |
| Phosmet                | 0.01                   |                |
| Methylbromide          |                        | 0.10           |
| Tetradifon             |                        |                |

## Appendix 2

Maximum residue levels fixed for spices as per the German legislation and Pesticide residue limits prescribed by Spain

| German*                              |                       | Spain                  |             |
|--------------------------------------|-----------------------|------------------------|-------------|
| Active Substance                     | Highest limit (mg/kg) | Name of the pesticides | MRL (mg/kg) |
| Aldrin and Dieldrin                  | 0.1                   | Acephate               | 0.10        |
| Chlordane                            | 0.05                  | Atrazine               | 0.10        |
| Sum of DDT Isomers                   | 1.0                   | Bendiocarb             | 0.05        |
| Endrin                               | 0.1                   | Carbaryl               | 0.10        |
| HCH without Lindane                  | 0.2                   | Carbosulfan            | 0.10        |
| Heptachlor and Epoxide               | 0.1                   | Chlorpyrifos           | 0.05        |
| Hexachlor Benzol                     | 0.1                   | Chlorpyrifos – methyl  | 0.05        |
| Lindane                              | 0.01                  | Cipermethrin           | 0.05        |
| HCN and Cyanides                     | 15.0                  | Diazinon               | 0.05        |
| Bromides                             | 400.0                 | Dicofol                | 0.02        |
| Carbaryl                             | 0.1                   | Dimethoate             | 0.05        |
| Carbofuran                           | 0.2                   | Etion                  | 0.10        |
| Chlorpyrifos                         | 0.05                  | Fentoato               | 0.05        |
| Methyl Chlorpyrifos                  | 0.05                  | Fenitrothion           | 0.05        |
| Cypermethrin**                       | 0.05                  | Fenthron               | 0.05        |
| Deltamethrin                         | 0.05                  | Melathron              | 0.50        |
| Diazinol                             | 0.02                  | Metalaxyl              | 0.05        |
| Dichlorvos                           | 0.1                   | Methamidophos          | 0.01        |
| Diclofop methyl                      | 0.1                   | Monocrotophos          | 0.02        |
| Dicofol**                            | 0.02                  | Omethoate              | 0.10        |
| Dimethoate                           | 0.5                   | Phosalone              | 0.10        |
| Disulfoton                           | 0.02                  | Pirimicarb             | 0.05        |
| Dithiocarbamate                      | 0.05                  | Pirimiphos – methyl    | 0.01        |
| Endosulfan**                         | 0.05                  | Profenofos             | 0.02        |
| Ethion                               | 0.05                  | Prothiofos             | 0.02        |
| Fenitrothion                         | 0.05                  | Pyrazophos             | 0.01        |
| Fenvalarate**                        | 0.05                  | Terbuconazole          | 0.05        |
| Copper based pesticides              | 40.0                  | Tolclophos – methyl    | 0.01        |
| Malathion                            | 0.05                  | Triazophos             | 0.01        |
| Methyl Bromide                       |                       | Vinclozolin            | 0.05        |
| Mevinphos                            | 0.05                  |                        |             |
| Omethoate                            | 0.05                  |                        |             |
| Parathion and Paraoxon               | 0.1                   |                        |             |
| Methyl Parathion and Methyl Paraoxon | 0.1                   |                        |             |
| Phorate                              | 0.05                  |                        |             |
| Phosalone                            | 0.05                  |                        |             |
| Phosphamidon                         | 0.05                  |                        |             |
| Pyrethrin                            | 0.5                   |                        |             |
| Quinalphos                           | 0.01                  |                        |             |
| Quintozen                            | 0.01                  |                        |             |

\* Note: Of the above, the limits mentioned against the pesticides 1 to 10 are specific for spices and the remaining are the general regulations for all plant foods.

\*\* Sum of Isomers

# 13

## Cumin

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**Abstract:** Cumin is a strong aromatic spice with a sweet aroma and a slightly bitter and pungent taste, made from the dried ripe fruit of *Cuminum cyminum* L. This chapter presents a full history of the different uses of cumin from ancient times to modern day. The chemical structure of cumin is described along with production methods for seed, essential oil and aromatic water. Cumin's uses in food processing, its functional properties in relation to both traditional and modern medicine and its nutritional properties are discussed and quality specifications for the seeds, powder and oil are presented.

**Key words:** cumin, *Cuminum cyminum*, zireh-e sabz, zira, kemon.

### 13.1 Introduction

Cumin is a strong aromatic of dried ripe fruit of *Cuminum cyminum* L. (Umbelliferae or Apiaceae family). Although the part of the plant which is used is generally called a seed, it is in fact a fruit (Fig. 13.1), composed of both the pericarp and the seed. It is variously known as: cumin, kummel, comino, zireh-e sabz, cumino, kemon, zira and kamun. The cumin which is sold in the market is a mixture of united and separated mericarps (cotyledons), which are yellowish green or yellowish brown, elongated ovoid and 3–6 mm in length. The surface has five primary ridges alternating with four less distinct secondary ridges bearing numerous short hairs. Some fruits have a short attached stalk (Fig. 13.1). These fruits (seeds) belong to a small annual herb, 15–50 cm in height, with long slender and white roots, bi-divaricated branching stem, long, narrow deep green slender leaves and small umbels of white or rose-colored flowers, covered with tiny hairs. The spice has a strong, sweet and spicy aroma with a slightly bitter and pungent taste, which is especially present in the ground spice. Dry-frying before grinding produces less pronounced flavor, and introduces overtones of nuttiness.

Cumin is indigenous to Egypt (upper Nile valley), the Mediterranean region, Iran and India. Today, it is cultivated in Mediterranean countries, Saudi Arabia, Iran, Turkey, India, Mexico, southern Russia, Indonesia, Japan and China. Nowadays, most cumin is grown in Iran, Sicily, India and Malta. Cumin seeds were used by the ancient Greeks and Romans in their cuisine. The popularity of cumin is thought partly to have been due to the fact that it was used as a less expensive alternative to black pepper. Cumin is mentioned in Isaiah xxvi 25, 27 and Matthew xxii 23, and



**Fig. 13.1** Fruit (seed) of cumin.

in the works of Hippocrates and Dioscorides. It was found in the pyramids, suggesting it was known to the Egyptians 5000 years ago. References to threshing cumin with a rod can be found in the bible, and this practice is still carried on today in remote parts of the eastern Mediterranean. From Pliny, we learn that the Romans consumed the ground seed for medicinal purposes with bread, water or wine. During the Middle Ages in Europe, cumin was one of the most common spices used. Around that time, it was considered a symbol of love and fidelity. Wedding guests carried cumin in their pockets, and married soldiers were sent off to war with a loaf of cumin bread baked by their wives. Cumin's use for fortifying is also represented in certain Arabic traditions in which a paste of ground cumin, pepper and honey is thought to have aphrodisiac properties.

While cumin is still used extensively in Indian and Middle Eastern cuisines, it became less popular in Europe after the Middle Ages. Interest in its health-promoting properties are, however, now increasing interest in this spice worldwide.

### **13.1.1 Etymology**

The English name of cumin is derived from the old English Cymene, from Latin 'Cuminum' which is the Romanization of the Greek 'Kuminon', cognate with Hebrew kammon, Arabic Kammun. Forms of this word are attested in several ancient semitic languages, including Kamunu in Akkadian. The ultimate source is the Sumerian word Gamun. The earliest attested form of the word Kuminon is the Mycenaean Greek Ku-mi-on, written in Linear B syllabic script.

### **13.1.2 Chemical structure**

Cumin contains about 2–4.5 % of volatile oil (average yield is about 3 %) and about 10 % fixed oil, together with tannins, oleoresin, mucilage, gum, protein and malts. The characteristic cumin odor is due to the presence of its light yellow to brown

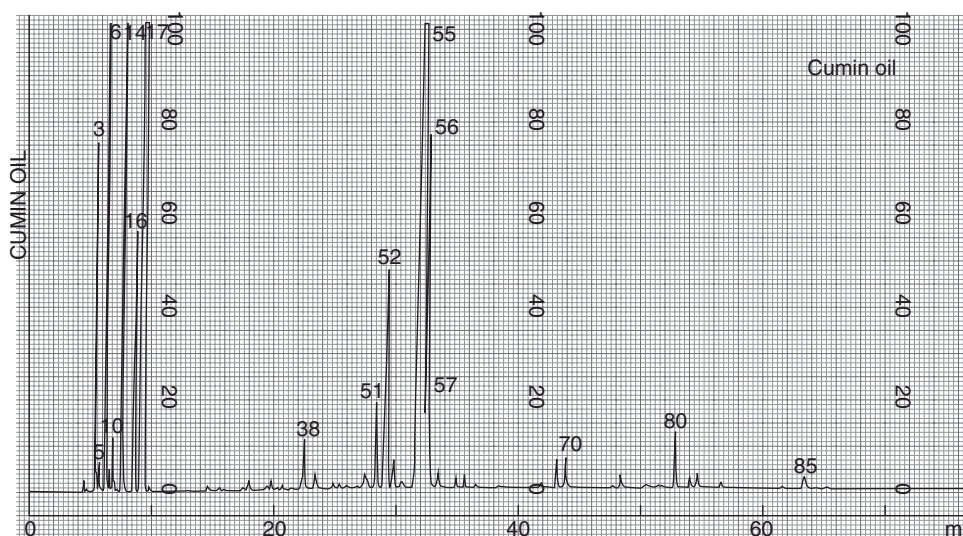


Fig. 13.2 Chromatogram of cumin oil.

liquid, having a strong and somewhat disagreeable essential oil. This odor and flavor is due principally to the aldehydes present (i.e., cumin aldehyde or cuminal, *p*-mentha-3-en-7-ol and *p*-mentha-1,3-dien-7-ol). Studies of the chemical composition of cumin oil showed the presence of the following components:  $\alpha$ -pinene (0.5 %), myrcene (0.3 %), limonene (9.5 %), 1-8-cineole (0.2 %), *p*-mentha-3-en-7-ol (0.7 %), *p*-mentha-1,3-dien-7-ol (5.6 %), caryophyllene (0.8 %),  $\beta$ -bisabolene (0.9 %),  $\beta$ -pinene (13.0 %), *p*-cymene (16.5 %),  $\beta$ -phellandrene (0.3 %),  $\gamma$ -terpinene (19.5 %), cumin aldehyde (32.4 %), perilla aldehyde (6.5 %), cuminal alcohol (2.8 %),  $\beta$ -farnesene (1.1 %) together with much smaller quantities of  $\alpha$ -phellandrene,  $\alpha$ -terpinene, *cis* and *trans* sabinene, myrtenol and  $\alpha$ -terpineol (Fig. 13.2). Some of major compounds are shown in Fig. 13.3.

## 13.2 Production of cumin

### 13.2.1 Seed production

Cumin is cultivated extensively as a cold season in plains areas and as a summer crop in the hills. The small white or pink flowers grow on small compound umbels like many of the plants in the Umbelliferae family. In the south part of the Khorasan province of Iran, it is the main crop in a dry farming system with an average production of about 12000 tons per year. It is also cultivated in northern India (the Himalayas, Punjab, Baluchistan, Kashmir) and in Eastern Europe. The three major types of cumin seed on the market, Iranian, Indian and Middle Eastern, differ in seed color, quantity of essential oil and flavor. Cumin has several varieties and some of them, such as Cumin (RZ345), are studied in India.

For the summer crop, the seeds are planted in early April. They should be sown in small pots, filled with light soil, and placed initially in a moderately warm bed to bring the plants on. They can then be hardened gradually in an open frame and

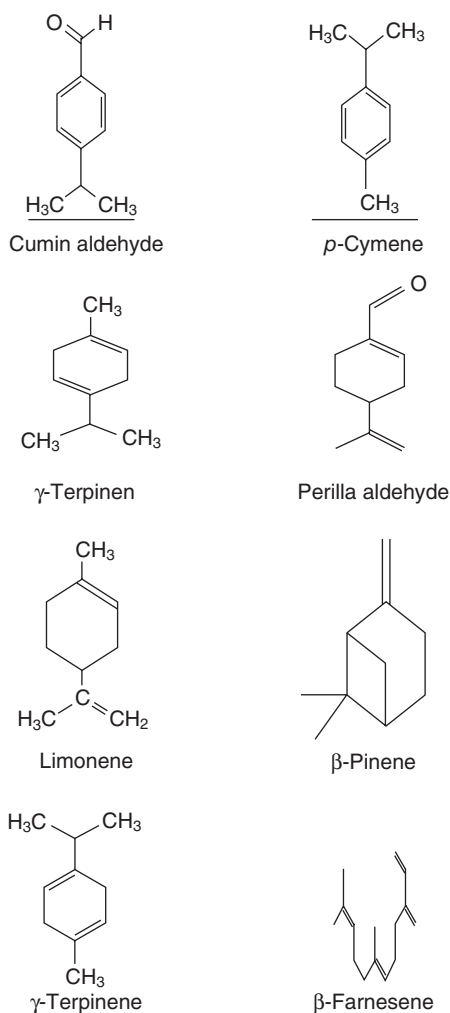


**Fig. 13.3** Chemical structures of some major components of cumin oil.

transplanted into a warm border of good soil. The plants bloom in June and July, and are harvested when 85 % of fruits are ripe. Harvesting takes place about 4 months after planting. The fresh seeds are spread out on cloth to dry and, after drying, are stored in cotton bags. Cumin seeds and cumin powder should be kept in tightly sealed glass containers in a dry, cool, dark place. Ground cumin will keep for about 6 months, while the whole seeds will stay fresh for about 1 year.

### 13.2.2 Seed oil production

The ripe seeds are used for oil production, as whole seeds or coarsely ground. If a freely alcohol-soluble oil is required, the whole seeds must be used. Hydrodistillation is used for essential oil extraction, producing a colorless or pale yellow oily liquid with a strong odor. The yield for oil production varies from 2.5–4.5 %, depending on whether the entire seed or the coarsely ground seed is distilled. Cumin oil



can be readily converted artificially into thymol. The volatile oil should be kept in well-sealed bottles or aluminum containers.

### **13.2.3 Aromatic cumin water production**

During cumin oil processing or as an independent process, the ripe seeds can be used to obtain cumin aromatic water or cumin distilled water. In many parts of cumin-producing countries, especially in central parts of Iran, there are many small traditional industries for this purpose.

Traditional equipment is employed. The ripe seeds are put in big vessels at a ratio of up to one part to ten parts water and heated. The boiling gaze is then cooled with water and the distilled water stored in an adjunct container. Commercially, distilled aromatic cumin water is obtained through cumin essential oil production.

## **13.3 Main uses of cumin in food processing**

Cumin seed is an ancient spice with a strong aromatic smell and warm, bitter taste. In Biblical times, cumin seeds were valued for their digestive properties and were used for flavoring bread and other dishes during the periods of ceremonial fasting, to make up for the lack of meat. Ground cumin is most popular in countries and regions in which the food is generally highly spiced, such as India, Iran and other parts of the Middle East, Mexico and North Africa. It is an essential ingredient in most Indian curry powders and garam masala. In Iran cumin is a very common spice flavor and fragrance agent for culinary and confectionery use; in Morocco it is used with lamb and in Mexico with chili con carne. It is frequently used in German classic foods, such as sauerkraut, pickles and sausages, and Munster cheese is also often served with cumin. Ground cumin can be added, for example, to lime or lemon-based marinades for chicken, turkey, lamb and pork, or added to curries or spicy meat stews. It can be added to olive oil when stir-frying vegetables. Whole cumin is used to make various pickles in Iran, Pakistan and India. Cumin is a common flavor in confectionery, sausage and bread manufacturing and is also used as a preservative in food processing. Cumin essential oil is also used in perfumes.

### **13.3.1 Functional properties**

Cumin seed and its distilled aromatic water are used as a stimulant, antispasmodic, carminative, antimicrobial, anti-inflammatory and wound-healing agent. Cumin was once widely employed in Europe as a digestive stimulant and a remedy for colic. Cumin is regarded as an appetite stimulant, and it is widely used to ease stomach disorders, diarrhea and flatulence. It is used as a carminative, particularly in veterinary practice, and has also been used as an aid for dyspepsia and jaundice. In Iranian traditional medicine, cumin has a hot and dry temperament and is employed as a carminative, diuretic, antibleeding and antidiarrhea agent and for obesity.

In terms of modern research into the functional properties of cumin, cumin oil has demonstrated antimicrobial and antifungal activity in laboratory tests. Antibacterial activity was tested on gram-positive and gram-negative bacteria species and was

**Table 13.1** The nutrition value of cumin seeds per 100 g

|                       |                     |
|-----------------------|---------------------|
| Energy                | 1.567 KJ (375 Kcal) |
| Carbohydrates         | 44.24 g             |
| Sugars                | 2.25 g              |
| Dietary fiber         | 10.5 g              |
| Fat                   | 22.27 g             |
| Saturated fatty acids | 1.535 g             |
| Protein               | 17.81 g             |
| Water                 | 8.06 g              |
| Vitamin A             | 64 µg (7 %)         |
| Riboflavin (vit. B2)  | 0.327 mg (22 %)     |
| Niacin (vit. B3)      | 4.579 mg (31 %)     |
| Vitamin B6            | 0.435 mg (33 %)     |
| Folate (vit. B9)      | 10 µg (3 %)         |
| Vitamin B12           | 0.00 µg (0.0 %)     |
| Vitamin C             | 7.7 mg (13 %)       |
| Vitamin E             | 3.33 mg (22 %)      |
| Vitamin K             | 5.4 µg (5 %)        |
| Calcium               | 931 mg (93 %)       |
| Iron                  | 66.36 mg (53 %)     |
| Magnesium             | 366 mg (99 %)       |
| Phosphorus            | 499 mg (71 %)       |
| Potassium             | 1788 mg (38 %)      |
| Sodium                | 168 mg (7 %)        |
| Zinc                  | 4.8 mg (48 %)       |

effective on the genera *Clavibacter*, *Curtobacterium*, *Rhodococcus*, *Erwinia*, *Xanthomonas*, *Ralstonia* and *Agrobacterium* isolated from clinical isolates and foods. The antioxidant properties of acetone extract and essential oil of cumin have been studied and shown to be strongly effective as DPPH radical scavengers, lipid peroxidation inhibitors and reducing agents, with IC(50) values of 4, 32, and 8 µg/ml. Cumin seeds may also have anticarcinogenic properties. In one study, cumin was shown to protect laboratory animals from developing stomach or liver tumors, possibly due to the potent free radical scavenging abilities of cumin. Cumin also stimulates bile acid secretion and pancreatic enzymes in laboratory animals. A typical dosage of cumin in traditional medicine is a decoction prepared with 15–30 grains and a 1/2 cup of cumin distilled water, diluted with a 1/2 cup of water. This is usually taken twice daily after meals. Contraindications to cumin have not yet been identified. Information regarding safety and efficacy in pregnancy and lactation is lacking; in fact, general information on toxicity is not available. Cumin generally is recognized as safe for human consumption as a spice and flavoring. Cumin seeds are not a commonly allergenic food and are not known to contain measurable amounts of oxalates or purines. The nutrition value of cumin seeds per 100 g is shown in Table 13.1.

## 13.4 Quality specifications

### 13.4.1 Specification for whole seeds

Seeds are oblong in shape, thicker in the middle, compressed laterally, about 3–6 mm long, resembling caraway seeds, but lighter in color and bristly instead of smooth,

almost straight instead of being curved. They have nine fine ridges, overlapping as many oil channels, or *vita*. The odor and taste are somewhat like caraway, but less agreeable. Specific quality indices are:

- seed moisture: less than 6 %
- total ash: 7 %
- acid insoluble ash: 1.5 %
- volatile oil: minimum 2 %
- foreign organic matter: 2 % (US maximum for harmless foreign matter: 5 %)

### 13.4.2 Specification for powdered seeds

The powdered seeds are yellowish-brown with an aromatic, slightly camphoraceous odor and taste. Distinctive characteristics are:

- The epicarp, composed of a layer of colorless cells, polygonal in surface view with thin sinuous walls and a faintly and irregularly striated cuticle; stomata are fairly frequent and, very occasionally, cicatrices may be present. Underlying the epicarp the thin-walled cells of the palisade are sometimes visible.
- The covering trichomes, usually found attached to small fragments of the epicarp; they are pluricellular, multiseriate and rounded at the apex; they vary in length and are composed of fairly thick-walled cells which sometimes show faint, scattered cuticular striations.
- The fairly numerous pale yellowish-brown fragments of the vittae, composed of fairly large, thin-walled cell, polygonal in surface view. The fragments which include the whole width of the vittae demonstrate that they are wider than in most of the other Umbelliferus fruits.
- The sclereids from the mesocarp, of two main types. Those of one type occur as a single layer of longitudinally elongated cells with moderately thickened walls and numerous regularly spaced, well-marked pits. Those of the second type are found in small groups and are composed of considerably elongated cells placed more or less end to end in a longitudinal direction. They are frequently associated with the vascular tissue or with fragments of the vittae. Most of these sclereids have moderately thickened walls, although occasional groups occur in which the walls are more heavily thickened and striated; there are few pits.
- The endocarp, composed of a layer of fairly large, thin-walled cells, elongated in surface view and arranged with their long axes parallel. This layer may be found associated with fragments of the vittae or with parenchymatous cells from the mesocarp.
- The very occasional fragments of the pericarp in sectional view showing the presence of a two-layered palisade under the epicarp.
- Endosperm, testa and fibrovascular tissue (Fig. 13.4).

### 13.4.3 Volatile oil specification

The specific characteristics of cumin oil are:

- colorless or pale yellow
- specific gravity (25°/25°C): 0.905–0.925

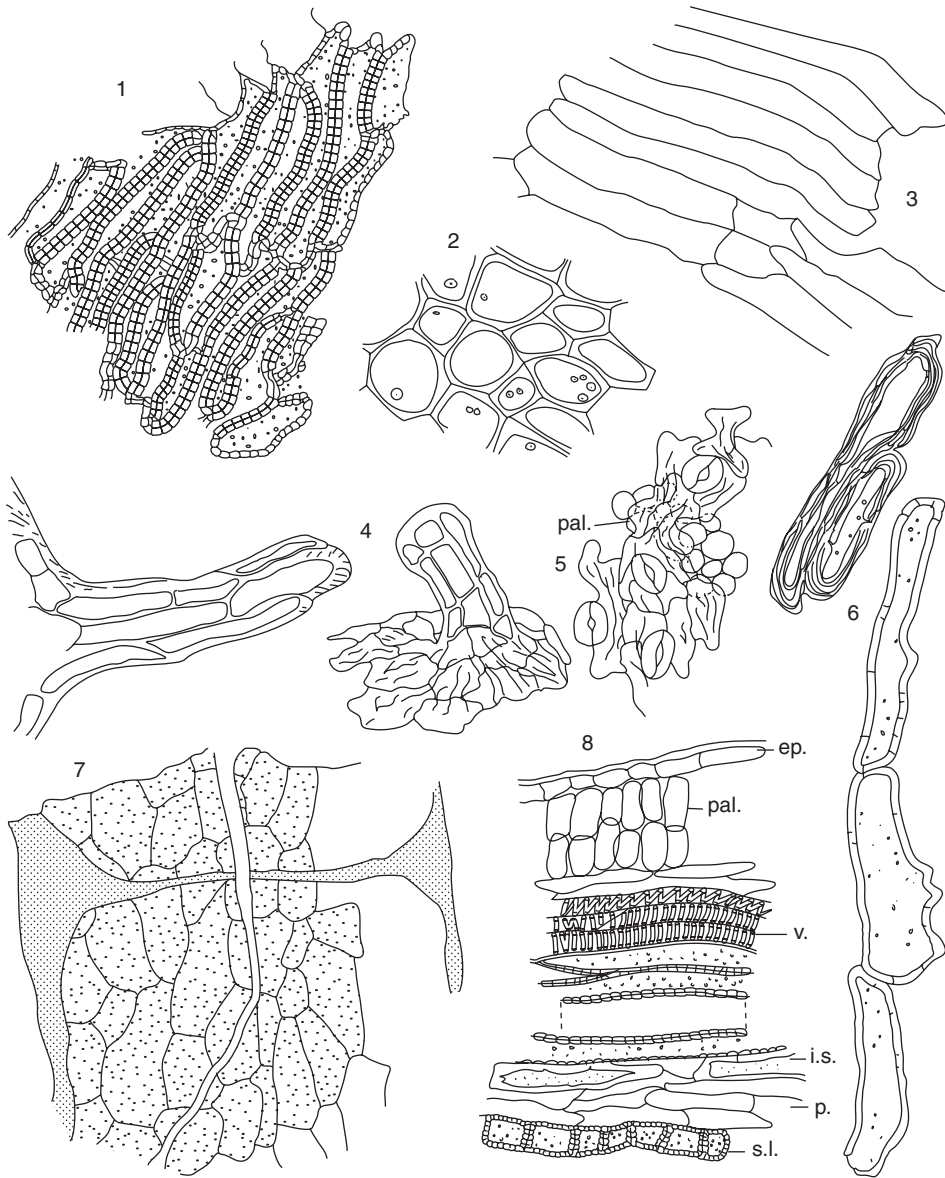


**Fig. 13.4** Microscopically enlarged components of cumin powder. 1 A group of sclereids from the sclerenchymatous layer of the mesocarp. 2 Endosperm containing microspheroidal crystals of calcium oxalate. 3 Endocarp in surface view. 4 Covering trichomes attached to fragments of epicarp in surface view. 5 Epicarp in surface view showing stomata, striated cuticle and part of the underlying palisade (pal.). 6 Groups of sclereids from the mesocarp. 7 Fragment of a vita showing a transverse septum. 8 Part of the pericarp in sectional view showing the epicarp (ep.), two-layered palisade (pal.), vascular tissue (v.), isolated sclereids (i.s.), parenchyma (p.) and the sclerenchymatous layer (s.l.).

**Table 13.2** Physicochemical properties of volatile oils of cumin from different origins

| Property                                      | Mediterranean | Mexico          | Iran   | India   | Pakistan |
|-----------------------------------------------|---------------|-----------------|--------|---------|----------|
| Specific gravity at 25°C                      | 0.917–0.924   | 0.936           | 0.911  | 0.8945  | 0.9290   |
| Optical rotation at 20°                       | +4'22 to +5'6 | +2'55           | +7'    | +3.6'   | +4.6'    |
| Refractive index at 20°                       | 1.501–1.504   | 1.507           | 1.498  | 1.491   | 1.501    |
| Cumin aldehyde (%)                            | 47.4–51.50    | 62.70           | 32.4   | 16.00   | 20.00    |
| Solubility in 80 %<br>alcohol (vol. required) | 5 vols        | 2 vols and more | 6 vols | 11 vols | 8 vols   |

- optical rotation (20°C): +3–+8
- refractive index: 1.501–1.506
- solubility (80 % ethanol): 8 vol
- aldehydes (as cuminic aldehyde): 40–52 %

The physicochemical properties of volatile oils of cumin from various parts of the world are shown in Table 13.2. Cumin essential oil can be adulterated in several ways. Synthetic cumin aldehyde is one of the most difficult to detect, although it can affect the optical rotation of the oil. Modern analytical techniques such as stable isotope ratio analysis (SIRA) and selective ion monitoring (SIM) are helpful in detecting adulteration of this kind.

#### 13.4.4 Confusion with other species

Cumin is often confused with caraway (*Carum carvi* L.), another Umbelliferus spice, because of the nearly similar size, odor and especially culinary and medical uses of the two spices. Some European languages even do not distinguish clearly between the two. However, there are many marked differences between them. Cumin is lighter in color, hotter in taste and larger than caraway. Some older cookbooks erroneously name ground coriander as cumin. The distantly related *Bunium persicum* and the unrelated *Nigella sativa* are both sometimes called black cumin. In Iran, a clear distinction is made between these four species with their different size, color and tests. Cumin, also called 'zireh sabz', is yellowish-green, narrowly boat shape, a little sweet and mild in taste and odor. Caraway, also called 'zireh siyah', is light black, a little flattened with pungent taste and odor. *Bunium persicum*, also called 'zireh kuhi', 'zireh kermani' or 'zireh siyah', is black, narrowly curved and very pungent in taste and odor. *Nigella sativa*, also called 'siyah daneh or shuniz', is black, irregular prismatic in shape and has a mild odor and pungent – bitter taste.

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## Curry leaf

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**Abstract:** Curry leaf (*Murraya koenigii* Spreng.), an aromatic deciduous tree, is a perennial leaf vegetable. The leaves of the plant are used extensively for seasoning and flavouring dishes. Although it is classed as a minor spice, the flavour enhancing qualities of this spice harmonize with eastern cooking and the leaves retain their flavour and aroma even after drying; hence it is widely used in culinary as well as Ayurvedic systems of medicine. In addition to the vast chemical constituents which are responsible for the plant's medicinal values, it is one of the richest sources of carbozole alkaloids. *M. koenigii* extracts induced peak fall in blood sugar as well as exhibiting strong toxicity against major pathogens. It is in this context that this review of the important characteristics of curry leaf plant, has been made.

**Key words:** chemical constituents, curry leaf, functional and medicinal properties, *Murraya koenigii* Spreng.

### 14.1 Introduction

Curry leaf (*Murraya koenigii* Spreng, Syn. *Bergera koenigii* Koen (N.O. Aurantiaceae), *Chaleos koenigii* Kurz ex Swingle) is a perennial leaf vegetable. It belongs to the family Rutaceae and is named 'Murraya' after John Adam Murray, Professor of Botany at Göttingen and editor of many of Linnaeus's works. It is a native of mountainous regions and grows up to an elevation of 1500 m. Curry leaf is found growing throughout India, including the Andaman Islands, up to an altitude of 1500 m (Anon., 1962). It has been recorded wild from Garhwal to Sikkim, in Bengal, Assam, the Deccan Plateau, Circar mountains, Western ghats, Coromandel (Tamil Nadu) and Travancore (Cochin, Kerala, India). The use of these leaves in Indian cuisine is cited in old Kannada texts.

Curry leaf is an aromatic deciduous tree which grows approximately 5 m tall and 15–40 cm in diameter. Curry leaf and curry leaf oil are exported from India to various parts of the globe, for use as a spice (Table 14.1). Although the curry leaf plant is found in the wild, it is also widely cultivated for its aromatic leaves and as an ornamental. It is mainly grown in homesteads (in fact, the plant is grown in the garden of almost every home in the south of India) but also on a larger scale in plantations. Curry leaf is grown commercially on farms in Tamil Nadu



**Table 14.1** Export statistics for curry leaf from India between the years 1993–4 and 1997–8 (quantity in tonnes, value in Rs. 1000)

| Product        | 1993–4 |       | 1994–5 |       | 1995–6 |        | 1996–7 |        | 1997–8 |        |
|----------------|--------|-------|--------|-------|--------|--------|--------|--------|--------|--------|
|                | Qty    | Value | Qty    | Value | Qty    | Value  | Qty    | Value  | Qty    | Value  |
| Curry leaf     | 15.2   | 989.7 | 10.9   | 618.8 | 39.0   | 1604.4 | 68.6   | 2185.1 | 152.8  | 3334.2 |
| Curry leaf oil | –      | –     | –      | –     | –      | –      | 0.4    | 115.4  | –      | –      |

Source: *Spices Statistics*, IVth edition by Spices Board, Ministry of Commerce, Government of India, Cochin, 682025.

**Table 14.2** Important constituents from different parts of *Murraya koenigii*

| Part          | Constituent                                                                                                                                                                                                                                                                                                                                                         |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Leaves</b> | <ul style="list-style-type: none"> <li>Free amino acids: asparagine, glycine, serine, aspartic acid, glutamic acid, theonine, alanine, proline, tyrosine, tryptophan, amino butyric acid, phenylalanine, leucine, isoleucine,</li> <li>Glucoside, koenigin</li> <li>Monoterpene and sesquiterpene hydrocarbons</li> <li><math>\beta</math>-caryophyllene</li> </ul> |
| <b>Stem</b>   | <ul style="list-style-type: none"> <li>C<sub>23</sub>-carbazole alkaloid, mahanimbinol</li> </ul>                                                                                                                                                                                                                                                                   |
| <b>Fruit</b>  | <ul style="list-style-type: none"> <li>Total sugars: 9.76 %</li> <li>Vitamin C: 13.35 mg/ 100 g pulp</li> <li>Protein: 1.97 g; phosphorus, 0.082 g; potassium, 0.811 g; calcium, 0.166 g; magnesium, 0.216 g; and iron, 0.007 g per 100 g</li> </ul>                                                                                                                |

and other states. Curry leaf is classed as a minor spice crop and accurate figures on its production are not available. About 893 tonnes of curry leaves were exported from India from 2002–3 ([http://www.kew.org/plant-cultures/plants/curry\\_leaf\\_production\\_trade.html](http://www.kew.org/plant-cultures/plants/curry_leaf_production_trade.html)). A strong odiferous oil occurs in the leaves and the seeds of *Murraya koenigii* (L.) Spreng. A chemical examination of this oil has been made by Goutam and Purohit (1974), who reported that this essential oil exhibited powerful antibacterial and antifungal properties. An alkaloid, murrayacinine, is also found in this plant (Chakraborty *et al.*, 1974).

### 14.1.1 Chemical composition

#### Leaves

The leaves contain the following free amino acids: asparagine, glycine, serine, aspartic acid, glutamic acid, theonine, alanine, proline, tyrosine, tryptophan, amino butyric acid, phenylalanine, leucine, isoleucine and traces of ornithine, lysine, arginine and histidine. The leaves also contain a crystalline glucoside, koenigin and a resin (Table 14.2).

Macleod and Pieris (1982) analysed concentrated essence of curry leaf, and mainly obtained terpenes. Other components made up less than 4 % of the concentrated essence, and included eight monoterpene hydrocarbons (C. 16 %) and 17

sesquiterpene hydrocarbons (C. 80 %). According to them, the most important constituents of *M. koenigii* are  $\beta$ -caryophyllene,  $\beta$ -gurjunene,  $\beta$ -elemene,  $\beta$ -phellandrene and  $\beta$ -thujene. Chowdhury (1999) reported that a fresh weight of leaves gave 0.5 % essential oil on hydrodistillation. This oil possesses a dark yellow colour, spicy odour and pungent clove-like taste. It has the following characteristics:

- specific gravity (25°): 0.9748
- refractive index (25°): 1.5021
- optical rotation (25°): +4.86
- saponification value: 5.2
- saponification value after acetylation: 54.6
- acid value: 3.8
- soluble in 80 % alcohol with slight opalescence

The constituents of the oil are:

- *d*l- $\alpha$ -phellandrene: 4.6 %
- *d*-sabinene: 9.2 %
- *d*- $\alpha$ -pinene: 5.5 %
- dipentene: 6.8 %
- *d*- $\alpha$ -terpineol: 3.2 %
- caryophyllene: 26.3 %
- isosafrol: 4.4 %
- cadinene: 18.2 %
- cadinol: 12.8 %
- lauric acid: 2.7 %
- palmitic acid: 3.4 %

On examination by gas chromatography–mass spectroscopy (GC–MS) the oil contained: aromadendrene,  $\beta$ -bisabolene, butyl myristate, carvomethone, *cis*-caryophyllene,  $\beta$ -costol, citral, *trans*-caryophyllene, isocaryophyllene, camphene, dehydroaromadendrene, dipentene,  $\beta$ -elemene,  $\beta$ -eudesmol, farnesol, junipene, linalyl acetate, isomenthone, menthol, spathulenol, stearyl alcohol, ateraldehyde, stearic acid,  $\alpha$ -terpineol, palmitic acid,  $\alpha$ -pinene and zingiberene.

Essential oils hydrodistilled from leaves of *M. koenigii* were analysed by GC and GC–MS. Both essential oils contained mainly monoterpenes and oxygenated monoterpenes. The main constituents are  $\alpha$ -pinene (19–19.7 %), sabinene (31.8–44.8 %),  $\beta$ -pinene (4.2–4.7 %),  $\alpha$ -terpinene (1.3–4.3 %),  $\beta$ -phellandrene (6.5–7.9 %), tauterpinene (3.9–7.1 %) and terpinen-4-ol (5.2–9.9 %), as reported by Mallavapura *et al.* (1999). Madalageri *et al.* (1996) reported 21 different compounds in the hydro-distilled essential oil out of which only seven were identified. A commercially important odiferous compound  $\beta$ -caryophyllene was among them.

The bioactive components of *M. koenigii* leaves have been evaluated using GC–MS, and the chemical compositions of the ethanol extract of *M. koenigii* were investigated using Perkin–Elmer GC–MS. The mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology (NIST) library. GC–MS analysis of ethanol extract of *M. koenigii* revealed the existence of 1-methyl-pyrrolidine-2-carboxylic acid (69.00 %), ethyl  $\alpha$ -dglucopyranoside

(13.36 %), isolongifolene, 4,5-dehydro- (3.68 %),  $\gamma$ -himachalene (2.88 %), 1,2-ethanediol monoacetate (2.79 %) 1,2-benzenedicarboxylic acid, diisooctyl ester (2.55 %). The results of the study offer a platform for using *M. koenigii* as herbal alternative to current synthetic antimicrobial agents (Hema *et al.*, 2011).

#### *Stem bark, fruit and seeds*

Bhattacharya *et al.* (1982) reported obtaining a carbazole alkaloid-isomurrayazoline from the stem of *M. koenigii* with a structure of 9a,10,11,12,13,13a-hexahydro-5, 9, 9, 12 tetramethyl-1, 12-epoxy-9 Hindolo(3, 2, 1 - de) phenanthridine ( $C_{23}H_{27}NO_2$ ). Alkaloids like muconicine, mahanimbine, koenimbine, koenigicine, cyclomahanimbine, mahanimbidine, girinimbine, isomahanimbine, murrayacine, mahanine, koenine, koenigine, koenidine and scopolin have also been reported in parts of the curry leaf plant by various workers (Rastogi and Mehrotra, 1993).

A new  $C_{23}$ -carbazole alkaloid, mahanimbinol, was isolated from the stem of *M. koenigii*. It is the key precursor in the biosynthesis of some 20 other carbazole alkaloids. Bhattacharya *et al.* (1994) identified two carbazole alkaloids, namely 2-methoxy carbazole-3-methyl carboxylate and 1-hydroxy-3-methyl carbazole from the stem bark extract of *M. koenigii*. Alkaloids like mahanimbinol, mukonal, murrayanine, murrayacinine and murrayazolidine have been isolated from the stem bark and characterized by various investigators (Rastogi and Mehrotra, 1993).

The fruit is edible and yields 0.76 % of a yellow volatile oil with neroli-like odour and pepper-like taste, accompanied by an agreeable sensation of coolness on the tongue. The characteristics of the oil are as follows:

- specific gravity (13°): 0.872
- refractive index (0°): 1.487
- optical rotation (0°): 27.24
- boiling point: 173–174°

The fruit is reported to contain koenigin. A clear yellow oil is procured from the seeds, known as limbole oil. The total soluble solids content of the fruit juice is 16.8 %. The pulp contains 64.9 % moisture, 9.76 % total sugars, 9.58 % reducing sugars, 0.17 % non-reducing sugars and an almost negligible amount of tannins and acidity. The vitamin C content of the fruit, which is 13.35 mg per 100 g of pulp, is higher than that of many fruits studied during the course of the present investigation. The mineral content of the edible portion of the fruit, as represented by its ash, is 2.162 %. Similarly, 100 g of the edible portion of the fruit contains: protein (1.97 g); phosphorus (0.082 g); potassium (0.811 g); calcium (0.166 g); magnesium (0.216 g); and iron (0.007 g). Purification and sequence analysis studies of proteins from the seeds of *M. koenigii* have been performed and reported (Shee and Sharma, 2007; Gahloth *et al.*, 2010).

## 14.2 Cultivation and production of curry leaves

### 14.2.1 Cultivation requirements

- **Soil and climate:** Red sandy loam soils with good drainage are ideal for good leaf yield. The optimum temperature requirement is 26–37°C.

- **Season of sowing and planting:** The main season of availability of curry leaf fruits is July–August. Within 3–4 days of collection, the seeds should be pulped and sown in nursery beds or polybags. One-year-old seedlings are suitable for planting. One seedling is planted at the centre of each pit.
- **Preparation of field:** The field is ploughed three to four times to get a fine tilth. Before last ploughing, well-decomposed farmyard manure (FYM) is applied at 20 t/ha. Pits of 30 × 30 × 30 cm in size are dug 1–2 months before planting at a spacing of 1.2–1.5 m.
- **Irrigation:** The pits should be irrigated immediately after planting, with the second irrigation given on the third day, and then once per week.
- **Application of fertilizers:** After each harvest 20 kg of FYM per plant is applied and mixed with soil. A foliar spray of 3 % panchagavya, used at 30-day intervals or twice per season, was found to improve fresh curry leaf yield and quality (Subha *et al.*, 2010).
- **Inter cultivation:** If the soil is given periodical hoeing then an intercrop, usually a pulse, can be grown in the first year.
- **Pruning and harvest:** After attaining 1 m in height, the terminal bud is cut off to encourage basal branching. In total five to six branches are maintained per bush. Ten to twelve months after planting, the first harvest starts.

#### 14.2.2 Propagation

Curry leaf is commonly propagated via stem cuttings. The lowermost leaves of twigs at the semi-ripe stage, which are neither very fleshy and green nor very hard and woody, are removed and the stem is planted into potting compost. Rooting usually takes place in about three weeks. Alternatively, curry leaf can also be propagated using seeds. Fresh curry leaves with ripe seeds require temperatures of about 20 °C to germinate, and germination may take a long time. For raising seedlings, well-ripened fruits are collected from high-yielding plants. The seeds are sown either in nursery or polybags filled with a mixture of 1:1:1 sand, soil and FYM. Seeds germinate in three weeks and one-year-old seedlings can be planted in the main field. Sprouting root suckers can also be harvested and propagated. In order to do this, the curry leaf should be watered well to simulate the end of a monsoon season. The root can then be dug out and severed from the rest of the plant to use as a propagule. Most potting mixes are adequate, but the use of some sand or perlite to increase drainage is beneficial. Sufficient heat, partial shade and occasional watering need to be provided.

In terms of recent scientific research, *in vitro* regeneration from immature seeds of *M. koenigii* was successfully accomplished by Uzma *et al.* (2010) on Murashige and Skoog medium supplemented with benzyladeninephosphate. Bhuyan *et al.* (1997) and Rout (2005) have reported *in vitro* propagation of curry leaf trees by axillary bud proliferation and from intact seedlings. Babu *et al.* (2000) have standardized micropropagation from mature explants in Woody Plants Medium, and Paul *et al.* (2011) have reported somatic embryogenesis. These protocols have reported high-frequency regeneration and would thus meet the pharmaceutical industry's demand for large numbers of plantlets.

### 14.2.3 Varieties

Improved varieties of curry leaf have been released by the University of Agricultural Sciences, Dharwad, viz., Dharwad-1(DWD1) and Dharwad-2(DWD2). Both of these have a good aroma and have an oil content of 5.22 % and 4.09 %, respectively. They are mainly cultivated in Karnataka. DWD 1 is sensitive to the winter season, whereas DWD 2 is winter insensitive. DWD 2 also gives higher yields than DWD 1. 'Senkaampu' is another promising local cultivar grown in many parts of Tamil Nadu.

Suwasini and DW-2 are promising varieties of curry leaf from Kerala Agricultural University. The leaves of Suwasini are dark green, shiny and highly aromatic. The leaves have an oil content of 5.2 %, and can be dehydrated at 50°C and made into powder. Though the leaves of DW-2 are pale green and slightly less aromatic, the rate of shoot growth is very high in this variety. Aside from these, many promising local types are also being cultivated (George, 2008).

Curry leaf trees come in three different commercial varieties. Curry leaf 'Regular' grows very tall and is the fastest growing plant amongst the three varieties. The leaves look like the ones you buy at the grocery store. Dwarf variety does not grow as tall but the branches spread. Gamthi variety is the most fragrant, with a very thick leaf structure, but is the slowest of all curry trees. All three varieties have this in common: they like full sun and very well-drained soil, which should be on the dry side, and they need fertiliser during the summer months.

### 14.2.4 Production

India is the largest producer and consumer of curry leaf, though it is also produced in Burma, Malaysia and South Africa. The state of Tamil Nadu, in the south of India, is one of the main curry leaf-producing areas. The leaves retain their flavour even after drying and hence these are marketed both in fresh and dried forms.

### 14.2.5 Diseases and pests

Curry leaf plants are reasonably resistant to pests, although they are more likely to become infested with mealybugs when they are grown indoors, because natural predators of those insects are not present. Curry leaf is commonly seen infested with pests like scales, bugs, and Citrus butterfly. The control methods employed include hand picking, destruction of the larvae and spraying with Malathion @ 1 ml/l. Psyllid bug and scales can be controlled by spraying with Dimethoate @ 1 ml/l. The most common disease affecting curry leaves is leaf spot disease, which can be controlled by spraying Carbendazim @ 1 g/l of water. Spraying sulphur compounds is generally to be avoided.

### 14.2.6 Harvest and yield

Under ambient conditions for cultivation, 250–400 kg of leaves/ha can be harvested by the end of the first year. From the second year onwards, leaves can be harvested once in 4 months, amounting to an annual yield of 5400 kg/ha. After the fifth year, the frequency of harvesting the leaves can be increased to once in 3 months, with

plants producing 5000 kg/ha at each harvest, resulting in a total yield of 20000 kg/ha/year.

Curry leaves are picked 15 months after planting. Commercial harvest can be started from when plants are 3 years old. With good management, normal yield can be obtained up to the age of 20–25 years. The leaves can be harvested at 2½–3 months intervals, the average yield being 20–25 tonnes/ha.

#### 14.2.7 Post-harvest techniques

All parts of the plant can be used in flavouring (Table 14.2). However, the leaves are the commercially useful part. Fresh curry leaves are frozen and vacuum-packed, or kept in a refrigerated condition for two weeks. Dried curry leaves are those which are washed, air-dried for 4 or 5 days, then kept in plastic bags, and can thus be utilized in the preparation of food for about a year. There is not much loss of volatile oil during drying either in sun or shade, or in a cross-flow dryer. Oven drying at 50 °C is recommended as the best technology for converting fresh leaves into dry powder (Madalageri *et al.*, 1996). Higher temperatures during drying deteriorate powder quality.

Curry leaves can also be dried in a microwave oven for 12–15 minutes at 70 °C, powdered and packed and stored at ambient temperature. In order to retain higher levels of  $\beta$ -carotene, ascorbic acid and chlorophyll, curry leaves should first be dehydrated in a cabinet dryer, then packaged and stored in HDPE (200 gauge), and afterwards kept at low temperatures of  $7 \pm 1$  °C (Singh and Sagar, 2010).

On steam distillation under pressure (90 lb/in<sup>2</sup>), fresh leaves yield 2.6 % volatile oil (curry leaf oil). According to Madalageri *et al.* (1996), hydrodistillation of fresh leaves at 70 °C is a cheap and non-cumbersome method of extracting essential oil. Leaf maturity influenced oil composition, the youngest leaves tested being the best. An extended period of extraction caused loss of or decrease in certain components while there was gain/increase in other components.

The post-harvest processing of curry leaf can help farmers in fetching better prices. Curry leaf essence, extracted from the fresh product, can provide city-dwellers and people living in countries where fresh leaf material is not available or very expensive with the aroma of fresh curry leaf. The Central Research Institute for Dryland Agriculture (CRIDA) pioneered the technology for extraction of essence from fresh curry leaves (Yadav *et al.*, 2007).

### 14.3 Functional properties and uses of curry leaves

Curry leaves are extremely high in antioxidants, plant sterols, amino acids, glycosides, proteins and flavanoids, and possess a wide range of health-promoting properties (Table 14.3).

#### 14.3.1 Nutritional properties

Curry leaves contain 2.5 % oil,  $\alpha$ -selinene,  $\beta$ -bisabolene,  $\beta$ -cadinene,  $\beta$ -caryophyllene,  $\beta$ -elemene,  $\beta$ -gurjenene,  $\beta$ -phellandrene,  $\beta$ -thujene and  $\beta$ -transocimene. On analysis, the leaves have been shown to contain nutrients as shown in Table 14.4.

**Table 14.3** Functional properties of curry leaf

| Properties                            | Reference                                                                                                                                                                     |
|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibiotic                            | Abhishek <i>et al.</i> (2010)                                                                                                                                                 |
| Antioxidant                           | Arulselvan and Subramanian (2007);<br>Farhath <i>et al.</i> (2000)                                                                                                            |
| Anti-inflammatory and analgesic       | Maiti <i>et al.</i> (2004)                                                                                                                                                    |
| Cancer prevention                     | Muthumani <i>et al.</i> (2009); Khan <i>et al.</i> (1996)                                                                                                                     |
| Tumor reduction                       | Trisha <i>et al.</i> (2003)                                                                                                                                                   |
| Blood cholesterol and glucose control | Xie <i>et al.</i> (2006); Vinuthan <i>et al.</i> (2004); Iyer and<br>Mani (1990); Arulselvan <i>et al.</i> (2006);<br>Yadav <i>et al.</i> (2002); Achyut <i>et al.</i> (2005) |
| Hepato-protective                     | Pande <i>et al.</i> (2009)                                                                                                                                                    |
| Onset of puberty                      | Nandini <i>et al.</i> (2010)                                                                                                                                                  |
| Reduction of halitosis                | Abhishek <i>et al.</i> (2010)                                                                                                                                                 |
| Sunscreen activity                    | Rekha <i>et al.</i> (2010)                                                                                                                                                    |
| Mosquito control                      | Ramsewak <i>et al.</i> (1999)                                                                                                                                                 |
| Production of value-added products    | Yadav <i>et al.</i> (2007)                                                                                                                                                    |

**Table 14.4** Nutritional value of curry leaves

| Nutritional information            | Value per 100 g |
|------------------------------------|-----------------|
| Moisture                           | 63.800 g        |
| Protein                            | 6.100 g         |
| Fat                                | 1.000 g         |
| Minerals                           | 4.000 g         |
| Fibre                              | 6.400 g         |
| Carbohydrates                      | 18.700 g        |
| Energy                             | 108.000 Kcal    |
| Calcium                            | 830.000 mg      |
| Phosphorus                         | 57.000 mg       |
| Iron                               | 0.930 mg        |
| <b>Vitamins</b>                    |                 |
| Carotene                           | 7560.000 µg     |
| Thiamine                           | 0.080 mg        |
| Riboflavin                         | 0.210 mg        |
| Niacin                             | 2.300 mg        |
| Folic Acid (Free)                  | 23.500 µg       |
| Folic Acid (Total)                 | 93.900 µg       |
| Vitamin C                          | 4.000 mg        |
| <b>Minerals and trace elements</b> |                 |
| Magnesium                          | 44.00 mg        |
| Copper                             | 0.100 mg        |
| Manganese                          | 0.150 mg        |
| Zinc                               | 0.200 mg        |
| Chromium                           | 0.006 mg        |
| S                                  | 81.000 mg       |
| Cl                                 | 198.000 mg      |
| Oxalic acid                        | 132.000 mg      |
| Phytin phosphorus                  | 35.000 mg       |

Source: National Institute of Nutrition, ICMR, Hyderabad.



The leaves are a fair source of vitamin A. They are also a rich source of calcium, but due to the presence of oxalic acid in high concentration (total oxalates, 1.35 %; soluble oxalates, 1.15 %), its nutritional availability is affected.

Three types of curry leaves are popular, *viz.*, fresh curry leaves, sauteed or fried curry leaves, and dried curry leaves. Sauteed or fried curry leaves are edible and thus are not removed from food before serving. These can also be sliced or ground to use as a garnish. They are generally used in the dishes of South India such as lentil and vegetable curries, etc.

### 14.3.2 Uses in foods

Curry powder, made by grinding curry leaves, was invented by the British in order to imitate the flavour of Indian cooking with little effort. The leaves of the plant are employed extensively as flavourant in curries like 'dal', 'South Indian Sambar', 'rasam' and chutneys and mulligatawny. Ground curry leaf with mature coconut kernel and spices forms an excellent preserve. 'Veppilakkatti', a very famous preparation from Southern India, can be made with the following ingredients: curry leaf (100 g); tender leaves of malta lemon (50 g); common salt (50 g); seedless tamarind (40 g); red chilli powder without seed (20 g); fenugreek powder (2 g); asafoetida (6 g); black pepper powder (2 g); gingelly oil (10 g). To prepare 'veppilakkatti', fry the asafoetida in gingelly oil and powder it. Mash the tender leaves of malta lemon in a mixer and remove all the fibres. Then add curry leaves, red chilli powder and other ingredients and grind well. If kept in airtight containers it can be stored for a long time.

### 14.3.3 Medicinal properties

Curry leaf is used in traditional medicine, for example Ayurvedic and Unani medicine (Kirthikar and Basu, 1935; Dastur, 1970; Drury, 1978). The plant is credited with tonic, stomachic and carminative properties. Ethanobotanical aspects have been explored in curry leaf and many indigenous formulations are available (Table 14.5).

#### *Diabetes*

Curry leaf has a potential role in the treatment of diabetes. Hypoglycaemic action on carbohydrate metabolism was reported in rats fed with curry leaves (Khan *et al.*, 1995). Hepatic glycogen and glycogenesis, as evident from the increased activity of glycogen synthetase, increased and glycogenolysis and gluconeogenesis decreased as evident from the decreased activity of glycogen phosphorylase and gluconeogenic enzymes. Studies at the Department of Biochemistry and Molecular Biology at the University of Madras in India have shown that extract of curry leaves reduces oxidative stress on pancreatic cells and has a therapeutic effect on damaged pancreatic cells in diabetic rats. The effects were comparable to those of the hypoglycaemic drug glibenclamide and have great promise for use in humans.

#### *Cancer prevention*

The effect of drinking water containing crude curry leaf aqueous extract on Swiss albino mice was investigated by Dasgupta *et al.* (2003). The investigated dose levels of curry leaf showed a significant reduction in tumour burden ( $P < 0.001$ ) as well

**Table 14.5** Use of curry leaf in indigenous formulations

| Condition                      | Treatment formulations                                                                                                                                                                                                                                                                                                     |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Indigestion and acidity        | Curry leaf juice (2–4 teaspoons) should be given every day to improve digestion and control acidity.                                                                                                                                                                                                                       |
| Urinary disorders              | Curry leaf juice (40 mg) with cardamom ( <i>Elettaria cardamomum</i> ) seed powder (1 g) can be given orally to treat urinary disorders.                                                                                                                                                                                   |
| Insect bite                    | A paste from the leaves is prepared and applied to the affected area to reduce pain and swelling.                                                                                                                                                                                                                          |
| Stomach ache                   | The indigenous people of Pataalkot prepare a decoction of the curry leaf root (5 ml) and ginger powder ( <i>Zingiber officinale</i> ) (1 pinch) to relieve stomach ache, which is given orally.                                                                                                                            |
| Eczema                         | To treat eczema, curry leaf seeds ( <i>M. koenigii</i> ) (5 nos.) and golden shower tree ( <i>Cassia fistula</i> ) fruit pulp (5 g) are mixed and crushed, curd (10 ml) is added, and the mixture applied externally.                                                                                                      |
| Perspiration                   | To reduce excessive perspiration, leaf powder (4 g) should be taken along with a glass of buttermilk every day. The preparation works as an anti-sudorific.                                                                                                                                                                |
| Breast tumour                  | According to Dangi healers, to treat breast tumours, the roots (10 g) of the plant should be scrubbed in water and applied externally all over the breast.                                                                                                                                                                 |
| Haemorrhoids                   | Root powder (5 g) should be dipped in goat urine for 3–5 minutes. The mixture is then dried and given to the patient twice a day along with one cup water. The patient is advised to take Brinjal ( <i>Solanum melongena</i> ), jaggery and chapatis with meals.                                                           |
| Tonic                          | Some indigenous people prepare a juice from the leaves, bark and roots which is said to be very effective as a tonic. Equal amounts of all the above are collected, washed and then dried in the shade. The plant materials are then ground into a powder. One teaspoon is added to one cup of water and taken once a day. |
| Gum bleeding and tooth cleaner | The twigs of <i>M. koenigii</i> are commonly used as Datun (tooth cleaning sticks) because they are said to strengthen teeth and gums                                                                                                                                                                                      |

Source: Acharya and Shrivastava (2008).

as tumour incidence in the tumour model system studied, suggesting that the curry leaf might be useful for the prevention of human stomach and skin cancers (Dasgupta *et al.*, 2003). The antioxidant potential of curry leaves in rats treated with a known chemical carcinogen, dimethylhydrazine hydrochloride (DMH), was investigated by Khanum *et al.* (2000). Food intake was reduced in the rats fed curry leaf-supplemented diet, but the body and the organ weights were not affected. Vitamin A content in the liver was significantly increased whereas glutathione (GSH) content was not altered. When the rats were fed a curry leaf-supplemented diet, a 50 % reduction was seen in the micronuclei induced by DMH, together with a 30 % reduction in the activity of glutamyl transpeptidase. These results indicate that curry leaves have high potential in reducing the toxicity of DMH.

### *Hormonal effects*

The effects of methanolic extracts of *M. koenigii* Spreng. (Curry leaf) on ovarian surface follicles in female rats were studied by Nandini *et al.* (2010). A significant advancement in the mean age of attainment of puberty was observed, along with increase in number of surface follicles on both the ovaries, which was attributed to

the effects of phytoestrogens present in the methanolic extracts of *M. koenigii* Spreng.

#### *Oral hygiene*

It has been observed that holding curry leaves in the mouth is helpful in reducing halitosis, and that the terpenes in curry leaf have been found to reduce airborne chemicals and bacteria. In addition to the presence of essential oils, the curry leaves contain chlorophyll,  $\beta$ -carotene and folic acid, riboflavin, calcium and zinc, all of which can help in maintaining good oral health. Chewing two to four fresh curry leaves with 10–15 ml water in the mouth, swishing for 5–7 minutes, then rinsing the mouth out with water can help in keeping good oral hygiene. Because curry leaf is a green leafy vegetable, it will be safe and cheap to use as a mouthwash (Mathur *et al.*, 2010). The branches of *M. koenigii* (L.) Spreng. are also very popular for cleaning the teeth and are said to strengthen the gums and the teeth.

#### *Gastrointestinal motility*

Curry leaf extracts have also been shown to increase gastrointestinal motility. The motility effects were felt by rats who both had and had not been given constipating agents. The results of the study indicate that curry leaf extracts show great promise as a remedy for abdominal pain, constipation and gastroparesis.

#### *Blood cholesterol*

According to a study conducted by Malmuthuge *et al.* (2007), daily intraperitoneal injection of curry leaf extract reduced blood cholesterol level in mice.

#### *Sunscreen activity*

Studies have also been designed to investigate the sunscreen activity of herbal formulation with essential oil of curry leaf oil via the COLIPA method. Curry leaf oil cream exhibited limited sunscreen activity ( $\text{SPF} = 2.04 \pm 0.02$ ), suggesting it could be used to help maintain the natural pigmentation of the skin, or as a supplement in other formulations to enhance their activity (Patil *et al.*, 2010).

### **14.3.4 Antimicrobial properties**

The undiluted essential oil exhibited strong antibacterial and antifungal activity when tested on microorganisms (Goutam and Purohit, 1974). Even the crude leaf extracts of curry leaf plant are reported to possess antibacterial activity (Thakare, 1980). A monomeric protein with a molecular mass of 35 kDa, isolated from curry leaves, showed potent antibacterial activity. The protein designated as APC (anti-oxidant protein from curry leaves) demonstrated potent antibacterial activity against all the human pathogenic strains tested: it inhibited *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Bacillus subtilis*. The inhibition is comparable to that of commercial antibiotics chloramphenicol, streptomycin and gentamycin. APC inhibited bacterial growth, with MIC values ranging from 13–24 lg/ml, which are comparable to MIC values of standard antibiotics. APC is non-toxic at tested doses and studies have shown that it is a potent therapeutic agent (Mylarappa *et al.*, 2009).

### 14.3.5 Antioxidant properties

Curry leaf was found to exert antioxidant properties in rats fed a high-fat diet (Khan *et al.*, 1997). There were lower levels of hydroperoxides, conjugated dienes and free fatty acids in the livers and hearts of rats supplemented with curry leaves compared to rats fed on the high-fat diet alone. Activities of superoxide dismutase, catalase and glutathione transferase increased in the hearts and livers of rats supplemented with curry leaves. Activities of glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase also increased in the liver, and the concentration of glutathione decreased. Thus, supplementing a high-fat diet with 10 % curry leaf can prevent the formation of free radicals and maintain tissue function at normal levels.

The *in vitro* antioxidant properties of different extracts (water, alcohol, alcohol:water, hexane or chloroform extract) of curry leaves (*M. koenigii* L.) were evaluated using various assays (Mylarappa *et al.*, 2008). The alcohol:water (1:1) extract of curry leaves (AWEC) showed the highest antioxidant and free radical scavenging activity. It reduced cytochrome *c*, ferric ion levels and chelated ferrous ions and inhibited ferrous sorbate: ascorbate-induced fragmentation and sugar oxidation of DNA. These results establish the antioxidant potential of AWEC, which could be used as a natural source of antioxidants. Antioxidant activities in ambient and hot conditions were studied by Sasidharan and Nirmala (2010). They showed that extracts at ambient temperature had higher antioxidant activity.

Patel and Rajorhia (1979) reported that ghee samples treated with 1 % curry leaves during clarification showed higher resistance to oxidation and higher sensory scores than those treated with a mixture of BHT (butylated hydroxy toluene) + BHA (butylated hydroxy anisole), due to the presence of naturally-occurring antioxidants. Curry leaves at 1 % concentration could be used instead of BHT and BHA for extending the shelf-life of ghee.

Ningappa and Srinivas (2008) characterized an antioxidant protein from curry leaves (*M. koenigii* L.), with an apparent molecular weight of 35 kDa. Its protein nature was established by the presence of amino acids and loss of antioxidant activity on heat and protease treatment.

### 14.3.6 Inhibition of corrosion on carbon steel

The inhibitive action of curry leaf extract on corrosion in carbon steel in 1N HCl has also been investigated. A protective film was formed on the surface of carbon steel by the adsorption of extracts of curry leaf; this was confirmed by SEM studies (Sharmila *et al.*, 2010).

## 14.4 Conclusion

Curry leaf is already frequently utilised for its various functional properties, and has the potential for wider pharmaceutical and industrial applications. For example, the plant is used effectively in mosquito control programmes. The leaves, bark and the root of the plant have medicinal properties, and are used in indigenous medicine as a tonic, stomachic, stimulant and carminative. The fresh and dried leaves as also

used extensively in foods. The use of essential oil as flavour or perfume has not yet been popularized. Given its properties, there is scope to create increased demand for curry leaf in the future.

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# 15

## Dill

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**Abstract:** Dill is a seed spice crop and an important aromatic herb, native to South-West Asia or South-East Europe and cultivated since ancient times. There are two closely related cultivated species of dill, i.e. European dill (*Anethum graveolens*) and Indian dill (*A. sowa*). This chapter looks at the history and description of dill before presenting its production, cultivation and harvesting. The extraction of essential oil and the chemical composition of oils from different dill varieties are described, along with culinary, medicinal (both traditional and modern), antimicrobial, insecticidal and other uses. Quality issues and applicable standards and specifications are described.

**Key words:** dill, seed spices, antimicrobial activity, *Anethum graveolens*, *Anethum sowa*.

### 15.1 Introduction

Dill (*Anethum graveolens* Linn.) is a seed spice crop and an important aromatic herb which is used for flavouring tea, pickles, and confectionery. Dill leaves are rich in minerals, proteins and fibre. The seeds are mainly used for spice, culinary and medicinal uses. It is also used as a vegetable and an aromatic (Sharma, 2004), and as an inhibitor of sprouting in stored potatoes (Score *et al.*, 1997). The aroma volatiles of seed and herb of dill have been identified (Huopalahti and Linko 1983; Shankaracharya *et al.*, 2000) along with several therapeutic properties, and the antimicrobial activity of carvone isolated from the dill seed oil has been reported (Aggarwal *et al.*, 2002). Dill contains carotenoids, vitamin C and polyphenols, the contents of which vary during different stages of its growth (Lisiewska *et al.*, 2006).

Dill is probably native to South West Asia or South East Europe, and has been cultivated since ancient times (Bailer *et al.*, 2001). Europe, Africa and Asia are considered as the centre of origin for dill crop. It is cultivated worldwide in countries like India, Germany, Hungary, the Netherlands, Pakistan and the USA. Principal dill production areas are India and Pakistan; but Egypt, Mexico, the Netherlands, the USA, Hungary, Germany and Holland also have commercially productive areas (Osman and El-Wahab, 2009). In India dill is grown with the name of sowa (*A. sowa*) in the states of Rajasthan, Gujarat, Maharashtra, Andhra Pradesh and Madhya Pradesh for its seeds (Tiwari and Agrawal, 2004).

The earliest reference to the use of dill seed in medicine goes back to 'Charak Samhita' (700 BC). It is an ancient Egyptian remedy described in the Ebers papyrus (1500 BC), where it is an ingredient in a pain-killing mixture. The Romans chewed dill seeds to promote digestion, and they hung dill garlands in their dining halls, believing the herb would prevent stomach upset. It was also spread on the floors of banquet halls so that its fragrance would counteract the heavy food smells. In the seventeenth century, Nicolas Culpeper listed dill as a 'tonic that strengthens the brain'. The Puritans were known to place dill seeds in their Bibles to discreetly chew so that their stomach would not rumble during the long church services (Anon., 2010).

Dill is an erect, hardy annual plant belonging to the family Apiaceae. The leaves are thin, wispy and fern like. The glossy stem is usually single, erect, dull-green cylindrical, subdichotomously branched and reaching up to 0.9–1.5 m in height. The umbels are large and contain small yellow flowers and yellowish brown, ridged, flattened, ovoid fruits (Drazic *et al.*, 2007). Unlike the flowers of most other umbelliferous crops, the small yellow dill flowers have a strong odour but contain little nectar. The flowers are homogamous and hermaphrodite. Some of them are entirely pistillate (female), some staminate (male), and a few are hermaphrodite. The primary umbels bear hermaphrodite flowers, the secondary and tertiary ones bear hermaphrodite ones on the margins and staminate ones in the centre, similar to coriander. The ovary of the pistillate flower normally contains two ovules. The yellow staminate flower has five stamens that arise between the petals. Nectar is visible on the staminate flowers, and very little or none on the pistillate flowers. The seed of dill is hard, light brown, winged and oval, with one side flat and the other convex. There are three ridges on the convex side with three vittae, or oil channels; the flat side bears two ridges. The ridges are more pronounced in Indian dill than in the European dill.

#### 15.1.1 Dill species and varieties

There are two closely related cultivated species of dill, i.e. European dill (*A. graveolens*) and Indian dill (*A. sowa*). European dill is widely distributed in the world. Indian dill, which grows in India, has a lower carvone content and a slightly different flavour. The seeds are longer and narrower, with paler ridges. In India, dill is considered a minor seed spice crop and its cultivation is limited to a few parts of the country. Farmers cultivate the local varieties using their own seed. Mehasana local and Ruby local are those used by farmers in Gujarat and Pratapgarh local by farmers in Rajasthan.

The National Research Centre on Seed Spices in India has developed and recommended two improved varieties of dill: Ajmer dill 1 (NRCSS-AD-1) and Ajmer dill 2 (NRCSS-AD-2) for general cultivation. Ajmer dill 1 is a European-type dill variety which is suitable for cultivation under irrigated conditions. The leaves are dark green in colour with good content of herbal constituents. The seeds are dillapiole-free and contain about 3.5 % essential oil. The average plant height of Ajmer dill 1 is 134 cm. It takes about 142 days to reach maturity and yields 14.7 q/ha of seed under irrigated conditions. Ajmer dill 2 is an Indian-type dill variety, with bold, compact and dark

brown seeds. It is suitable for the cultivation under irrigated and rain-fed conditions. The average plant height is 90 cm and it takes about 135 days to reach maturity. The average seed yield is 14.6 q/ha under irrigated conditions and 5.8 q/ha under rainfed conditions. Seed of this variety contains about 3.2 % essential oil.

## 15.2 Production and cultivation of dill

Dill is primarily a summer crop of the temperate regions, but it has also adapted to grow in warmer areas. A dry and cool climate is suitable for dill cultivation. In the Northern Indian plains, it is grown during the *rabi* (winter) season. It requires cold weather for early vegetative growth and warm sunny days for seed formation and seed maturity. Temperatures above 30°C and below 7°C are not favourable for its growth and development. A humid climate is not suitable for this crop as this favours the attack of diseases and pests. It can tolerate higher rainfall, but waterlogging is not favourable for growth. Dill can be grown in a wide range of soils, including heavy black soils with high moisture retention capacity, in rain-fed cultivation and light soils as an irrigated crop. The soil should be rich in organic matter with a good fertility level and proper drainage for a higher yield. Saline soils are not suitable for dill cultivation.

### 15.2.1 Sowing details

The soil should be well-prepared and in good tilth before sowing. It should be prepared by one deep ploughing with a soil-turning disc plough followed by two ploughings with a harrow. For rain-fed/unirrigated crop production, moisture can be conserved by ploughing and planking after it has rained. The field should be well levelled before sowing for better germination.

Time of sowing has a significant effect on plant growth and seed yield. However, it may vary with the climatic conditions of the area. It is grown during the spring season (February–March) in temperate climates and October in tropical conditions. In India, dill is cultivated as an annual crop sown generally during the *rabi* (winter) and the early *rabi* (post-rainy) season. The rain-fed crop is sown early in the season, in the months of August–September. The irrigated main *rabi* season crop is sown in the month of October, preferably between the 15<sup>th</sup> and 30<sup>th</sup>. Delayed sowing after 30<sup>th</sup> October reduces seed yield as well as volatile oil content. Zehtab-Salmasi *et al.* (2006) also reported that early sowing resulted in higher and faster seed germination with heavier seedling dry weight as compared to late sown crop. Little is known about intercropping and crop rotation with dill. In general, dill should be grown as a sole crop to get the good results in terms of seed yield. However, it can be intercropped with onion and carrot during the *rabi* season. Dill should be grown in a three to four year crop rotation for healthy cultivation.

The general practice of sowing of dill is by broadcasting the seeds in the field, but line sowing is better, being an effective system for facilitating intercultural operations in the field. The seed should be sown about 1.5–2.0 cm deep. The row

spacing should be 50–60 cm for the European-type dill and 40–50 cm for the Indian-type dill. Plant to plant distance should be kept to 20 cm in both cases. Light irrigation is required just after sowing if the initial moisture in the soil is not sufficient. The seeds take 10–12 days to germinate. The soil temperature should be around 30°C.

Clean and healthy seeds, preferably less than two years old, should be selected so that they germinate easily. Soaking seeds in water for a day before sowing; accelerates germination. The seed rate depends on the sowing method used and the irrigation conditions. A higher seed rate is required for the broadcasting method under rain-fed cultivation as compared to line sowing and irrigated conditions. The seed requirement is 3 kg/ha for irrigated and 5 kg/ha for rain-fed conditions. The seed should be treated with seed dresser fungicides before sowing to get rid of soilborne and seedborne pathogens. There should be uniform and sufficient coating of the fungicides on the seed surface.

### 15.2.2 Manures and fertilizers

Dill requires soil with a good fertility status that provides all nutrients; hence, it responds well to fertilizer application. The application of fertilizers can affect seed yield and seed oil composition, so a balanced dose of nutrients in the form of fertilizers must be applied at the right time. The soil in the field should be tested before sowing for its nutrient status, and nutrients should be applied as required in the form of manures and fertilizers. Under irrigated conditions, well-decomposed farmyard manure (FYM) or compost at 10 t/ha should be applied at the time of field preparation. Additional nutrients should be applied by using fertilizers. The application of 60–90 kg nitrogen, 40–45 kg phosphorus and 20 kg potash has been recommended for higher seed, straw and biological yield under semi-arid conditions. One third of the dose of nitrogen should be applied as a basal dose at the time of sowing with the total quantities of phosphorus and potassium. The remaining quantity of nitrogen should then be applied in two split doses, the first at 30 days after sowing and the second at the flowering stage as a top dressing. An application of sheep manure at 10 t/ha with biofertilizer is recommended for higher seed yield of dill (Meena *et al.*, 2007). Under rain-fed conditions, manure should be applied in the form of well-decomposed FYM or compost (10 t/ha) once every two years. The fertilizer requirement is 40 kg N, 30 kg P<sub>2</sub>O<sub>5</sub> and 20 kg K<sub>2</sub>O per hectare. The fertilizers should be applied at the time of sowing.

### 15.2.3 Irrigation

Dill should be irrigated as required for better growth during the cropping period. The crop is sown under irrigated conditions in light soil and in unirrigated condition (i.e. as a rain-fed crop) in black cotton soils. Under irrigated conditions, pre-planting irrigation is required to wet the seed bed. If the soil moisture is not sufficient at the time of sowing, a light irrigation can be given just after sowing for proper germination. Sufficient water should be provided during the seed filling stage for the production of high-quality seeds (Zehtab-Salmasi *et al.*, 2006). In all, five to eight light irrigations are required to raise the crop.

### 15.2.4 Weed management

The initial growth of dill is slow; therefore, it is necessary to keep the field clean and weed free by proper weeding and hoeing. In general, two or three manual or mechanical weeding are required. The first weeding and hoeing should be done in about three to four weeks after sowing. The next weeding should be done whenever required at 30-day intervals. Weeds can also be controlled by pre-emergence application of chemicals. There should be sufficient moisture in the soil to ensure better weed control by chemicals.

### 15.2.5 Disease and pest management

Dill is susceptible to a number of plant pathogenic fungi, but the damage caused by root rot and foliar diseases like powdery mildew and blight is extensive. The disease incidence and damage on dill crop depends on the climate and soil conditions. Humid and moist conditions generally favour the appearance and spread of diseases. Powdery mildew and root rot are the major diseases of dill. Powdery mildew in dill is caused by the fungus *Erysiphe* spp. It has been reported from India by Saxena and Saksena (1981) on *A. sowa*. The symptoms of the disease appear on all green plant parts including leaves, stem and inflorescence as white powdery mass. The symptoms later on extend to other parts of plants, including seeds, which affect the yield as well as the quality of seeds. Root rot is caused by the fungus *Fusarium* spp. The disease symptoms can appear in all growth stages, but the early vegetative growth stage is more affected with the disease. The leaves showing chlorosis initially become yellow and ultimately the plant dries and dies prematurely. The affected plant roots show browning and rotting. The disease can cause severe yield losses if it appears early in the season.

Dill crop is also affected by several other diseases of minor importance, i.e. damping off – *Pythium* spp., *Rhizoctonia* spp., seedling blight – *Alternaria radicina*, leaf spot – *Ascochyta anethicola*, head rot – *Botrytis* spp., *Alternaria* spp., scab – *Fusicladium* spp., rust – *Puccinia petroselini* and celery mosaic virus.

Dill is frequently attacked by insect pests of the other umbelliferae crops, but the damage is comparatively less. The crop is generally attacked by insects like aphid, cutworm and leaf-eating caterpillars. Aphid is a major sucking pest of dill, which generally appears during the flowering stage of the crop. It sucks tender parts of the plant and flowers resulting in yellowing of the crop and shrivelled grains. It can be controlled by spraying of chemical and biopesticides and by natural enemies (predators and parasites) appearing naturally during the cropping season.

### 15.2.6 Harvesting

The harvesting time of dill depends upon the purpose for which it is grown. When it is grown for vegetable purpose it can be harvested at the four to five leaves stage. When it is grown for seed purpose the crop is harvested at maturity. The crop matures in 130–150 days and can be harvested when the seeds of the main umbel turn brown. Delay in harvesting results in shattering of grains in the field. Boonsong and Sutevee (2008) recommended that mature dill seed could be safely harvested between 45 and 70 days after anthesis, when the seed moisture content is between 9 and 12 %. This

corresponds to the time that fruits are fully dried but not shed. Harvesting is done by cutting the plants with a sickle 40 cm above ground level. Moustafa *et al.* (1990) recommended that cutting dill plants after 75 days from sowing (for the 1st cut) and 45 days after the 1st cut (for the 2nd cut) gives the highest herb and fruit yields with best quality. However, according to Osman and El-Wahab (2009), harvesting dill one or two times to obtain a fresh herb causes reduction in plant weight at the flowering stage, and consequently less umbel numbers, seed and oil yield, as compared to non-harvested plants. On average, the yield is 10–15 q/ha under irrigated conditions and 6–7 q/ha under rain-fed conditions. Good crop management practices will enable yields such as these to be achieved.

### 15.2.7 Post-harvest processing and value addition

Harvested dill plant bundles should be dried under shade and threshed either manually by beating with sticks or mechanically in a seed spice thresher. Threshed seeds should be cleaned, dried and stored in ventilated bins to minimize the losses during storage.

Oil of dill can be extracted from both herbs and seeds. For the production of dill herb oil, fresh plants are chopped into two to three pieces and distilled immediately. Oil is extracted from dried fresh seeds through steam distillation. The quality of oil received from these seeds is superior. An essential oil yield of 0.9–1.5 % and 2.5–4 % can be achieved from herbs and seeds, respectively. The volatile oil and oleoresins extracted from dill seed are in demand from the pharmaceutical, cosmetics and food industries. The important processed products of dill are herb essential oil, the dried fruit or spice dill seed, seed essential oil and oleoresin.

## 15.3 Chemical composition

The chemical composition of dill volatile oil varies depending on the plant part from which it was extracted (Valeria *et al.*, 2010). In the leaves oil, monoterpenic hydrocarbons are predominant, amounting to 79.14 % (62.71 %  $\alpha$ -phellandrene and 13.28 % limonene). In the flowers oil, the content of  $\alpha$ -phellandrene and limonene is 32.26 % and 33.22 %, respectively. Anethofuran (dill ether) is present in leaves and flowers with 16.42 % and 22 %, respectively, but is missing in the fruit oil. The main compound in seed essential oil is carvone (75.21 %), while the  $\alpha$ -phellandrene content is only 0.12 % and the limonene content is 21.56 %. Recently, Kaur and Arora (2010) reviewed the phytoconstituents of dill and reported that seeds of *A. graveolens* contain 1–4 % essential oil comprising major compounds: carvone (30–60 %), limonene (33 %),  $\alpha$ -phellandrene (20.61 %), including pinene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, isomyristicin, myristicin, myristin, apiole and dillapiole. Furanocoumarin, 5-(4''-hydroxy-3''methyl-2''-butenyloxy)-6,7-furocoumarin, oxypeucedanin, oxypeucedanin hydrate and faltarindiol are other constituents of dill essential oil. Badar *et al.* (2008) separated capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid as heterogeneous components from *A. graveolens* seed oils using gas chromatography.

The chemical composition also varies depending on the type of dill. Saleh-e-In Moshfekus *et al.* (2010) analysed the oil obtained by hydrodistillation from the fresh leaves and stems of *A. sowa* by gas chromatography–mass spectroscopy (GC-MS) and isolated and identified 20 compounds. The major constituents were apiole (25.39 %), *o*-cymene (15.25 %),  $\alpha$ -thujene (14.84 %),  $\alpha$ -phellandrene (7.17 %), 6,6-dimethyl-2-(3-oxobutyl)bicyclo[3.1.1]heptan-3-one (6.90 %), *exo*-2-hydroxycineol (5.03 %), limonene (4.13 %), 3-isopropyl-4-methyl-1-pentyn-3-ol (2.89 %), myristicine (2.46 %) and dihydroumbellulone (2.14 %). Dillapiole is usually found in much smaller quantities in European dill oil than in Indian dill oil. However, European dill oil was found to contain up to 3.0 % of dillapiole (Baslas *et al.*, 1971) when grown under tropical conditions. Gupta (1982) explained that this was due to more sunlight hours combined with solar intensity in the tropics.

## 15.4 Main uses of dill

The dill plant has diverse uses. The young tender leaves and dried leaves are used for culinary purposes and the seeds are used as spices. It is also used for medicinal purposes.

### 15.4.1 Culinary uses

The taste of dill leaves resembles that of caraway, while the seeds are pungent and aromatic. Freshly cut, chopped leaves enhance the flavour of dishes such as dips, herb butter, soups, fish-based meals and salads. The seeds are used in pickling and can also improve the taste of roasts, soups, stews, rice dishes and vegetables. The difference between dill leaf and dill seed lies in the degree of pungency. There are occasions when the seed is better because of its sharper flavour. The dried leaf can also be used in many dishes, and dill oils are used extensively in alcoholic and soft drinks.

### 15.4.2 Medicinal uses

Both seeds and oil are used in the preparation of various indigenous medicines. In traditional medicine, it has a prominent place for stomach problems, especially in the ailments of children and women. The essential oil, dill oil or its emulsion in water commonly known as dill water are considered to be aromatic and carminative and especially useful in the control of flatulence, colic pain, hyperacidity, vomiting, diarrhoea and hiccups due to indigestion in infants. The application of dill water with turmeric powder is said to prevent the formation of ulcers and heal them quickly. Leaves boiled in sesame oil are said to make an excellent liniment for reducing swelling and pain of the joints. The seeds are considered effective in respiratory disorders like colds, influenza and bronchitis. It is used together with vacha as fumigation therapy for piles. It is thought to be very useful for women during delivery for expulsion of placenta and for promotion of milk secretion.

There has also been scientific research into the properties of dill. Hosseinzadeh *et al.* (2002) and Rifat-uz-Zaman *et al.* (2006) reported the anti-ulcer activity of dill



fruits. *A. graveolens* also possesses potent hypocholesterolaemic effects in rats, probably mediated through the suppression of endogenous cholesterol biosynthesis by inhibition of the activity of HMG-CoA reductase (Yazdanparast and Bahramikia, 2008). Myristin in dill is psychoactive and hallucinogenic and the apiole content may be responsible for the diuretic properties (Mazyad *et al.*, 1999; Delaquis *et al.*, 2002; Singh *et al.*, 2002; Lopez *et al.*, 2005).

#### 15.4.3 Antimicrobial activity

Some studies have shown the antimicrobial activity of *A. graveolens*. Essential oils of *A. graveolens* had antimicrobial activity against *Saccharomyces cerevisia*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* sp. and *Escherichia coli* (Pascal *et al.*, 2002; Stefanini *et al.*, 2003). Volatile oils extracted by steam distillation method from roots, stem and leaves of dill did not show antibacterial or anticandidal activities; however, seed extracts from dill exhibited varying degrees of growth inhibition of *Candida albicans*, *C. tropicalis* and *C. glabrata* (Abed, 2007). According to Badar *et al.* (2008), essential oils extracted from dill seed by steam distillation also showed antimicrobial activity (100 %) as compared to oxytetracycline (100 %), chloramphenicol (35 %), gentamicin (81.82 %) and penicillin G (57.14 %) against *E. coli*, *S. typhi*, *Bacillus subtilis* and *S. aureus*, respectively. Singh *et al.* (2005) considered the essential oils an alternative source of natural antioxidants as well as antimicrobials. Oxypeucedanin, oxypeucedanin hydrate and falcariindiol isolated from *A. graveolens* plant exhibited antibacterial activity against a panel of rapidly growing mycobacteria with minimum inhibitory concentration (MIC) values in the range 2–128 µg/ml (Stavri and Gibbons, 2005). In addition, essential oil of *A. graveolens* also possesses antimicrobial activity against the mould *Aspergillus niger* and the yeasts *Saccharomyces cerevisiae* and *Candida albicans* (Jirovets, *et al.*, 2003). Bayat *et al.* (2008) reported that essence of *A. graveolens* was effective in inhibiting the growth of most important microorganisms in Leucorrhoea including *C. albicans* and *Gardnerella vaginalis*.

#### 15.5.4 Other uses

Carvone from dill increases the effectiveness of the garden insecticide parathion. Studies showed that only 8 % of fruit flies exposed to parathion die, but 99 % die when exposed to the same amount of parathion plus carvone. Myristin, apiole and dillapiole present in dill essential oil are also effective, naturally-occurring insecticides or insecticide synergists. The oil of Indian dill contains a large proportion (20 %) of dillapiole and, compared to European dill, has a lower carvone content (30–45 %). Dillapiole is a viscid colourless substance that is found to act in synergy with pyrethrin (which is used in insecticides), making it more effective. Dillapiole isolated from the essential oil of dill inhibited aflatoxin G<sub>1</sub> production by *A. parasiticus* with an IC<sub>50</sub> value of 0.15 µm (Razzaghi-Abyaneh *et al.*, 2007). Dill oil is also used as a fragrance component in detergents, cosmetics, perfumes and especially soaps.

## 15.5 Quality issues and standards

All the importing countries of the world strongly prefer clean, high-quality spices, and when selecting spices to purchase they consider issues including pesticide residues, levels of mycotoxins (including aflatoxins), heavy metals and microbial contaminants, and adulterants including artificial colours among other materials. The quality standards mostly used as international guidelines for importing spices are: the Bureau of Indian Standards (BIS) specifications, the American Spice Trade Association (ASTA) specifications, the United States Food & Drug Administration (US FDA) specifications, the International Organization for Standardization (ISO) guidelines and the European Spice Association (ESA) specifications. The analytical test methods used are those of the Association of Official Analytical Chemists (AOAC).

According to the BIS, the specification for oil of Indian dill seed (*A. sowa*) is as follows: specific gravity (27°C) 0.9360–0.9800, optical rotation +50° to 65°, refractive index (20°C) 1.426–1.495, ester value before acetylation 35–42, ester value after acetylation 50–65, total ketone (calc. as carvones) min. 35 %, soluble in 0.5 and more vols of 90 % alcohol (BIS, 1992). The ASTA specifications for dill seed, which are phrased in terms of suggested limits for each contaminant, are: whole dead insects count: 4, mammalian excreta: 3 mg/lb, other excreta: 2 mg/lb, mould: 1 % by weight (wt), insect defiled/infested: 1 % by wt and extragenous/foreign matter: 0.5 % by wt. According to the European Spice Association (ESA), the prescribed quality specification for dill are: content of total ash maximum 10 % w/w, acid-insoluble ash maximum 2.5 % w/w, H<sub>2</sub>O maximum 12 % w/w, volatile oil minimum 1 % v/w.

Pesticides residues, mycotoxin, heavy metals, pathogenic microorganisms and adulterants are considered the main factors that reduce dill quality. Badar *et al.* (2008) reported that the oil extracted from the European dill seed smelled like grass, was pale yellow in colour and had a watery viscosity. Percentage yield, specific gravity, refractive index and acid value of oils were 0.66 %, 1.51, 1.49 and 0.58, respectively. In dill oil a common adulterant employed is distilled orange terpenes (Singhal *et al.*, 2001). The iodine value for pure dill oil is recommended to be 265–307. The presence of a minimum of 5.0 % 3, 9, epoxy-*p*-menthene in dill herb oil is also a good indicator of its purity as there is no commercial source of this compound. Lawrence (1981) opined that examination of the percentage ratio of  $\alpha$ -phellandrene to limonene to  $\beta$ -phellandrene is another test for quality determination, the acceptable ratio in dill oil being 20:25:3. Indian dill is a common biological adulterant for the European dill (Dhanya and Sasikumar, 2010).

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## Fenugreek

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**Abstract:** Fenugreek (*Trigonella foenum-graecum* L.) is a widely cultivated annual herb. The leaves and seeds of fenugreek are commonly used for flavouring and as a spice in curries due to their strong flavour and aroma. This chapter presents the history and description of fenugreek before describing its production, cultivation and harvesting and chemical composition. The functional properties of fenugreek leaves and seeds, including their culinary and medicinal (both traditional and modern) uses, are presented together with the nutrient levels, heat treatments and toxicity. Quality issues and applicable standards and specifications are also described.

**Key words:** fenugreek, *Trigonella foenum-graecum* L., health food.

### 16.1 Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb belonging to the Fabaceae family. The genus *Trigonella*, meaning ‘little triangle’, refers to the shape of the leaflets. The genus *Trigonella* consists of more than 50 species. The most cultivated and widely spread species of this genus is *foenum-graecum*, fenugreek, which literally means ‘Greek hay’ indicating its use as forage in the past. Fenugreek is a native of South-Eastern Europe and West Asia, i.e. the Mediterranean region (Petropoulos, 2002), where it is found growing wild and has been cultivated for a long time. The Indian sub-continent is thought to be its secondary centre of origin as there is wide diversity among the fenugreeks found in this area and wild forms are also found. It is one of the oldest known crops in India. Carbonized fenugreek seed has been recovered from Rohilla village in the Sangrur district of Punjab, India, indicating its use and trade in the Harappan civilization as far back as 2000–1700 BC (Saraswat, 1984). References for utilization of fenugreek as far back as 1578 have been reported by Hidvegi *et al.* (1984) and uses of fenugreek seed as incense and to embalm mummies were documented in ancient Egypt (Basch *et al.*, 2003). Fenugreek leaves and seeds are commonly used for flavouring and as a spice in curries due to their strong flavour and aroma. In addition, Araee *et al.* (2009) reported a wide range of medicinal uses, including for the treatment of inflammation, tumours, cardiovascular diseases, renal insufficiency, infections and metabolic disorders.

Fenugreek plants are usually 20–80 cm tall, spreading and moderately branched, but erect and straight forms are also available. The stem is circular to slightly quadrangular, sparsely pubescent, usually hollow and light green with or without an anthocyanin tinge. The leaves are simple and trifoliate, distinctly petiolate and stipulate. The mature plant leaves are trifoliate. The leaflets are ovate–orbicular to oblong lanceolate in shape, 1–4 cm long, sometimes finely haired. These may be tinged with anthocyanin to a varying degree of green. Petioles are pale green, pubescent and often anthocyanin tinged. The common type of fenugreek shows indeterminate behaviour with axillary flowers, but some cultivars are also available which show determinate behaviour with terminal flowers. Flowers are typically leguminous and situated in leaf axils singly or in pairs. They have pale yellow corolla when young but are white on maturity. Pods are beaked, 10–18 cm long, curved or straight with hairs. Young pods are green or pigmented, but mature ones are light straw to brown coloured. The mature pod contains 10–20 seeds. Seeds are usually golden yellow coloured, but other variants such as light cream, light greyish, brown and olive green are also present. Seeds vary from rectangular to rounded in shape. Their length is 3.5–6 mm and their width is 2.5–4 mm. Fenugreek seed is a good source of protein (29 %), volatile and fix oil (6 %), vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, niacin, pantonine, the alkaloids trigonelline, saponine, diosgenin, methionine and trimethylamines. It also contains other constituents like iron, phosphorus, several enzymes, hormones and gums.

## 16.2 Production and cultivation of fenugreek

Fenugreek is widely distributed throughout the world in wild or cultivated forms. Fenugreek is now grown in six continents, namely Europe, Africa, Asia, South America, North America and Australia. The distribution of fenugreek (*Trigonella foenum-graecum* L.) is given in Table 16.1. Two species of *Trigonella* are reported to be cultivated and economically important. These are *T. foenum-graecum* (the common seed type) and *T. corniculata* (the leaf type) fenugreek. Fenugreek has been cultivated as an economically important crop in India, Morocco, China, Pakistan, Spain, Tunisia, Turkey, Lebanon, Israel, Egypt, Ethiopia, Kenya and Tanzania. (Edison, 1995). Besides these countries, it is also cultivated in Portugal, the UK, Germany, Austria, Switzerland, Greece, Sudan, Japan, Russia, Argentina, Australia, Canada and the USA.

**Table 16.1** Distribution of fenugreek in world by continents

| Continents    | Countries                                                                                 |
|---------------|-------------------------------------------------------------------------------------------|
| Asia          | India, China, Iran, Israel, Japan, Russia, Lebanon, Nepal and Pakistan                    |
| Africa        | Egypt, Ethiopia, Kenya, Morocco, Sudan, Tanzania and Tunisia                              |
| Europe        | Austria, France, Germany, Greece, Portugal, Russia, Spain, Switzerland, Turkey and the UK |
| South America | Argentina                                                                                 |
| North America | Canada and the USA                                                                        |
| Australia     | Some parts of Australia                                                                   |



**Table 16.2** Area production and export of fenugreek seed from India

| Year       | Area (ha) | Production (tonnes) | Export from India |                         |
|------------|-----------|---------------------|-------------------|-------------------------|
|            |           |                     | Quantity (tonnes) | Value (Rs. in millions) |
| 2005–6     | 33 393    | 38 990              | 15 525            | 340.3                   |
| 2006–7     | 44 984    | 55 780              | 8616              | 271.1                   |
| 2007–8     | 55 000    | 55 400              | 11 100            | 330.0                   |
| 2008–9     | NA        | NA                  | 20 750            | 717.5                   |
| 2009–10    | NA        | NA                  | 21 000            | 697.2                   |
| 2010–11(E) | NA        | NA                  | 18 500            | 654.8                   |

Source: Spices Board, Cochin, Kerala.

### 16.2.1 Cultivated areas

Global statistics for the size of the fenugreek cultivation area and amount of seed produced are not available. Many countries have only a very small area under cultivation according to their agricultural statistics. As per the information available, Petropoulos (2002) estimated that the annual cultivated area of fenugreek amounts to roughly 57 000 ha with a seed production of 68 000 tonnes. The average cultivated area during 1975–95 was around 34 500 ha producing 41 500 tonnes of seeds. India is the largest producer, consumer and exporter of fenugreek in the world. In 2007–8, India's total cultivated area of fenugreek reached 55 170 ha with a production of 55 450 tonnes of seeds. Size of production area and statistics on fenugreek cultivation in India are given in Table 16.2. Export statistics show that India has exported 20 750 tonnes of fenugreek with value of 718 million rupees during 2008–9 (Anon., 2009). Major markets for Indian fenugreek are France, Germany, Canada, Israel, Japan, Korea, Kuwait, Malaysia, the Netherlands, Saudi Arabia, Singapore, South Africa, Switzerland, UAE, the UK and the USA.

### 16.2.2 Production technology

#### *Climate*

Fenugreek is very adaptable, meaning that the crop can be grown successfully both in tropical and temperate regions up to an altitude of 2000 m above mean sea level. A cool growing season without extremes of temperature is favourable for best development. Though, in India, it is mainly grown as a *rabi* (winter) season crop but, in South India, it is also grown as rainy season crop. Dry weather during crop maturity is essential for a good seed yield.

#### *Soil*

Fenugreek can be grown in almost all types of soils that are well drained, but it grows best on well-drained loamy soils. Organic matter-rich clay-loam soil may also be used if adequate drainage facilities are available. The crop does not thrive particularly well in sandy or gravelly soils. Although the crop is tolerant to salinity up to 8.4 pH, it always gives higher yield and has better quality leaves in neutral soils with a pH range of 6.0–7.0.



*Preparation of land*

The land should be well prepared for better germination and growth of fenugreek. A total of three to four ploughings are required. The first ploughing should be done by a soil-turning plough followed by two to three ploughings with a harrow to bring the soil to a fine tilth. At the time of sowing, there should be good moisture in the soil for better germination of seeds.

*Sowing time*

Fenugreek, being a cool season crop, is sown from October–November in Northern plains of India, whereas, in hilly tracts, it is sown from March–May, depending on the altitude. As already mentioned, for higher yield, it is advisable to adjust the sowing time so that the pod development and seed maturity phase may coincide with a dry period.

*Seed rate and crop geometry*

The quantity of seed required for the common type of fenugreek is 20–25 kg/ha. The crop geometry should be 25–30 cm × 10–12 cm.

*Fertilizer requirement*

The fenugreek crop has been reported to absorb both macro as well as micro-nutrients well. The crop removes N, P and K in the ratio of 2:1:1 from the soil, in the uptake order of 10, 3.5, 8.2 kg, respectively. The doses of fertilizer that are required depend on the fertility status of the soil, the soil type and fenugreek variety. The doses are in the range of 40–60 kg nitrogen, 40–60 kg phosphorus and 20–40 kg potash.

*Irrigation*

Fenugreek, being primarily an irrigated crop, requires light irrigation at frequent intervals for quick growth. Irrigation is given at 12–15 day intervals, depending on soil type, season, rainfall and other temporary weather conditions. The early growth period and seed setting are the critical stages at which irrigation is particular required. Application of seven irrigations at irrigation water/cumulative pan evaporation (IW/CPE) ratio of 1.00/0.75 was found to be optimum. Normally six to seven irrigations are required in light soil and four to five irrigations in heavy soil.

*Interculture operations*

Fenugreek, being a leguminous crop, needs proper soil aeration for normal development of its root system; thus, hoeing and weeding during early stages of plant growth are very essential to make the soil loose around the roots and to control the weeds. Weeds may pose problems in the initial stages due to the slow growth of fenugreek seedlings. However, in the later stages, when the crop's canopy is fully developed, weeding is not at all required as the crop suppresses the weeds itself.

*Crop protection*

Fenugreek crop is generally less affected by insect-pests and diseases. However, a few that sporadically harm the crop are aphids (*Acrythosiphon pisum*, Henis. *Myzus*

*persicae*, Sulzer and *Aphis craccivora*, Koch.), leaf-eating caterpillars (*Spilarcitia oblique* and *Spodoptera litura* Fabricius), pod borers (*Helicoverpa armigera* Hubner), jassids (*Empoasea* spp.), white fly (*Bemisia tabaci*), leaf minor (*Empoasea* spp.), mites (*Pertobia latens*), powdery mildew (*Erysiphe polygoni* D.C. and *Laveil-lula tourica* Lev.), downy mildew (*Peronospora trigonella* Gaum.), root rot (*Rhizoc-tonia solani* Kuhn.), leaf spot (*Cercospora traversiana* SOCC) and rust (*Uromyces anthyllidis*). Plant protection measures for reducing the incidence of diseases and pests and adoption of control measures should include selection of resistant varieties, crop management practices such as correct time of sowing, balanced nutrition, crop rotation, green manuring, etc.

#### *Harvesting and yield*

Depending upon variety and season of growing, the seed crop takes about 80–165 days from sowing to harvest. A good crop can yield 2.0–2.5 tonnes of seeds per hectare (Kakani *et al.*, 2009).

### **16.2.3 Post-harvest processing and value addition**

It is strongly advised to keep the requirements of the target market in mind during post-harvest operations. Fenugreek seeds should be properly cleaned and then stored with an initial moisture level of 7–8 % and at an equilibrium relative humidity of 40 %. Well-packed fenugreek seeds should be stored in a ventilated dry and cool place. Fenugreek powder is also prepared as there is a market for it in different countries. Oleoresin is also extracted from fenugreek seed and is used in many industries. Fenugreek powder can be produced by milling of whole seed to a certain degree of fineness as required by the food processor. Oleoresins are generally produced by the solvent extraction method.

## **16.3 Chemical composition**

Fenugreek seeds contain 6.87 % moisture, 28.4 % crude protein, 7.14 % crude lipids, 9.3 % crude fibre, 3.28 % ash and 47.4 % carbohydrates (Nasri and Tinay, 2007). Nour and Magoul (1986) reported that 100 g of fenugreek seed contained 49.3 mg sodium, 1306 mg potassium, 22.5 mg iron, 158 mg calcium, 415 mg phosphorus, 1550 mg manganese, 9.9 mg zinc and 331 mg copper. They also reported that 1 g of fenugreek protein contains 38.5 mg isoleucine, 53.5 mg leucine, 51.4 mg lysine, 5.6 mg methionine, 58 mg phenylalanine, 26.6 mg threonine, 50 mg valine, 19.6 mg histidine, 24.5 mg tyrosine, 29.7 mg alanine, 82.2 mg aspartic acid, 115 mg glutamic acid, 39.8 mg glycine, 32.1 mg proline, 35.3 mg serine, 3.1 mg ornithine and 10.8 mg phosphoethanolamine. Lipids in fenugreek seeds are 85 % neutral lipids, 10 % phospholipids and 5 % glycolipids. The fatty acid profile is dominated by unsaturated fatty acids comprising mainly 40 % linoleic, 25 % linolenic and 25 % oleic acids (Baccou *et al.* 1978; Sulieman *et al.* 2000). Carbohydrates are mainly present as galactomannans. Trigonelline is an important alkaloidal component of the seed. Aroma in the seeds of fenugreek arises from *n*-alkanes, sesquiterpenes and non-lactone. Dawidar *et al.* (1973) reported that seeds are also rich in saponins like

rigogenin, neorigogenin, diosgenin, gitogenin, yamogenin and tigogenin. Diosgenin is the main steroid saponin which varies from 0.78 % to 1.9 % depending on the genotype and cultural practices (Bochannon *et al.*, 1974; Bhavasar *et al.*, 1980; and Sharma and Kamal, 1982). Fenugreek leaves are also a good source of protein, minerals and vitamin A and C. They contain 86.19 % moisture, 4.49 % protein, 0.99 % fat, 6.09 % carbohydrate, 1.19 % fibre, 1.59 % total ash. One hundred grams of fenugreek leaves contain 51 mg phosphorus, 76.1 mg sodium, 360 mg calcium, 17.2 mg iron, 51 mg potassium, 167 mg sulphur, 165 mg chlorine, 6450 IU vitamin A, 0.05 mg vitamin B<sub>1</sub>, 54 mg vitamin C and 13 mg oxalic acid (Aykroyd *et al.*, 1963).

## 16.4 Functional properties and main uses of fenugreek

### 16.4.1 Culinary uses

Fenugreek seeds have been used as a spice and its leaves eaten as a vegetable in India, for example in salads, soups, stews and in cauliflower, bean, potato and mango-based dishes, in China and the Middle East for centuries. The most well-known use of fenugreek as a food is as a flavouring agent in curry dishes, but the ground seeds are not only a vital ingredient of curry powders, but also of oriental sauces and halva as well. It is also used in chutneys, pickles and other condiments, chewing gum and confectionery, soft drinks, cakes, puddings, ice creams and syrups (maple, caramel, butterscotch and vanilla). Bakr (1997) reported that supplementation of 4 % fenugreek flour in wheat flour resulted in bread with high nutritional characteristics and higher acceptability in Egypt. Galal (2001) reported the use of corn bread mixed with about 3 % fenugreek as a staple food in Egypt. Sharma and Chauhan (2002) reported that supplementation of fenugreek flour in rice bran improved the physical and sensory properties and qualities of bread and cookies. The sprouted seeds are used in salads and sandwiches. In India and the Arabic world, the seeds are roasted and used to make fenugreek coffee, a highly regarded beverage. In other parts of the world, fenugreek tea is drunk as a soothing beverage.

### 16.4.2 Medicinal uses

Fenugreek seeds have long been prized for their medicinal properties. A wide variety of medicinal properties associated with fenugreek have been recorded in literature, travel records and religious texts from different parts of the world and periods of human history (Lust, 1986). The ripe, dry seeds have long been used in Arabian, Indian and Chinese medicines. Fenugreek was esteemed also by the ancient Egyptians, Greeks and Romans as a remarkably versatile medicinal plant that could be used as a remedy for a wide variety of conditions. Over the centuries it has particularly become known as a powerful galactagogue (a substance which increases a mother's milk supply) and digestive aid, for example to resolve gastric inflammation and as a general digestive tonic. In traditional Chinese medicine, fenugreek is prescribed for: kidney abnormalities, backache, liver ailments, loss of libido, impotence, premature ejaculation, testicular pain, hernias, painful menstruation, menopausal symptoms, lymphadenitis, microbial and fungal infections and oedema of the legs.

In Ayurvedic medicine it is used for rejuvenation, as an aphrodisiac, a diuretic, for digestive complaints, dyspepsia, coughs, colic, bronchitis, constipation, gout, arthritis, enlargement of liver and spleen and as a breast enhancer. Externally it is used in poultices for local inflammation of the skin, cellulitis, boils, ulcers, abscesses, burns, eczema, dermatitis and chapped lips. Scientific studies suggest the potential to use fenugreek as an antidiabetic and to lower cholesterol (Chaturvedi and Pant, 1998; Devi *et al.*, 2003; Hannan *et al.*, 2003; Thakran *et al.*, 2003; Thompson Coon and Ernst, 2003; Vats *et al.*, 2003; Venkatesan *et al.*, 2003; Suboh *et al.*, 2004), against hyperthyroidism (Tahiliani and Kar, 2003a), against thyroxine-induced hyperglycaemia (Tahiliani and Kar, 2003b), for its anticancer (Devasena and Menon, 2003) and gastroprotective effects (Pandian *et al.*, 2002), as an antioxidant (Raskin *et al.*, 2002), antinociceptive (Javan *et al.*, 1997), antimicrobial (Bhatti *et al.*, 1996) and antihelminthic (Ghafgazi *et al.*, 1980), against sterility and as an anti-androgenic (Kamal *et al.*, 1993), for wound healing (Taranalli and Kuppast, 1996) and as an anti-inflammatory and antipyretic (Ahmadiani *et al.*, 2001).

#### *Chemicals with medicinal value in fenugreek seeds and leaves*

Petropoulos (2002) listed a number of important chemicals found in fenugreek seeds and leaves along with their medicinal value. In general, fenugreek contains three important constituents with medicinal value: (i) 4-hydroxyisoleucine, (ii) galactomannans and (iii) steroidal saponins. These constituents have placed fenugreek among the most commonly recognized 'nutraceutical' or health food products.

- **4-hydroxyisoleucine:** 4-hydroxyisoleucin, an amino acid structurally related to branched-chain amino acids, is not present in mammalian tissues, but it is present in the fenugreek seed (Gupta *et al.*, 1999). It is known to regulate the secretion of insulin in animals (Sauvaire *et al.* 1998; Broca *et al.* 1999, 2000).
- **Galactomanans:** Galactomannans, the polymers of galactose and manose (G:M), are present in fenugreek seeds at a ratio of 1:1 (Raghuram *et al.* 1994). They make up an integral component of the cell wall in the seed endosperm (Meier and Reid 1977). The 1:1 G:M ratio makes fenugreek galactomannans unique. The high ratio of galactose substitution contributes to their hydrophilic properties making them superior in terms of gel-forming characteristics. Raghuram *et al.* (1994) also suggested that this higher hydrophilic activity reduces glucose absorption in the digestive tract.
- **Steroidal saponins:** Saponins are naturally-occurring glycoside compounds found in many foods and forage plants. The fenugreek seed is an important source of steroidal saponins such as diosgenin which are used extensively by both the pharmaceutical and nutraceutical industries. The saponins mainly consist of an aglycone linked with one or more sugars. Aglycones are hydrophobic and sugars are hydrophilic making saponins an effective emulsifiers. Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone (Fazli and Hardman, 1968; Raghuram *et al.*, 1994). Fenugreek may be a viable alternative source for production of diosgenin because of its shorter growing cycle, lower production costs and consistent yield and quality (Hardman 1969; Petropoulos 1973).

*Antidiabetic activity and related effects*

Fenugreek galactomannans appear to be beneficial for control of type 2 diabetes in animals (Ribes *et al.* 1986; Raju *et al.* 2001; Tayyaba *et al.* 2001; Puri *et al.* 2002; Vats *et al.* 2002, 2003) and humans (Sharma 1986; Madar *et al.* 1988; Sharma and Raghuram 1990; Raghuram *et al.* 1994; Sharma *et al.* 1996a; Puri *et al.* 2002) by reducing hyperglycaemia. When these fibres are taken as supplements, they are found to be effective in lowering lipids and systolic blood pressure (Vuksan *et al.*, 2001). Fenugreek's antidiabetic activity is thought to be due not only to its high fibre content, but also to other seed components as well. These components work synergistically inhibiting glucose absorption and promoting pancreatic functions. For example, the presence of 4-hydroxyisoleucine together with dietary fibre in fenugreek seed produces a hypoglycaemic and anti-hyperglycaemic effect (Madar 1984; Ribes *et al.* 1986; Moorthy *et al.* 1989; Sauvaire *et al.* 1996; Petropoulos 2002). Vijaykumar and Bhat (2008) in a study using mice reported that fenugreek seed extract corrects metabolic alterations associated with diabetes by exhibiting insulin-like properties and has potential for clinical applications. McAnuff *et al.* (2002) also reported that steroidal saponins are effective agents for the treatment of hypocholesterolemia, a disorder often associated with diabetes. In addition to its hypocholesterolemic effects, Basu and Srichamroen (2010) concluded that extensive research done so far suggests that 4-hydroxyisoleucine, galactomannan and saponins from fenugreek lower blood glucose, cholesterol, triglyceride and free fatty acids levels and reduce abdominal fat.

*Anti-inflammatory and analgesic activity*

The analgesic and anti-inflammatory effects of fenugreek were examined by Vyas *et al.* (2008) using a partially purified fraction of the seed extract. The fenugreek extract showed highly significant, dose-dependent analgesic activity against thermally as well as chemically induced pain when compared to the control group. The seed extract at a dose of 40 mg/kg showed significant analgesic activity as compared to diclofenac sodium and pentazocine. Fenugreek also produced marked acute anti-inflammatory activity in rats (Vyas *et al.*, 2008). These results suggest that water-soluble fraction of fenugreek has significant analgesic and anti-inflammatory potential.

*Antioxidative activity*

Bukhari *et al.* (2008) reported that methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate extract of fenugreek exhibit antioxidant activity as studied using the free radical scavenging (DPPH<sup>•</sup>) and ferric reducing antioxidant power (FRAP) assays. Maximum activity was shown by the ethanol extract followed by the methanol extract. The lowest activity was shown by the hexane extract. This antioxidant activity correlates with the polyphenolic compounds present in the extracts. The steroidal saponins have also shown antioxidant activities (McAnuff *et al.* 2002).

**16.4.3 Changes in nutrient levels in fenugreek seeds**

Fenugreek seed are also consumed cooked, roasted or after germination. The processing of fenugreek seed has various effects on its nutrients, protein fractions and nitrogenous constituents.

*Effect of germination*

After 96 hours germination, the dry weight of fenugreek seeds decreased by 18 % and there was a slight increase in total nitrogen, a decrease in protein nitrogen and an increase in non-protein nitrogen and free amino acid nitrogen, amido nitrogen and ammonia nitrogen. The ratio of protein fractions, namely albumin, globulin, prolamin and glutelin, in ungerminated seeds was 4:3.5:2.8:1 and became 2:6.5:7.7:1 after germination as calculated on the basis of protein nitrogen. Germination also resulted in a slight increase in pancreatic digestibility, a 33.7 % decrease in digestibility of pepsin followed by pancreatin (El-Mahdy and El-Sebaiy, 1981a). Phytic acid values diminish and water-soluble inorganic phosphorus value increase during germination. Phytase activity, which is absent in ungerminated seeds, originates after germination. Phosphatase activity also increased in the germinated seed (El-Mahdy and El-Sebaiy, 1981b).

*Effect of water boiling and roasting*

Heat treatments when cooking and roasting fenugreek seeds have less effect on phytate elimination than germination. Changes in calcium, magnesium, iron, manganese, copper and zinc were found to be dependent on the loss of dry weight (El-Mahdy and El-Sebaiy, 1981b). Abdel-Nabey and Damir (1990) reported an increase in both weight and volume as well as the *in vitro* digestibility of fenugreek seeds, especially after the first 5 minutes of boiling, and a decrease in the content of total sugars, protein compounds, calcium, magnesium, phytic acid, phosphorus and amino acid.

**16.4.4 Toxicity**

Fenugreek natural extracts, oleoresins and essential oils have GRAS (generally recognized as safe) status (21 CFR 182.20), are included in the list of approved flavouring substances by the Council of Europe (COE No. 460) and are considered GRAS by the Flavor and Extract Manufacturer's Association (FEMA No. 2485). Flammang *et al.* (2004) reported negative results in the genetic toxicity battery demonstrating that fenugreek extract is devoid of any significant genotoxic activity under the conditions of the assays. Toxicological evaluation of 60 diabetic patients who took powdered fenugreek seeds at a dose of 25 g per day for 24 weeks disclosed no clinical hepatic or renal toxicity and no haematological abnormalities (Sharma *et al.*, 1996b). Therefore the use of fenugreek is expected to be safe and extracts may be useful ingredient in foodstuffs.

**16.5 Quality issues and standards**

The Government of India has prescribed standards for almost all spices, which are graded compulsorily under law before export. The grades adopted for various spices are those prescribed under the Agricultural Produce Grading and Marking Act (1937) and are popularly known as Agmark Grades. Agmark quality parameters and grades have been published for whole fenugreek seed and



fenugreek powder. Besides this, the Bureau of Indian Standard (BIS) has also fixed the quality standard for fenugreek seed whole and powder in IS 3795:1993 (Spice Board India, 2011).

In addition, quality standards for fenugreek have been described by the International Standard Organisation (ISO). According to ISO the whole seed should not contain more than 1 % extraneous matter, 2 % foreign matter, 7 % ash level, 2 % acid insoluble ash level, and 12 % water content. Besides this, the fenugreek seed should comply with national and or EU legislations for heavy metals and pesticide residue limits. As per the microbiology test, salmonella should be absent, yeast and moulds should not be more than  $10^5$ /g and *E. coli* should also not exceed  $10^3$ /g limits. Seeds should be free from any infestation in practical terms, free from dead insects, insect fragments and rodent contaminations visible to naked eye and also free from any adulteration.

## 16.6 References

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## Garlic

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**Abstract:** This chapter discusses the chemical structure, production, processing, uses, functional properties and toxicology as well as the quality of dehydrated garlic. The chapter first reviews the nutritional composition and chemical structure of key compounds before looking at production and processing technologies in order to understand the compounds present in garlic and also the major production and processing technologies required to achieve a good yield and quality products. The chapter then discusses uses, functional properties and toxicologies as well as the quality of dehydrated garlic in order to enable judicious use of different products with a view to managing health.

**Key words:** chemical structure, production, processing, functional properties, quality of dehydrated garlic.

### 17.1 Introduction

Garlic (*Allium sativum* L.) is the second most widely cultivated *Allium* after onion and has been used throughout history for culinary and medicinal purposes. The Chinese, Sumerians, Indians and Ancient Egyptians are well known to have consumed garlic over 4000 years ago. Among others, Hippocrates (430 BC) and Theophrastus (322 BC) described the consumption of garlic in the Greek and Roman period (Rabinowitch and Brewster, 1990). Garlic is thought to have originally come from Central Asia and Southern Europe, especially the Mediterranean region (Thompson and Kelly, 1957). Some authorities consider that *A. longicuspis* Regel, which is endemic to Central Asia, is the wild ancestor and spread in ancient times to the Mediterranean region.

Garlic was carried to the western hemisphere by the Spanish, Portuguese and French (Purseglove, 1975). The bulb of the garlic plant is the most commonly used part of the plant. With the exception of the single-clove types, the bulb is divided into numerous fleshy sections called cloves. Used in cooked or raw form, garlic has a characteristic pungent, spicy flavour that mellows and sweetens considerably with cooking (Gernot, 2005).

There is lot of controversy regarding the taxonomic position of *Allium* and related genera. In earlier classification, garlic was placed in the Liliaceae family. However, some British and American botanists included garlic in Amyryllidaceae family. The botanical classification of *Allium* was reviewed and summarized by

Hanelt (1990), and the comments here are largely based on this account together with the well-known earlier summary by Jones and Mann (1963).

class: Monocotyledons  
 super order: Liliiflorae  
 order: Asparagales  
 family: Alliaceae  
 tribe: Alliae  
 genus: *Allium*  
 species: *A. sativum*

Garlic is grown worldwide in all temperate to subtropical and tropical hilly areas as an important spice and medicinal plant. According to FAO estimates for the year 2007–8, the world area cultivated is 1.217 million ha and production is 16.41 million tonnes. China, India, Korea, Egypt and Russian Federations are major garlic-growing countries. China ranks first in area (0.694 million ha) and production (12.58 million tonnes). India, having 0.147 million ha area and production 0.665 million tonnes is in second position. In productivity, the Netherlands though tops the list (45.45 tonnes/ha), but it is not major garlic-producing country. In major garlic-growing countries, the yield in Egypt is the highest, i.e. 21.93 tonnes/ha followed by the USA (18.86 tonnes/ha) and China (18.12 tonnes/ha).

The data on area, production and productivity of garlic are given in the Table 17.1.

**Table 17.1** Area, production and productivity of garlic in the major countries of the world during 2008

| Country                 | Area (ha)        | Production (tonnes) | Productivity (tonnes/ha) |
|-------------------------|------------------|---------------------|--------------------------|
| China                   | 694 040          | 12 575 036          | 18.12                    |
| India                   | 147 000          | 645 000             | 4.39                     |
| Republic of Korea       | 28 416           | 375 467             | 13.21                    |
| Egypt                   | 11 794           | 258 608             | 21.93                    |
| Russian Federation      | 25 740           | 226 677             | 8.90                     |
| USA                     | 10 300           | 194 230             | 18.86                    |
| Mayanmar                | 24 500           | 147 000             | 6.00                     |
| Bangladesh              | 33 622           | 144 817             | 4.31                     |
| Spain                   | 15 473           | 142 500             | 9.21                     |
| Argentina               | 15 600           | 140 000             | 8.97                     |
| Ukraine                 | 17 300           | 136 800             | 7.91                     |
| Turkey                  | 16 000           | 104 970             | 6.56                     |
| Ethopia                 | 9 317            | 103 542             | 11.11                    |
| Democratic Peoples Rep. | 8 500            | 95 000              | 11.18                    |
| Brazil                  | 10 228           | 91 714              | 8.97                     |
| Thailand                | 13 821           | 85 648              | 6.20                     |
| Romania                 | 13 773           | 72 333              | 5.25                     |
| <b>World</b>            | <b>1 216 663</b> | <b>16 410 374</b>   | <b>13.49</b>             |

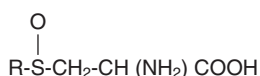
Source: FAO (2010).

## 17.2 Chemical structure of garlic

Garlic has higher nutritive value than other bulb crops. It is rich in proteins, phosphorous, potash, calcium, magnesium and carbohydrates. The nutritional composition of fresh peeled garlic cloves and dehydrated garlic powder is given in Table 17.2.

Garlic, like any other *Allium*, is characterized by the remarkable sulphur-containing compound present in it, which gives the product its distinctive smell and pungency. The chemical composition of garlic is expertly described by Perotto *et al.*, (2006). The description below is based on this source.

The key compounds in intact garlic tissues which serve as precursors of the flavour and odour compounds are the alkyl and alkenylcysteine sulfoxides. The major compound in garlic is (+)-S-allyle-L-cysteine sulfoxide (allylcyste sulfoxide or alliin) with smaller amounts of (+)-S-methyl-L-cysteine sulfoxide (methylcysteine sulfoxide) and (+)-S-*trans*-(1-propenyl)-L-cysteine sulfoxide (*trans*-1-propenylcysteine sulfoxide or isoalliin). The general structure of flavour precursors is:



In garlic, alliinase catalyses the formation of allicin which gives fresh garlic its characteristic smell (Brewster, 1994):

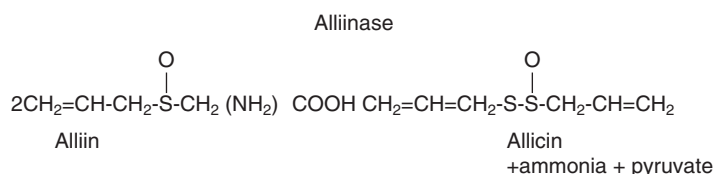


**Table 17.2** Nutrition composition of fresh peeled garlic cloves and garlic powder

| Nutrients                 | Fresh peeled garlic cloves | Garlic powder  |
|---------------------------|----------------------------|----------------|
| Moisture (%)              | 62.8                       | 5.2            |
| Protein (%)               | 6.3                        | 17.5           |
| Fat (%)                   | 0.01                       | 0.6            |
| Mineral                   | 1.0                        | 3.2            |
| Fibre (%)                 | 0.8                        | 1.9            |
| Carbohydrate (%)          | 29.0                       | 71.4           |
| Calcium (%)               | 0.03                       | 0.1            |
| Phosphorous (%)           | 0.31                       | 0.42           |
| Iron (%)                  | 0.001                      | 0.004          |
| Sodium (%)                | –                          | 0.01           |
| Potassium (%)             | –                          | 1.1            |
| Niacin (%)                | –                          | 0.7            |
| Vitamin A (lu)            | 0                          | 175.00 / 100 g |
| Vitamin B (mg/100 g)      | 0                          | 0.68           |
| Vitamin B2(mg/100 g)      | 0                          | 0.08           |
| Vitamin C (mg/100 g)      | 13.00                      | 12.00          |
| Nicotinic acid (mg/100 g) | 0.40                       | –              |

Caloric value (food energy) 14.2 calories/100 g 380 calories/100 g

Source: Pruthi (1987).

The enzyme alliinase is a pyridoxal 5'-phosphate-dependent  $\alpha$ ,  $\beta$ -eliminating lyase which acts upon the S-substituted-L-cysteine sulfoxides when intact tissue is disrupted. The enzymatic reaction gives rise initially to a group of flavour intermediates, the sulphenic acids, which are very unstable and undergo condensation to form thiosulphinates, the primary flavour compounds of garlic.

The co-factor, pyridoxal phosphate, has been shown to act on the alkyl- and alkenylcysteine sulfoxide substrates so that a complex is formed with the enzyme (Block, 1985). It has been suggested that there are two different alliinase activities for garlic (Rabinowitch and Brewster, 1990), one being specific for substrate (+)-S-2-propenyl-L-cysteine sulfoxide and (+)-S-1-propenyl-L-cysteine sulfoxide at an optimum pH of 4.5 and the other specific for substrate (+)-S-methyl-L-cysteine sulfoxide at an optimum pH of 6.5. Both of these alliinases are irreversibly deactivated at pH 1.5–3 and > 9.

Although alliinase can act on both (+) and (–)-sulfoxides, it shows a preference for the (+) isomers (Whitaker, 1976; Block, 1985). The transformation rate of thiosulphinates from their relative precursors is also different (Yu and Ho, 1993). While maximum formation of diallyl thiosulphinate (allicin) can reach 100 % within 1 minute, it takes more than 5 minutes for allyl methyl thiosulphinate to reach 100 %.

The activity of alliinase is also pH dependent. Below pH 4, alliinase is partially deactivated while at pH 4.5–7, alliinase has very high activity (Stoll and Seebeck, 1948; Yu and Wu, 1989a, b). The effect of pH adjustment during the blending of garlic cloves on the formation of flavour compounds as studied by Yu and Wu (1989a), who showed that maximum allicin formation occurred at around pH 6.5.

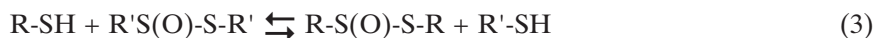
Purified alliinase loses its activity after 14 days of storage at 3°C (pH 6.4) (Matsukawa *et al.*, 1953) and cannot be stored at all at 10°C, particularly in diluted concentration. However, the addition of glycerol to dilute concentrations (final concentration 10 % V/V) stabilizes activity completely for at least 30 days (Magelis and Crews, 1968).

When intact garlic tissue is disrupted, alliinase acts upon the flavour precursor to generate sulphenic acids, the flavour intermediates. Being very unstable, the sulphenic acids undergo rapid condensation to form thiosulphinates, the principal flavour compounds in garlic, pyruvic acid and ammonia. Being an enzymatic decomposition product of the cysteine sulfoxides, pyruvic acid has been used as a measure of garlic flavour (Jager, 1955; Alfonso and Lopez, 1959).

Symmetrical thiosulphinates are considered to result from bimolecular condensation of sulphenic acids with the alk(en)yl radicals. Thiosulphinates with unsymmetrical alk(en)yl groups also occur by:

- the condensation of two different sulphenic acids
- mixing two different thiosulphinates
- the catalytic effect of thiols

as shown below (Giovanelli *et al.*, 1980; Rennonberg, 1982):





Fresh homogenates of garlic contain 3–6 mg/g total thiosulphinates (Lawson *et al.*, 1991).

Thiosulphinates are in themselves unstable and tend to transform spontaneously at room temperature to thiosulphonates and disulphides. Thiosulphonates are considered to be less significant as intermediate compounds than the corresponding thiosulphinates (Fenwick and Hanley, 1985). Derived from thiosulphinates, disulphides can also undergo transformation to form trisulphides and monosulphides.

A study on the stability of allicin at room temperature (Lawson, 1992) showed that the transformation half-life of allicin and the transformation products from allicin were both pH and solvent dependent. Its half-life in water was 96 hours which increased to 240 hours in mild acid (pH 2-3), the main transformation products being diallyl trisulphide and diallyl disulphide in both cases.

### 17.3 Production and processing of garlic

Proper cultivation of any crop depends upon factors such as variety, climate, soil, cultural practices, nutrition and moisture, etc. These factors differ from region to region. Proper production techniques need to be followed to get maximum yield of quality products. Garlic is a frost-hardy plant requiring a cool and moist period during growth and a relatively dry period during maturity of bulbs. It can be grown on variety of soils but thrives on fertile well-drained loamy soils. Heavy clay soils may result in misshapen bulbs and make harvesting difficult. A soil pH between 6 and 7 is suitable for a good crop. Irrigation should also be carried out according to the requirements of the crop at different stages. In general, this varies from 25 mm (good soil) to 50 mm (sandy soil) per week. More water at the maturity stage causes the roots and bulb scales to rot. This also causes discolouration of the bulbs and exposes the outer cloves. Irrigation after long spell of drought results in splitting of bulbs. Successful garlic crops with increased yield and better quality can be achieved by using drip irrigation and planting the cloves on raised beds. The Directorate of Onion and Garlic Research (ICAR) at Rajgurunagar, Maharashtra in India in its trial (Bhonde and Prakash, 2006) found that drip irrigation at 100 % Pan evaporation recorded the highest yield of 14.5 t/ha in garlic with a higher proportion of grade A bulbs, and there was water saving of 55 %. Drip irrigation and fertigation gave higher yield and better quality bulbs in the trial conducted by the National Horticultural Research and Development Foundation at its Karnal Centre in Haryana, India (Bhonde and Prakash, 2006). To achieve a good yield and high-quality garlic bulbs it is necessary to manage nutrient requirements. Since nutrient requirements differ from soil to soil, it is better to get the soil tested and then apply the nutrient as per the recommendations of the soil testing laboratory.

Bulbing takes place during longer days and at high temperature; exposure to low temperatures subsequent to bulb formation favours the process, as does the presence of adequate vegetative growth. The critical day length for bulbing is 12 hours. Exposures of dormant cloves or young plants to a temperature around 20°C or lower, depending upon the variety for 1–2 months hastens subsequent bulbing.

Several garlic varieties have been developed in India. The Godavari and Sweta varieties have been developed by Mahatma Phule Agriculture University (MPAU),

Rahuri, Maharashtra; HG1 and HG2 by Chaudhari Charan Singh Haryana Agriculture University (CCSHAU) Hisar, Haryana; ARU-52 by Agriculture Research Unit (ARU) Almora, Uttarakhand; VL-6, VL-7 by Vivekanand Krishi Anusandhan Sansthan (VPKS), Almora, Uttarakhand; and Agrifound White, Yamuna Safed, Yamuna Safed-2, Yamuna Safed-3 and Yamuna Safed-4 for short day conditions and Agrifound Parvati for long day conditions by the National Horticultural Research and Development Foundation, Nasik, Maharashtra.

It is appropriate to consider the advantages and disadvantages of raw as compared to processed flavour ingredients in the food industry. Raw alliums possess the ideal balanced flavour profile and provide desirable textural and water-retaining properties when incorporated in food products. On the other hand, the bulk of the raw material is water and thus lacks flavour and aroma. Moreover, handling and storage costs are greater when garlic is used in raw form. Dehydration or flavour extraction reduces the weight which ultimately lowers the transportation and storage cost. However, it also introduces undesirable changes in appearance and modifies the natural balance between aroma and flavour. The spectrum of flavour-determining volatiles in a garlic-derived product depends very much on temperature, timing and duration of various cooking and manufacturing processes (Lancaster and Bolard, 1990). Among processed ingredients currently available are dehydrated products, essential oils, oleoresins and products employing a carrier.

### 17.3.1 Garlic oils and flavour oils

Concentrated garlic oil can be used to impart the flavour of garlic to processed products without the difficulties of handling a large bulk of fresh bulbs. Garlic oil is produced by boiling a mixture of the minced bulbs after allowing it to stand for several hours and then extracting the oils from the condensed steam, a process known as steam distillation. Garlic oil recovered in this way is a reddish brown overpowering liquid. Yields of 0.10–0.25 g of garlic oil/100 g of fresh garlic bulbs are obtained. The pungency of the product makes it difficult to use directly, and it is commonly diluted in vegetable oil or encapsulated. Encapsulated garlic oil and other products are discussed by Raghavan *et al.* (1983). Garlic oil is used in beverages, ice cream, baked foods, confectionery, chewing gum and condiments (Furia and Bellanca, 1975). Below is a list of the uses and concentration (ppm) of garlic oil in various food stuffs:

|                 |         |
|-----------------|---------|
| baked products: | 6.0     |
| beverages:      | 0.1–0.3 |
| condiments:     | 16      |
| chewing gum:    | 12      |
| confectionery:  | 2.9     |
| ice cream:      | 40      |

Recently, individual techniques for extracting the aromatic flavour constituents from unheated homogenates of bulbs have been developed. The soluble aromatics are extracted into food-grade solvents, and this can be evaporated to concentrate the flavour substances and recover the solvent. Because the aromatic oils are so concentrated, they are sometimes dispersed into a solid phase using salt, dextrose

or flour as a carrier. Alternatively, they can be microencapsulated using edible polymers, e.g. gum arabic. These forms of dilution and dispersal simplify the mixing and incorporation of the flavour oils into manufactured foods.

### 17.3.2 Dehydrated products

Garlic is widely used in dehydrated forms. The processing involves first washing the bulbs, separating the cloves and removing the skin and a small portion of the roots and tops, surface sterilization by flame or alkali, washing and then slicing or chopping. The slices are dried in hot air at 60 °C. Drying at too high a temperature cause darkening. Hot air drying is followed by a final drying to 4 % moisture while flakes are agitated in a blast of warm air. Dried flakes may be further processed into powder. A schematic representation of the process for garlic dehydration is shown in Fig.17.1. Garlic powder and flakes are widely used in manufacturing soups, ketchups, sauces and mayonnaises.

### 17.3.3 Garlic salt and other products employing solid carrier materials

In some instances, the product formulation may necessitate the use of solid material, rather than a flavour oil, and one such material is garlic salt. The approximate composition of garlic salt according to Farrell (1985) is given in Table 17.3. Garlic salt should not contain more than 2.5 % moisture or more than 31 % salt and should include moisture-free white garlic powder between 18–19 % and 1–2 %. It has much wider culinary potential than garlic powder and one tablespoon is equivalent to a clove of fresh garlic. For use in catering, garlic salt may be produced by blending 70–80 % salt plus 1–2 % calcium stearate. Alternatively, the essential oils or oleoresins may be fixed by dispersal onto salt and blended until a uniform dispersion is produced. Other suitable supports such as dextrose or sugar can also be used if the inclusion of salt is not desired.

Another technique to produce garlic products employing solid carrier materials which has found extensive use is microencapsulation (Blenford, 1986). This involves the coating of the flavour active components with a continuous film of polymer, the particle size of the capsules thus produced being between 0.1 and 4000/μm. Not only does this process facilitate ease of handling and standardization, but it also provides protection against oxidative and light-induced chemical changes. A number of polymers may be used in micro encapsulation and these, and the means of processing, enable specific characteristics (e.g. heat resistance, pressure rupturing, slow release) to be achieved. For use in food industry the polymers must be edible and non-toxic; and among the most commonly used are modified starches, gelatin and gums. Encapsulated products are relatively expensive. The exact flavour strength depends upon the conditions of manufacture, but it is generally two to four times that of garlic powder.

### 17.3.4 Other products

Garlic is also processed in the form of garlic juice. Garlic oleoresin is a dark viscous liquid having 12 times the flavour of dehydrated garlic or 50 times that of

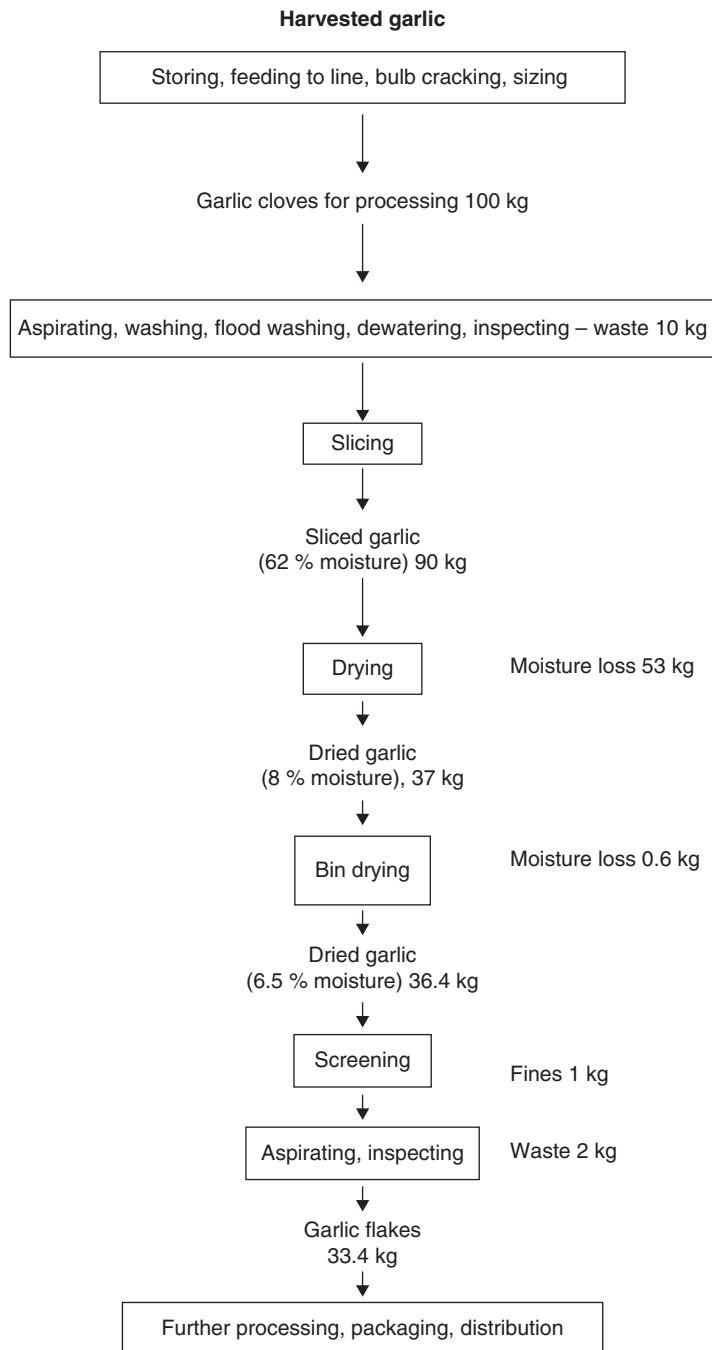


**Fig. 17.1** A schematic representation of the process for garlic dehydration (Van Arsdell *et al.*, 1973).

**Table 17.3** Approximate composition (per 100 g) of garlic salt

| Nutrient     | Garlic salt |
|--------------|-------------|
| Water        | 1.4 g       |
| Energy       | 63 kcal     |
| Protein      | 3.2 g       |
| Fat          | 0.1 g       |
| Carbohydrate | 13.8 g      |
| Fibre        | 0.4 g       |
| Ash          | 81.5 g      |
| Ca           | 220 mg      |
| Fe           | 1 mg        |
| Mg           | 11 mg       |
| P            | 79 mg       |
| K            | 252 mg      |
| Na           | 31.4 g      |
| In           | 1 mg        |
| Vitamins     | —           |

*Source:* Farrell (1985).

fresh garlic cloves. Garlic paste is formulated from suitable flavours and a viscous edible base.

### 17.3.5 Main uses of garlic

#### *Applications in foods*

Garlic is widely used around the world for its pungent flavour as seasoning or condiment. In the USA about 50 % of the entire output of fresh garlic is dehydrated and sold to food processors for use in mayonnaise products, salad dressing, tomato products and in several meat preparations. Raw garlic is used in preparation of garlic powder, garlic salt, garlic vinegar, garlic cheese croutons, potato chips, garlic breads, garlicked meat tit-bits and garlicked bacon. In India and other Asian and Middle Eastern countries, garlic is used in several food preparations, notably in pickles, curry powders, curried vegetables, meat preparations and tomato ketchup, etc. There has been increasing demand by the food industry for garlic powder as a condiment. Oil of garlic is appreciated as a valuable flavouring agent for use in all kinds of meat preparations, soups, canned foods and sauces (Pruthi, 1987).

#### *Uses in traditional medicine*

Garlic also has a long history of medicinal use by cultures around the world for a wide variety of conditions. Garlic was included in the Codex Ebers (1550 BC) (Castleman, 1991; Small, 1997). It has been a vital ingredient in Chinese medicine for millenia and was reportedly used by the ancient Egyptians to treat cavities in teeth (Heinerman, 1995). In Ayurvedic medicine, garlic is considered a diuretic, diaphoretic, expectorant, carminative, anticoagulant and anthelmintic (Palniswamy, 2003). Other uses in folk medicine include treatment for bronchitis and respiratory problems, gastrointestinal problems, flatulence, leprosy, menstrual cramp, high blood

pressure, diabetes, warts, corns, arthritis muscle pain, neuralgia and sciatica (Simon *et al.*, 1984; Heinerman, 1995; Holder and Gails 1996; Gruenwald *et al.*, 2000; PFAF, 2012).

In addition, garlic has a long folk tradition as an insect and pest repellent. Diallyl disulphide and diallyl trisulphide, two compounds in garlic oil, have been found to be insecticidal (Koch and Lawson, 1996). In scientific studies, garlic has successfully destroyed mosquito larvae and certain species of ticks and has repelled mosquitoes, black flies, fruit flies and fleas. It can be used to prevent the spread of mould and insects in stored fruit (Palniswamy, 2003) and some people even place garlic in drawers to repel moths (Holder and Gails, 1996).

## 17.4 Functional properties and toxicology

### 17.4.1 Modern research into functional properties

Studies suggest that garlic, which contains more than 200 different compounds, has biological activities that can have medically important effects. Garlic has been used in traditional medicine for centuries, for example as an excellent carminative, nerve tonic and antiseptic agent in Hindu medicine (Aman, 1969). However, it has been only recently that its medical benefits have been explored in detail. The functional properties of garlic have been reviewed by Agarwal (1996), Koch and Lawson (1996) and Lawson (1998a) and more recently by Banerjee and Maulik (2002), Khanum *et al.* (2004), Rahman and Lowe (2006), Ngo *et al.* (2007), Ried *et al.* (2008) and Kim and Kwon (2009). These properties include the following:

- Cholesterol lowering properties have been reported by numerous studies (including Reuter and Sendl, 1994; Han *et al.*, 1995; Adler and Holub, 1997). These studies have reported an average 10 % reduction in total serum cholesterol. Evidence suggests that these effects are due to allicin or allicin-derived compounds (Yeh and Yeh, 1994; Gebhardt and Beck, 1996). Some studies, however, have produced contradictory results (Berthold and Sudhop, 1998), although this may be due to the composition of the garlic supplements studied (Lawson, 1998b). More recent studies in this area have also produced contradictory results. Studies by Sovova and Sova (2004) and Durak *et al.* (2002) found that garlic supplementation reduced the accumulation of cholesterol on the vascular walls of animals; however, a randomized clinical trial found that the consumption of garlic in any form did not reduce blood cholesterol levels in patients with moderately high baseline cholesterol levels (Charlson and Mefferen, 2007).
- Garlic significantly lowers blood pressure (Silagy and Neil, 1994; Das *et al.*, 1995a, b; Capraz *et al.*, 2006; Ried *et al.*, 2010).
- Garlic has an influence on platelet aggregation, an important factor in cardiovascular disease (Lawson *et al.*, 1992; Han *et al.*, 1995; Batirel *et al.*, 1996; Steiner and Lin, 1998; Borrelli *et al.*, 2007; Chau *et al.*, 2007; Rahman, 2007). It also has an effect on blood coagulation and fibrinolytic activity which are factors in the development of thrombosis (Han *et al.*, 1995; Breithaupt-Grogler *et al.*, 1997).
- Epidemiological studies have suggested a link between garlic consumption and a reduced risk of stomach cancer (Steinmetz *et al.*, 1994; Han *et al.*, 1995; Milner,

1996; Gao *et al.*, 1999; Setiawan *et al.*, 2006). Various constituents of garlic have been identified as inhibiting tumour growth, notably garlic-derived organosulphides (Hu and Singh, 1997; Pinto *et al.*, 1997; Riggs *et al.*, 1997; Sakamoto *et al.*, 1997; Srivastava *et al.*, 1997; Pinto and Rivlin, 2001; Herman Antosiewicz and Singh, 2005).

- Garlic also has antioxidant properties which are helpful in preventing cancer and cardiovascular disease (Horie *et al.*, 1992; Phelps and Harris, 1993; Imai *et al.*, 1994; Ackermann *et al.*, 2001; Rahman, 2001; Gardener *et al.*, 2003).
- Garlic has antibiotic properties and has been used to treat wounds when other antibiotics were not available (Fenwick and Hanley, 1985; Han *et al.*, 1995; Groppo *et al.*, 2007). Garlic also have antimicrobial properties discussed Lemer *et al.* (2005) and Shuford *et al.*, (2005).

Although anticarcinogenic activity of garlic has been well documented (Lau *et al.*, 1990), the mechanism by which garlic compounds prevent carcinogenesis of many chemicals is not entirely clear. One possible mechanism is the ability of sulphur-containing garlic compounds to block the activation of procarcinogens to carcinogens by hepatic mixed function oxidases. It is interesting to note that diallyl sulphide has been found to inhibit hepatic mixed function oxidases at high doses (Dalvi and Salunkhe, 1993).

#### 17.4.2 Adverse effects and toxicology

Since the consumption and level of sulphur-containing ingredients of garlic that are supposed to be toxic are so low, acute or fatal garlic poisoning in humans is very rare. A case of wild garlic poisoning in sheep was reported by Stevens (1984). Garlic can cause indigestion, nausea, vomiting and diarrhoea and may interact with various drugs including warfarin, antiplatelet drugs, saquinavir, antihypertensives, calcium channel blockers and hypoglycemic drugs (Hogg, 2002). It also reduces the ability of the blood to clot (Anon., 2006). Compounds such as di(prop-2-enyl) disulphide present in garlic have been found to cause contact dermatitis (Hjorth and Roed-Peterson, 1976; Mitchell, 1980) and may also be responsible for occupational allergy. There have been many reports of serious burns resulting from garlic applied topically (Baruchin *et al.*, 2001; Dietz *et al.* 2004). Garlic is also known for causing halitosis as well as causing sweat to have a pungent smell. A study has shown that drinking milk at the same time as consuming garlic can significantly neutralize bad breath (Hansanugrum and Barringer, 2010).

### 17.5 Quality issues of dehydrated garlic

The quality of the raw material used in the processing industry determines to a large extent the quality of the finished product. Raw materials of poor quality cannot be expected to result in a final product of high quality even with the best processing methods. Therefore, gaining an understanding of the nature of the raw material and of its possible defects is an essential step in building quality into a product. Likewise, thorough knowledge of the likely effects of defective raw material on both



processing efficiency and quality of finished product is important. The garlic bulbs should be harvested at the proper maturity stage. Irrigation by drip or any method should cease as the crop reaches maturity to allow the field to dry out. Continued irrigation as the crop matures causes the roots and bulb scales to rot. This discolours the bulbs and exposes the outer cloves. Harvesting is done at the 100 % neck fall stage and then the bulbs are cured for about a week. When the tops have fully dried, they are removed by cutting leaves above the bulb and roots with shears. The harvested and dried products are then sorted in the field to remove diseased and damaged bulbs. They should be graded in a proper fashion at a later stage. Packages of dehydrated garlic should be stored in covered premises, well protected from sun, rain and excessive heat. The store room should be dry, free from unpleasant odours and protected against the entry of insects and other vermin. For transport, the containers should be clearly marked with warnings against careless handling which might lead to perforation of the containers. The containers should be dry and cool and should be stored well away from ships' boilers and bilges during sea transport.

According to International Standard ISO 5560 garlic cloves should be sound and practically free from mould, disease, soil, outer skins, stems, leaves and roots (ISO, 1997). Specifications for dehydrated garlic according to this standard are outlined in the sections below.

### **17.5.1 Organoleptic specifications**

#### *General*

Dehydrated garlic products shall conform to the requirement of this International Standard and on dehydration shall regain characteristics similar to those of fresh garlic.

#### *Colour*

The colour of dehydrated garlic shall be characteristic of the cultivar used, i.e. between white and pale cream. The product shall be practically free from scorched, toasted and baked particles.

#### *Odour*

Dehydrated garlic after dehydration by the method specified under the International Standard shall have a characteristic, pungent odour, free from foreign odour and off odour such as those coming from mouldy, rancid, fermented or burnt particles.

#### *Flavour*

The flavour of the dehydrated garlic is assessed after rehydration in accordance with the method specified under the International Standard. It shall be the characteristic of parboiled garlic and free from foreign flavour and off flavour such as those coming from mouldy, rancid or burnt particles.

#### *Freedom from insects, moulds, etc.*

Dehydrated garlic shall be free from live insects and practically free from moulds, dead insects, insect fragments and rodent contamination visible to the naked eye or

with such magnification as may be necessary in any particular case. If the magnification exceeds  $\times 10$ , this fact shall be mentioned in the test report. In case of dispute, the contamination of garlic in powder form shall be determined by using the method specified in ISO 1208.

#### *Extraneous matter*

The total percentage of extraneous matter, i.e. vegetable matter originating exclusively from plants, such as particles from skins and roots, shall not exceed 0.5 % (m/m).

### 17.5.2 Chemical requirements

Dehydrated garlic shall comply with the requirements specified in the Table 17.4.

### 17.5.3 Quality of dehydrated garlic products

Dehydrated garlic may be divided into the broad product categories given below:

- **Dehydrated garlic slices:** This is a product obtained by cutting garlic cloves into slices and removing broken pieces smaller than 4 mm by sieving.
- **Dehydrated garlic flakes or pieces:** These are the products that pass through a sieve of aperture size 1.25–4 mm. The particles do not have any definite shape.
- **Dehydrated garlic grits:** This is a product that passes through a sieve of aperture size 250  $\mu\text{m}$ –1.25 mm.
- **Powdered garlic:** This is a homogeneous product 95 % of which passes through a sieve of aperture size 250  $\mu\text{m}$ .

### 17.5.4 Sampling

- **Dehydrated garlic powder or grits:** Sampling of the product is done in accordance with ISO 948 using a conical sampler or other suitable implement to remove aseptically a representative sample.
- **Dehydrated garlic slices, flakes or pieces:** Certain problems arise as a result of the friability of the product and the danger of settling within the container. It

**Table 17.4** Chemical requirements of dehydrated garlic

| Characteristics                                                       | Requirement | Test method |
|-----------------------------------------------------------------------|-------------|-------------|
| Moisture content % (m/m) max.                                         | 8           | ISO 939     |
| Total ash % (m/m), on dry basis, max.                                 | 5.5         | ISO 928     |
| Acid insoluble ash, % (m/m) on dry basis max.                         | 0.5         | ISO 930     |
| Volatile organic sulphur compounds content, % (m/m) on dry basis min. | 0.3         | ISO 5567    |
| Cold-water-soluble extract, % (m/m) on dry basis                      |             |             |
| Min.                                                                  | 70          | ISO 941     |
| Max.                                                                  | 90          |             |

may, therefore, be necessary to take the entire contents of a single container as in the transport, the garlic may settle with the larger pieces towards the top and the smaller pieces towards the bottom.

- **Number of containers to be taken:** From a lot, between 0.5 % and 1 % of the containers are taken using a table of random numbers agreed between the interested parties. If no table of random numbers is available, every fourth container is taken. At least one full container is necessary.
- **Preparation of bulk sample:** The contents of each container is sieved to the commercial form considered appropriate. A bulk sample is prepared by mixing the different fractions in proportion determined by sieving. The size of the bulk samples shall be at least three times the quantity of product necessary to carry out all the tests required by this International Standard.

### 17.5.5 Methods of rehydration and sensory evaluation of dehydrated garlic

Methods of rehydration and sensory evaluation of dehydrated garlic products are given below:

- **Garlic slices:** A vessel of about 500 ml capacity made of a material which will not impart a foreign taste or affect the colour of the preparation dish made of porcelain or white earthenware and a stainless steel spoon are the apparatus used in rehydration. A sample of  $10\text{ g} \pm 0.1\text{ g}$  is weighed and transferred to a vessel containing 500 ml of cold water. The water containing the sample is then boiled at  $99^\circ\text{C}$  keeping the vessel covered for  $10\text{ minutes} \pm 1\text{ minute}$ . The volume is made up to 500 ml with cold water and then poured into the dish. Sensory evaluation of appearance of the cooking water (colour and clarity), colour of the preparation, odour, tenderness and flavour is carried out.
- **Garlic powder, grits, flakes or pieces:** A vessel of about 1000 ml capacity made of a material which will not impart a foreign taste or affect the colour of the preparation, a dish made of porcelain or white earthenware, and a stainless steel spoon are the apparatus used in rehydration. Flour made from durum as the most recent harvest and known to be of a good quality is used along with potable water as neutral as possible. 1000 ml of cold water is transferred to the vessel and 30 g of the flour is added while stirring continuously. The mixture is then heated and stirred continuously until it reaches boiling point and simmered for 2 minutes. 0.4 g of the garlic is placed in a dish; 250 ml of the prepared medium is added and allowed to stand for 5 minutes. It is stirred from time to time. Sensory evaluation is then done for odour and flavour.

### 17.5.6 Test methods

The samples of dehydrated garlic are tested for conformity with the requirement of this International Standard by following the methods of physical, organoleptic and chemical analysis specified above and in Table 17.4.

### 17.5.7 Packing and marking

Dehydrated garlic should be packed in clean, sound and dry containers made of a material which does not affect the product but which protects it from light and from

**Table 17.5** Quality standards for garlic dehydrated products as per ADOGA standards

| Properties                                                                       | Type of garlic                       | Measurement      |
|----------------------------------------------------------------------------------|--------------------------------------|------------------|
| Moisture (max.)                                                                  | Roasted / toasted garlic products    | 5.0 %            |
|                                                                                  | All other garlic (products)          | 6.8 %            |
| Extractable colour-optical index (max.)                                          | Untoasted garlic products            |                  |
|                                                                                  | Fancy powdered garlic                | 150              |
|                                                                                  | Agglomerated garlic coarse and fine  | 200              |
|                                                                                  | Standard powdered garlic             | 200              |
|                                                                                  | All other dehydrated garlic products | 200              |
|                                                                                  | Roasted / toasted garlic products    | Range            |
| Surface colour (scan)                                                            | All roasted garlic products          | 1000 + 500       |
|                                                                                  | Roasted garlic products              | Range            |
|                                                                                  | Garlic powders                       | 65–90            |
|                                                                                  | Granulated garlic                    | 40–70            |
| Hot water insoluble solids (max.)                                                | Chopped and minced garlic            | 50–75            |
|                                                                                  | Standard powdered garlic             | 20 %             |
| Defect tolerances                                                                | All other garlic products            | 12.50 %          |
| Speck counts (max.)<br>(garlic products from coarse agglomerated through minced) | Coarse agglomerated garlic           | 13/0.1 g         |
|                                                                                  | Granulated garlic                    | 20/0.1 g         |
|                                                                                  | Ground garlic                        | 20/0.5 g         |
|                                                                                  | Minced garlic                        | 13/10 g          |
| Defected pieces                                                                  | Chopped garlic                       | Major (max.) 1 % |
|                                                                                  |                                      | Minor (max.) –   |

the ingress of moisture. The packaging needs to comply with any national legislation relating to environmental protection.

The following particulars are required to be marked directly on each package or on a label attached to the package:

- name of the product and botanical name and trade names if any
- name and address of the producer or packer, or trademark if any
- code or batch number
- net mass
- producing country.
- any other information requested by the purchaser such as year of production and date of packing if known
- reference to this international standard
- whether product contains additives and, if so, which ones, in the case of countries where they are permitted.

The American Dehydrated Onion and Garlic Association (ADOGA) which was established in 1956 has also standardized qualities and grade specifications within the American dehydrated onion and garlic industry (ADOGA, 2005).

Quality standards for garlic dehydrated products as per ADOGA standards are given in Table 17.5.

**Table 17.6** Microbiological characteristics of dehydrated garlic

| Characteristic                                  | Recommended Specification |                 | Test method |
|-------------------------------------------------|---------------------------|-----------------|-------------|
|                                                 | M                         | M               |             |
| Microorganisms at 30°C per g, max.              | 10 <sup>5</sup>           | 10 <sup>6</sup> | ISO 4833    |
| Presumptive <i>Escherichia coli</i> per g, max. | 10                        | 10 <sup>2</sup> | ISO 7251    |
| Yeasts and moulds at 25°C per g, max.           | 10 <sup>3</sup>           | 10 <sup>4</sup> | ISO 7954    |
| <i>Clostridium perfringens</i> , per g, max.    | 10                        | 10 <sup>2</sup> | ISO 7937    |
| <i>Staphylococcus aureus</i> in 1 g             | Absent                    |                 | ISO 6888    |
| <i>Salmonella</i> in 25 g                       | Absent                    |                 | ISO 6579    |

### 17.5.8 Microbial characteristics

Tests conducted in laboratories representing producers and users of dehydrated garlic have shown that the microbiological characteristics given in Table 17.6 can be considered as acceptable. These are for information only.

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# Ginger

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**Abstract:** Spice ginger is obtained from the underground stems or rhizomes of *Zingiber officinale*, a herbaceous tropical perennial belonging to the family Zingiberaceae. The chapter begins with a description of the ginger plant and its chemical structure before going on to discuss production, varieties and cultivars. Products of ginger rhizomes – fresh ginger, preserved ginger, dry ginger, ginger powder, ginger oil, ginger oleoresin and ginger paste – are detailed. The main uses and functional properties of ginger, culinary and medicinal (both traditional and modern) are outlined before the chapter finishes with a look at quality specifications, organic ginger and some biotechnology studies.

**Key words:** ginger, *Zingiber officinale*, rhizomes, functional properties, quality, biotechnology.

## 18.1 Introduction

The spice ginger is obtained from the underground stems or rhizomes of *Zingiber officinale* (Rosc.), a herbaceous tropical perennial belonging to the family Zingiberaceae. In cultivation, it is usually grown as an annual. The whole plant is refreshingly aromatic, but it is the underground rhizome, raw or processed, that is valued as spice. Its medical value is increasingly being recognized. Ginger originated in South-East Asia, probably in India (Burkill, 1966; Purseglove *et al.*, 1981). The name itself supports this view. The Sanskrit name 'Sringavera' gave rise to Greek 'Zingiberi' and later the generic name Zingiber.

Ginger (*Zingiber officinale* Rosc.) is a monocotyledon,  $2n = 2x = 22$ , belonging to the family Zingiberaceae and to the order Zingiberales. In the Zingiberaceae, it belongs to the subfamily Zingiberoideae, whose members are aromatic with unbranched aerial stems, distichous leaves, open sheaths and hypogeal germination, mainly confined to the old world tropics with the centre of distribution in Indo-Malaysia (Purseglove *et al.*, 1981). Among them, ginger is a slender perennial herb, 30–100 cm tall with palmately branched rhizome bearing leafy shoots. The leafy shoot is the pseudostem formed by leaf sheath and bears 8–12 distichous leaves. The inflorescence is a spike which generally springs directly from the rhizome. The subfamily Zingiberoideae is also noted for two important spice crops, turmeric (*Curcuma longa* L.) and cardamom (*Elettaria cardamomum* Maton). It also includes a number

of subsidiary spice plants belonging to the genera *Aframomum*, *Amomum*, *Kaempferia*, *Languos* (*Alpinia*) and *Phaeomena* (*Nicolaia*). The genus *Zingiber* Boehm has about 80–90 species of perennial rhizomatous herbs distributed throughout South-East Asia and extending to Queensland and Japan (Purseglove *et al.*, 1981).

### 18.1.1 Chemical structure

The ginger rhizome contains steam volatile oil, fixed fatty oil, pungent compounds, resins, proteins, cellulose, pentosans, starch and mineral elements. The composition of these components varies with type of cultivar, region, agroclimatic conditions, maturity and nature of rhizome, i.e. fresh or processed (Purseglove *et al.*, 1981). Dry ginger on average contains moisture (10.85 %), volatile oil (1.8 %), oleoresin (acetone extract) (6.5 %), water extract (19.6 %), cold alcohol extract (6.0 %), starch (53 %), crude fibre (7.17 %), crude protein (12.4 %), total ash (6.64 %), water-soluble ash (5.48 %) and acid insoluble ash (0.14 %) (Peter and Kandiannan, 1999).

The characteristic organoleptic properties of ginger are due to steam volatile oil and non-volatile solvent-extractable pungent components. The pleasant aroma of ginger is caused by more than 70 constituents present in the steam volatile oil. Among them the sesquiterpene hydrocarbon (-)- $\alpha$ -zingiberene predominates and accounts for 20–30 % of the oil obtained from dry ginger (Purseglove *et al.*, 1981). The other important sesquiterpene hydrocarbons related with typical flavour of ginger are  $\alpha$ -curcumene,  $\beta$ -bisabolene and  $\beta$ -sesquiphellandrene (Zachariah, 2008). The warm pungent taste is caused by a number of components, predominately gingerols followed by shogaols and zingerone (Kulka, 1967). The aroma and flavour of fresh ginger will be different from dry ginger as some of the volatile oils will be lost by evaporation during drying (Purseglove *et al.*, 1981). Zingerone and shogals are found in small amounts in fresh ginger and in larger amounts in dried or extracted products (Govindarajan, 1982). The chemical constituents contributing to flavour and pungency show variation depending upon geographical region (Zachariah, 2008).

### 18.1.2 Production

Ginger is cultivated in several parts of the world, the most important producing regions being India, China, Indonesia, Nepal, Thailand, Nigeria, Bangladesh, Japan and the Philippines. Among them, India and China are the dominant suppliers to the world market. The world area and production of ginger shows an increasing trend from 1990 to 2008 (Table 18.1).

In terms of quality, Jamaican and Indian ginger are considered superior followed by West African ginger. Jamaican ginger possesses delicate aroma and flavour and is sometimes considered as first grade. Indian ginger, entering the world market as 'Cochin' and 'Calicut' ginger, has a lemon-like byenote which some prefer to Jamaican ginger. Chinese dried ginger is exported as whole peeled and sliced unpeeled. It is whiter than the Indian ginger and tends to be more fibrous and bitter (Plotto, 2011). Nigerian and Sierra Leone dried gingers possess somewhat camphoraceous and coarser odour and is rich in aroma and pungency factors. There is demand for them for oil distillation and oleoresin extraction.

**Table 18.1** Area and production of ginger in the important ginger-producing countries (1990, 2000, 2008)

| Country       | 1990      |                    | 2000      |                   | 2008      |                   |
|---------------|-----------|--------------------|-----------|-------------------|-----------|-------------------|
|               | Area (ha) | Production (tons)* | Area (ha) | production (tons) | Area (ha) | Production (tons) |
| India         | 54000     | 1 54 000           | 70000     | 2 35 000          | 104 100   | 3 82 600          |
| China         | 5 333     | 54 284             | 19 173    | 2 28 056          | 28 198    | 3 28 810          |
| Indonesia     | 9 612     | 79 891             | 18 576    | 1 49 300          | 42 000    | 1 92 341          |
| Nepal         | 3 200     | 30 000             | 8 314     | 74 994            | 13 332    | 1 76 602          |
| Thailand      | 9 000     | 22 000             | 13 000    | 32 500            | 9 855     | 1 61 505          |
| Nigeria       | 84 000    | 42 000             | 1 58 000  | 98 000            | 55 690    | 1 40 000          |
| Bangladesh    | 6 967     | 42 830             | 6 879     | 38 000            | 9 416     | 77 046            |
| Japan         | 1 200     | 25 000             | 1 034     | 30 000            | 1 920     | 49 800            |
| Philippines   | 3 519     | 21 811             | 3 583     | 22 679            | 3 926     | 27 538            |
| Rest of world | 8 079     | 37 326             | 8 296     | 45 242            | 10 673    | 67 906            |
| Total         | 184 910   | 509 142            | 306 855   | 953 771           | 279 110   | 1 604 148         |

\* Dry ginger.

Source: <http://faostat.fao.org/site/567/DesktopDefault.aspx> [accessed March 2012].

### 18.1.3 Varieties and cultivars

The yield and quality parameters of ginger, such as plumpness of rhizome, dry recovery percentage, fibre, oleoresin and oil content, are highly influenced by environmental factors, i.e. the soil and climate prevailing in the region. Variety selection is important with respect to end-product requirements. For dry ginger production varieties with high dry recovery percentage (>20 %), bold fingers and low fibre content (<4 %) are required. Cochin and calicut dry ginger exported from Kerala are produced from local cultivars, i.e. Kuruppampady, Cheranad, Erenad and Wayand local (Shanmugavelu *et al.*, 2005). Kuruppampady ginger is famous for its superlative superior quality with more fingers than average and a high oleoresin content. The medium plump low-fibre Assam variety 'Maran' and the plump 'Himachal' variety from Himachal Pradesh are also good for dry ginger production in Kerala. Improved varieties 'Varada', 'Rejatha' and 'Mahima' released by the Indian Institute of Spices Research from Kozikode are also good for fresh and dry ginger production in Kerala. Suprabha, Suruchi, Surabhi, V<sub>3</sub> S<sub>1</sub>-8, V<sub>1</sub>E<sub>8</sub>-2 are improved varieties released for Orissa state and Himagiri is recommended for Himachal Pradesh state of India for fresh ginger production (Nybe *et al.*, 2007). Rio de Janeiro is good for fresh ginger production and oleoresin extraction. Athira and Karthika are two ginger varieties released by Kerala Agricultural University for large-scale fresh and dry ginger production in Kerala (Shylaja *et al.*, 2010).

## 18.2 Products of ginger rhizomes

The primary products of ginger rhizomes for flavouring purposes are fresh ginger, preserved ginger in syrup or brine and dried ginger. Secondary products are ginger powder, oils and oleoresins from dry ginger.

### 18.2.1 Fresh ginger

Fresh ginger is outstanding for flavouring as it contains the full note of the spice compared to other products made from it. Fresh rhizomes with low fibre content but which are rich in aroma, pungency, fat and protein are preferred for green ginger purposes. The crop for this purpose can be harvested from 180–95 days after planting. Further maturity causes a progressive increase in crude fibre and decrease in protein and fat content.

### 18.2.2 Preserved ginger

Immature green ginger is preserved in brine or sugar syrup. Crystallized ginger is also made from the ginger in sugar syrup through further processing. Crystallized ginger is peeled ginger that has been impregnated with sugar syrup, dried and coated with crystalline sugar. Succulent ginger rhizomes with very little fibre and less pungency are preferred for preserved ginger. The raw material has to be harvested within 195 days of planting. The processing technology for preserved ginger varies with country. Chinese ginger has been the standard for ginger preserved in syrup. Australia also exports candied rhizomes reported to have superior and consistent quality.

### 18.2.3 Dry ginger

Dry ginger obtained by drying of fresh ginger enters the spice trade for the preparation of ground ginger, extraction of oleoresin and oil. It finds use in Ayurvedic formulations and is available in a number of physical forms. It can be peeled (scraped or uncoated) or non-scraped (coated or non-peeled) and is sometimes partially peeled or rough scraped. In the scraped grade, the corks skin has been removed cleanly without damaging the underlying tissue. Grades designated as 'bleached' or 'limed' are also available. They are prepared from clean-peeled or partially peeled whole rhizomes by treating them with lime or sulphurous acid to achieve a white colour. Physical grades like black ginger, splits and slices are also available.

Dried ginger is prepared from mature rhizomes which have developed full aroma, flavour and pungency, and harvesting is usually carried out at between 8 and 9 months after planting. For dry ginger making, cultivars with medium-sized rhizomes with high curing percentage are preferred. The processing of dry ginger involves the following steps:

1. Removal of roots and thorough washing of rhizomes.
2. Preparation of rhizomes for drying, which involves either peeling, splitting or slicing. When whole-coated rhizomes are to be dried, preparation is by immersing in boiling water for about 10 minutes. Black ginger is prepared like this.
3. Sun drying: during drying, rhizomes lose moisture about 60–70 % of their weight, achieving a final moisture of 7–12 %.

Jamaican ginger is clean peeled using a special knife and, after thorough washing, sun dried. Indian ginger is rough scraped, non-bleached or bleached

(limed). In this, the skin is partially scraped off with a sharpened piece of bamboo. Steel knives are not used as they stain the produce. Mechanization is possible in dry ginger processing, in grading, peeling and drying, but mechanical grading and peeling result in an inferior quality product. Most of the producing countries resort to traditional methods which employ mainly manual labour.

The appearance, the content of volatile oil and fibre, the pungency level and a subjective assessment of aroma and flavour are important in the quality evaluation of dried ginger. Clean-peeled dried ginger in the whole form possessing the best appearance generally finds a place in the grocery trade. Lower grades of clean-peeled, coated whole, split and sliced types are used for blending in the preparation of powdered mixed spices. All types may be used for oil distillation and oleoresin extraction, but the coated types are the most extensively used for these purposes.

#### **18.2.4 Ginger powder**

Ginger powder is made by pulverizing dry ginger to a mesh size of 50–60. Ginger is ground to release the flavour. The finer the powder, the more readily available the flavour and readily dispensable it is in the matrix. Some flavour may be lost by heat development during grinding. This can be minimized by adopting cryomilling and freeze grinding.

#### **18.2.5 Ginger oil**

Ginger oil is produced commercially by steam distillation of freshly ground dry ginger. The yield of oil varies from 1.5–3.0% with an average of 2.0%. The oil obtained is a green or yellow mobile liquid which becomes viscous on ageing (Purseglove *et al.*, 1981). The most suitable material for oil distillation is coated African ginger, followed by Nigerian splits and Cochin ginger. Ginger oil can also be recovered by steam distilling fresh ginger peelings, and the yield is 1.5–2.8%. Ginger oil is used as a flavourant in food processing, pharmaceuticals and also in perfumery (Nybe *et al.* 2007).

#### **18.2.6 Ginger oleoresin**

Ginger oleoresin is obtained by extraction of powdered dry ginger with suitable organic solvents such as alcohol, acetone and ethylene dichloride, etc. Concentration of solvent extract under vacuum and complete removal of traces of solvent yields 'oleoresin of ginger'. The yield, flavour and pungency of extracted oleoresin vary with cultivars, maturity of rhizome, choice of solvent and the method of extraction employed. Generally a yield of 3.9–9.3% with an average of 6.5% on dry weight of ginger is obtained. Commercial ginger oleoresin usually has a volatile oil content of 25–30% and a replacement strength of 1 kg oleoresin for 28 kg good-quality ground spice. They are offered to the consumer in liquid form or dispensed on sugar or salt.



### 18.2.7 Ginger paste

In India a 'ginger–garlic paste' for culinary purpose is traditionally made with 50 % sliced and macerated ginger, 35 % garlic and 15 % salt. Nowadays, it is available to the consumers in suitable packing with improved shelf-life.

## 18.3 Main uses and functional properties of ginger

### 18.3.1 Uses in food processing

The refreshing pleasant aroma, biting taste and carminative property of ginger make it an indispensable food ingredient throughout the world. Fresh ginger, ginger powder from dry ginger, oleoresin and oil are all used for this purpose. Fresh ginger is unique for its flowery flavour and spicy taste. Hygienic oleoresin and oil in convenient consumer-friendly packing and dispenser systems find a place in the culinary art of developed countries and the upper society strata of developing countries. Ginger preserve and candy are also in great demand for use in confectionery. Chocolate manufacturers utilize the preserve for enrobing. It is also used in jams and marmalades. The syrup in which ginger is preserved is valued for pickle and sauce making. It is also used in the production of gingerbread (Pruthi, 1993). In western countries, ginger is used widely for culinary purposes in gingerbread, biscuits, cakes, puddings, soups and pickles. It is also used in the production of alcoholic beverages like ginger beer, ginger ale, and ginger wine. Earlier, it was much favoured for spicing wines and possets (Purseglove *et al.*, 1981). In the east, fresh ginger chopped into small bits and in the ground form is very much used in vegetarian and non-vegetarian food preparations. It is also used in pickling, soft drink making, confectionery and curry powder preparations. Chinese cookery owes very much to the use of fresh ginger as ground paste. In India, in meat and fish dishes it is indispensable in making them palatable and digestible. Buttermilk containing crushed fresh ginger, green chillies, salt and curry leaf is a delicious drink and appetizer of South India. 'Puliyangi', a curry prepared from finely chopped fresh ginger and ripe tamarind fruit extract as main ingredients, is unique in taste and is indispensable in social and festival feasts of the Malabar coast. Certain Indian recipes for ginger wine, fish, meat and tomato curry preparations are given below:

#### ***Ginger wine***

##### *Ingredients*

|                               |             |
|-------------------------------|-------------|
| Fresh ginger cut into pieces: | 1 kg        |
| Yeast:                        | small pinch |
| Sugar:                        | 1 kg        |
| Water:                        | 1.5 l       |

##### *Method*

1. Cook the ginger pieces in a pressure cooker for 10 minutes.
2. When cold, keep for fermentation along with  $\frac{3}{4}$  kg sugar and yeast for 21 days.
3. Every other day the contents are turned.
4. On completion of fermentation, wine is squeezed out from the fermented ginger.

5. To improve the appearance, the remaining  $\frac{1}{4}$  kg sugar is burned to brown colour and mixed with the wine.

Consuming one teaspoon of ginger wine daily is good for digestion and health.

### ***Spicy Lamb Fry Masala***

#### *Ingredients*

|                      |                        |
|----------------------|------------------------|
| Lamb:                | 750 g                  |
| Onions:              | 2 medium size          |
| Ginger:              | 20 g                   |
| Garlic:              | 4 cloves               |
| Green chillies:      | 1 teaspoon             |
| Garam masala powder: | 1 teaspoon             |
| Curry leaves:        | 10 g                   |
| Coriander powder:    | 1 tablespoon           |
| Red chilli powder:   | 4 teaspoon             |
| Turmeric powder:     | $\frac{1}{2}$ teaspoon |
| Capsicum:            | $\frac{1}{4}$ teaspoon |
| Fresh tomatoes:      | 2                      |
| Ground black pepper: | 2 teaspoons            |
| Coriander leaves:    | to garnish             |
| Lemon juice:         | to taste               |
| Salt:                | to taste               |
| Oil:                 | 20 g                   |
| Fresh lemon:         | 1                      |

#### *Method*

1. Cut the meat into half-inch cubes and wash them.
2. Cook meat with salt along with sliced onions, ginger, garlic, red chilli powder, turmeric powder, garam masala powder and coriander powder on a slow fire until meat is tender and all water has evaporated.
3. Slice some onions and sauté them in a little oil.
4. Add cooked meat and sauté it until well fried.
5. Add sliced tomatoes, capsicum, green chillies and fry them.
6. Add ground black pepper, chopped coriander leaves and lemon juice.
7. Serve with a garnish of onion rings, curry leaves and sliced fresh lemon.

### ***Garcinia fish curry***

#### *Ingredients*

|                  |                  |
|------------------|------------------|
| Fish:            | $\frac{1}{2}$ kg |
| Chilli powder:   | 4 tablespoons    |
| Turmeric powder: | 1 teaspoon       |
| Garlic flakes:   | 10               |
| Ginger julienne: | 1 inch piece     |
| Garcinia:        | 4 pods           |
| Coconut oil:     | 2 tablespoons    |
| Mustard:         | 1 tablespoon     |
| Fenugreek:       | 4 seeds          |
| Curry leaves:    | 1 spring         |

Salt: to taste  
Water: 2 glasses

*Method*

1. Cut the fish into dices and wash under running water.
2. Heat up an earthenware pot, pour the coconut oil, crackle mustard, fenugreek.
3. Sauté garlic, ginger and curry leaves and add chilli powder and turmeric powder.
4. Stir till it gets cooked and add water to it.
5. Add cleaned garcinia pods and place the diced fish into it.
6. Cover the pot and boil it until fish gets cooked.
7. Check for the seasoning and finish it off with a dash of coconut oil.

**Tomato curry**

*Ingredients*

Onions: 2 medium  
Tomatoes: 4 medium  
Cumin powder: 1 teaspoon  
Ginger: 10 g  
Coriander powder: 1 teaspoon  
Turmeric powder: 1 teaspoon  
Dry red chillies: 4  
Mustard: 1 teaspoon  
Oil: 2 tablespoons  
Cashew nut: 10 g  
Chilli powder: 1 tablespoon  
Yoghurt: 2 tablespoons

*Method*

1. Fry sliced onions until brown.
2. Grind coriander powder, chilli powder and turmeric powder with sliced ginger into a smooth paste along with yoghurt.
3. Add ground condiments to browned onions.
4. Cut tomatoes into quarters and add to brown mixture.
5. Fry for 2 minutes.
6. Add 2 cups of water and boil for 5 minutes.
7. Add salt.
8. Give a tempering of mustard, red chillies and cashew nuts.

**18.3.2 Functional properties**

The nutritive value of ginger is given in Table 18.2.

**Medicinal uses**

Plant-derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80 % of the world population relies on botanical preparations as medicines to meet their health needs. Herbs and spices are generally

**Table 18.2** Nutritional composition of dry ginger (per 100 g)

| Composition           | Quantity |
|-----------------------|----------|
| Water (g)             | 7.0      |
| Food energy (k cal)   | 380      |
| Protein (g)           | 8.5      |
| Fat (g)               | 6.4      |
| Carbohydrates (g)     | 72.4     |
| Ash (g)               | 5.7      |
| Calcium (g)           | 0.1      |
| Phosphorus (mg)       | 150      |
| Sodium (mg)           | 30       |
| Potassium (mg)        | 1400     |
| Iron (mg)             | 11.3     |
| Thiamine (mg)         | 0.05     |
| Riboflavin (mg)       | 0.13     |
| Niacin (mg)           | 1.90     |
| Vitamin activity (RE) | 1.5      |

considered as safe and have been proved effective against certain ailments. (Kalpagam and Nirmala, 2003). Ginger is much used in traditional Indian (Ayurveda) and Chinese medicine (Sivarajan and Balachandran, 1994). In India, ginger has been used as medicine from the Vedic period and is called ‘maha aushadhi’, which means the great medicine. In Ayurveda, ginger is recommended for use as a carminative, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic and digestive aid (Warrier, 1989). Ginger is also an essential ingredient in many traditional Chinese medicines and has been used in that part of the world since the fourth century BC. The Chinese take ginger for a variety of medical problems such as stomach ache, diarrhoea, nausea, cholera, asthma, heart conditions, respiratory disorders, tooth ache and rheumatic complaints (Wagner and Hikino, 1965). In the USA and Europe, ginger preparations are sold as nutraceuticals or over-the-counter remedies against nausea, motion sickness and migraine. Ginger is listed in the German commission E Monograph as an approved phytomedicine against dyspepsia and to prevent motion sickness (Plotto, 2011).

### *Dosage*

For most purposes, a typical dose of ginger is 1–4 g daily taken in divided doses. To prevent motion sickness, it is best to begin treatment 1–2 days before the scheduled trip and continue dosing throughout the duration of travel. For nausea and vomiting during pregnancy, ginger tea made from fresh ginger rhizome boiled and diluted to taste appears to work best.

### *Modern scientific research into functional properties*

Recent research has supported its functional value in the following areas:

- Ginger has excellent antioxidant properties. Antioxidants are increasingly linked to the prevention of certain cancers (Kikuzaki *et al.*, 1994) and coronary heart disease, and are also used to preserve lipid-based foods. Studies include the role

of components such as gingerol in inhibiting linoleic acid autoxidation (Kikuzaki and Nakatani, 1993), extending the shelf-life of meat (Ziauddin *et al.*, 1995), dehydrated pork (Fuijo *et al.*, 1969) and fermented meat sausage (Al-Jalay *et al.*, 1987). Incorporation of 5 % ginger into 'ogi', a Nigerian traditional fermented food, improved its sensory attributes and shelf stability by reducing microbial load (Adesokan, 2010).

- There are a number of studies about the antimicrobial activity of gingerols, for example in relation to *Bacillus subtilis* and *E. coli* (Yamada *et al.*, 1992) and *Mycobacterium* (Galal, 1996; Hiserodt *et al.*, 1998).
- Ginger has a known influence on the eicosanoid cascade which influences such functions as wound healing, inflammation and platelet aggregation, and is involved in conditions such as arteriosclerosis (Srivasta, 1986; Sankawa, 1987; Kiuchi *et al.*, 1992; Kawakishi *et al.*, 1994).
- In Ayurveda, ginger is reported to be useful in treating inflammation and rheumatism. One of the mechanisms by which ginger exerts its ameliorative effects could be related to inhibition of prostaglandin and leukotriene biosynthesis (Kiuchi *et al.*, 1992). A study conducted in Denmark revealed that an average intake of 5 g of fresh ginger or 0.5–1 g powdered ginger reduced pain, swelling and morning sickness in patients suffering from arthritis.
- In traditional Chinese medicine, ginger is used to improve the flow of body fluids. It stimulates blood circulation through the body by powerful stimulatory effect on the heart muscle and by diluting blood. A Japanese study showed that active constituents in ginger reduced blood pressure and decreased cardiac workload (Tanabe *et al.*, 1993).
- Ginger has beneficial effects on the digestive system, enhancing gastrointestinal motility, and is used traditionally for the treatment of stomach ache, vomiting and indigestion (Yamahara *et al.*, 1990). It has also been investigated for its gastroprotectant and anti-ulcer activity (Yamahara *et al.*, 1988, 1992; Yoshikawa *et al.*, 1994).
- A recent study has investigated antitumour properties in gingerol, notably in inhibiting skin cancer (Park *et al.*, 1998). Observations from studies conducted at the National Institute of Nutrition, Hyderabad, in rats suggest that ginger has the ability to stimulate the levels of glutathione-s-transferase (GST), a group of enzymes which are known as cellular detoxification enzymes. There is a high correlation between the induction of these enzymes and inhibition of carcinogenesis (Kalpagam and Nirmala, 2003).
- Ginger checks cholesterol biosynthesis and thereby inhibits hypercholesterolaemia (Tanabe *et al.*, 1993). Its role in Chinese herbal medicine in controlling obesity has also been investigated (Wijaya and Wu, 1995).
- Ginger powder (500–600 mg) taken at the onset of migraine aura, followed by 4 hourly intake for 3–4 days, is reported to provide relief from migraine attacks (Kalpagam and Nirmala, 2003).
- Ernst and Pittler (2000) found that, in preventing motion sickness, ginger (940 mg/day) is superior to dimenhydrinate (100 mg/day). Studies also support that it is effective in preventing post-operative nausea and vomiting. Administration of powdered ginger (250 mg four times daily) significantly reduced symptoms of nausea and vomiting in pregnant women.

- The compounds of 6-gingerol and 6-shogaol have been shown to have a number of pharmacological activities including antipyretic, analgesic, antitussive and hypotensive effects (Suckawa, 1984).

The various functional properties of ginger are discussed in detail in Kikukazi (2000).

### 18.3.3 Safety

Ginger has been listed as generally recognized as safe (GRAS) by the US FDA. A dose of 0.5–1 g ginger powder ingested two to three times for periods ranging from 3 months to 2.5 years did not cause any adverse effects. *The British Herbal Compendium* documents no adverse effects of ginger (Bradley, 1990). Despite widespread uses of ginger by pregnant women, the safety of this herb has not been formally established, and the Complete German Commission E Monographs recommends against the use of ginger rhizome for nausea and vomiting in pregnancy.

## 18.4 Quality specifications

India and China are the world's largest producers and exporters of ginger. Other important producers are Indonesia, Nepal, Nigeria, Thailand, Bangladesh, Japan and the Philippines. The UK, Germany, Japan, Saudi Arabia, Singapore, Hong Kong and Canada are the major importers of ginger. The importing countries give top priority to the health of their citizens and it is important that the ginger imported conforms to the quality standards prescribed by the particular country. Exporting countries also fix standards to supply required quality products to the consumers.

### 18.4.1 Indian standards

In India, the world leader in ginger export, the Bureau of Indian Standards (BIS) has AGMARK grading system for dry ginger and ginger powder. It categorizes Calicut ginger and Cochin ginger to different grades as given in Table 18.3 based on the size of rhizome, extraneous matter, lime content as calcium oxide and very light pieces present. Among them only garbled non-bleached 'Cochin' and Calicut meet the UK Standards.

### 18.4.2 United States standards

The US Government standard defines ginger as the washed and dried or decorticated and dried rhizome of *Zingiber officinale* Rosc. The standards stipulated for ginger are given in Table 18.4. The ginger imported to the USA should also conform to the cleanliness specifications stipulated by the American Spice Trade Association (ASTA) (Table 18.5) and also the regulations enforced by the Food and Drug Administration (FDA). The FDA has fixed defect action levels for whole ginger as shown in Table 18.6.

**Table 18.3** Grades of whole dry ginger with specifications as per the ISI specification No. IS:1908–1961

| Grade                                                   | Special characteristics                                                                                            | Size of rhizome               | Extraneous matter in percentage (max.) | Light pieces by count in percentage (max.) | Lime as CaO by weight in percentage (max.) |
|---------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|-------------------------------|----------------------------------------|--------------------------------------------|--------------------------------------------|
| 1. NGK/NGC<br>Garbled,<br>nonbleached<br>Calicut/Cochin | Pieces irregular in shape and size with peel not entirely removed. Light pieces removed by garbling                | Not less than 20 mm in length | 2                                      | Not allowed                                | Nil                                        |
| 2. BGK/BGC<br>Garbled<br>bleached<br>Calicut/Cochin     | Pieces irregular in shape and size with peel not entirely removed. Lime bleached. Light pieces removed by garbling | Not less than 20 mm in length | 2                                      | Not allowed                                | 2.5                                        |
| 3. NUGK/K<br>Ungarbled<br>nonbleached<br>Calicut/Cochin | Pieces irregular in shape and size with peel not entirely removed.                                                 | Not less than 20 mm in length |                                        |                                            |                                            |
| a) Special                                              |                                                                                                                    |                               | 3                                      |                                            | 5                                          |
| b) Good                                                 |                                                                                                                    |                               | 4                                      |                                            | 10                                         |
| c) Nonspecified                                         |                                                                                                                    |                               | N S*                                   |                                            |                                            |
| 4. BUGK/C<br>Ungarbled<br>Bleached<br>Calicut/Cochin    | Pieces irregular in shape and size with peel not entirely removed. Lime bleached.                                  | Not less than 20 mm in length |                                        |                                            |                                            |
| a) Special                                              |                                                                                                                    |                               | 3                                      | 5                                          | 4                                          |
| b) Good                                                 |                                                                                                                    |                               | 4                                      | 10                                         | 6                                          |
| c) Nonspecified                                         |                                                                                                                    |                               | NS                                     |                                            |                                            |

NS = not specified.

**Table 18.4** US government specifications for dry ginger and powder

|                                                                   |        |
|-------------------------------------------------------------------|--------|
| Total ash per cent not >                                          | 7.0    |
| Acid insoluble ash per cent >                                     | 1.0    |
| Crude fibre per cent not >                                        | 8.0    |
| Volatile oil expressed as ml per 100 g not<                       | 1.5    |
| Moisture per cent not >                                           | 12.0   |
| Starch per cent not <                                             | 42.0   |
| Sieve test – US standard sieve size<br>(for powdered ginger only) | 110.30 |
| Percentage required to pass through not <                         | 95.0   |

Source: from US Federal Specifications: Spices ground and whole and spice blends, No.EE-S-531 H, June 5, 1975 (Purseglove *et al.*, 1981).



**Table 18.5** ASTA cleanliness specifications

| Factors specified (not >)                   | Ginger |
|---------------------------------------------|--------|
| Mammalian excreta, mg per 1b                | 3      |
| Other excreta, mg per 1b                    | 3.0    |
| Extraneous matter, per cent by weight       | 1.00   |
| Whole dead insects, per 1b by count         | 4      |
| Insect defiled/infested, per cent by weight | SF (3) |
| Mouldy ginger, per cent by weight           | SF (3) |

*Source:* Sivadasan (1998).

**Table 18.6** Defect action levels fixed by FDA

| Defect                                     | Action level                                                                 |
|--------------------------------------------|------------------------------------------------------------------------------|
| 1) Insect and mould infestation (MPM-V 32) | Average of 3 % or more of pieces by weight are insect infested and/or mouldy |
| 2) Mammalian excreta (MPM-V 32)            | Average of 3 mg or more of mammalian excreta per pound                       |

*Source:* Sivadasan (1998).

**Table 18.7** Tolerance limits for certain pesticides and aflatoxin

| Pesticides   | Tolerance limit (ppm) |
|--------------|-----------------------|
| Aldrin       | 0.05                  |
| Dieldrin     | 0.05                  |
| BHC          | 0.05                  |
| Chlordane    | 0.1                   |
| Heptachlor   | 0.1                   |
| Malathion    | 0.1                   |
| Parathion    | 0.3                   |
| Carbonde     | 0.3                   |
| DDT, Endrin  | Not permitted         |
| Aflatoxin B1 | 2 ppb (max.)          |
| B1+B2+G1+G2  | 4 ppb (max.)          |

*Source:* Spices Board (1995).

Besides the above, the FDA has also specified tolerance limits for pesticide and aflatoxin as shown in Table 18.7.

### 18.4.3 European standards

Importers in Germany, the Netherlands, the UK and the ESA have laid down specifications for whole dry ginger and powder regarding commercial cleanliness and health requirements (Table 18.8).

**Table 18.8** Cleanliness and commercial specifications for whole dry ginger imported to some European countries

| Sl. No | Factors                    | Germany | The Netherlands | UK   | ESA  |
|--------|----------------------------|---------|-----------------|------|------|
| 1      | Extraneous matter (% wt)   |         |                 | 1.0  | 1.0  |
| 2      | Moisture (% wt)            | 12.5    | 10.0            | 12.0 | 12.0 |
| 3      | Total ash (% wt)           | 7.0     | 8.0             | 6.0  | 8.0  |
| 4      | Acid insoluble ash (% wt)  | 1.0     | 3.0             | 1.0  | 2.0  |
| 5      | Volatile oil (% wt) (min.) | 1.4     | 0               | 0    | 1.5  |

Source: Kalyanaraman (2002); Nair (2002).

#### 18.4.4 Oleoresin standards by the US Essential Oil Association

Volatile oil content: 8–35 ml/100 g

Refractive index: 1.488–1.498

Optical rotation: –30°–60°

Ginger oleoresin can be standardized to meet specific product requirements, but the product must be labeled WONF (with other natural flavours) with the added flavour identified.

#### 18.4.5 Requirements for organic ginger

Plotto (2011):

To be sold as ‘organic’ the product must be certified by an accredited certification body. IFOAM, the International Federation of Organic Agricultural Movement, has established organic production, processing and trading standards, and tried to harmonize certification systems worldwide. National and regional governments are also trying to work under a compatible minimum set of standards. The EU has established basic regulations for organic products in 1991 (Council Regulation 2092/91) which apply to all products marketed as organic. To be labeled as ‘organic’ the products must be grown following organic agricultural practices. Post-harvest handling and processing must be done in certified facilities whether on the farm or in food packing or processing facilities. Only mechanical, thermal or biological methods can be used in organic processing. The use of genetically modified organisms (GMO) and products of GMO is prohibited in organic cultivation. Ionizing radiation and sewage sludge are prohibited from organic agricultural practices. Labels of organic products must identify the certification body. For spice and oleoresin production, ionizing radiation and the use of synthetic solvents are prohibited in the processing of organic products.

#### 18.4.6 Biotechnology studies in ginger

Clonal propagation of ginger under *in vitro* conditions through enhanced release of axillary buds has reported by many (Hosoki and Sagawa, 1977; Bhagyalakshmi and Singh, 1988). The technique is useful for large-scale multiplication of elite planting materials and for the production of disease-free nuclear seed material. Tissue culture plants require 2 years to produce good yield, so tissue culture technology cannot be used for large-scale production of planting material for routine cultivation. Callus

mediated organogenesis has been reported by many (Malamug *et al.*, 1991, Babu *et al.*, 1996; Suma and Kesvachandran, 2005; Paul, 2006). The protocol is useful for ginger breeding through the exploitation of somaclonal variations. Kerala Agricultural University has released two varieties, Athira and Karthika, using this biotech breeding tool for large-scale cultivation in Kerala (Shylaja *et al.*, 2010). In ginger, seed set and seed germination is scarce or absent in most of the ginger-growing geographical regions. So the spectrum of genetic variability available for crop improvement is limited. An *in vitro* pollination and fertilization technique has been standardized in ginger for seed set and development (Valsala, 1994). Bindu (1997) and Rethidevi (2005) reported embryo rescue and germination studies in ginger. Plant regeneration by embryo culture of *in vitro* developed seed was reported by Valsala *et al.* (2009).

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## Marjoram

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**Abstract:** This chapter begins with an introduction to marjoram, followed by a description of the various species. It then goes on to review different research works which have looked into production, harvesting and post-harvest management. The essential oil of marjoram is discussed in the following sections, including composition, methods for extraction, storage and uses in food as well as in traditional medicines. Various functional properties of marjoram, including antimicrobial and antioxidants, quality issues, including quality control and adulteration, chemical and physical specifications, are also discussed.

**Key words:** sweet marjoram (*Origanum majorana* L.), chemotypes, essential oil composition, antimicrobial properties, antioxidant properties, quality issues.

### 19.1 Introduction

According to Skoula and Harborne (2002) and Lukas *et al.* (2009), the genus *Origanum* (Lamiaceae) consists of 43 species and 18 hybrids arranged in three groups and 10 sections. Members of this genus comprise some of the most important aromatic plants throughout the world such as: sweet marjoram (*O. majorana* L.), dittany from Crete (*O. dictamnus* L.), Italian oregano or pot marjoram (*O. onites* L.), Greek oregano or marjoram (*O. heracleoticum* L.) and Turkish wild oregano (*O. vulgare*) and bible hyssop or Syrian oregano (*O. syriacum*). These are all commercially available and exportable plants with appreciable market values (Kintzios, 2002).

Sweet marjoram (*O. majorana* L.) is indigenous to Mediterranean countries and was known to the ancient Egyptians, Greeks and Romans (Tainter and Grenis, 1993). They cultivated it as a pot herb and not only used it to flavour food but also prized it as a miraculous herb with the power to heal practically all diseases, especially colds and chills. The Greeks considered it a symbol of happiness and thought that if grown on the grave, the deceased would be eternally happy. Hemphill and Hemphill (1984) mentioned that marjoram was one of the strewing herbs once used to give houses a pleasant, clean smell, and it was a favourite in sweet bags for the linen cupboard. Marjoram was popular during the Middle Ages as a medicine and as a culinary herb in England during the sixteenth century. Now marjoram is grown in central Germany, Hungary, southern France and in the USA. It is also grown in

western Asia, South and North America, France, Spain, Portugal, the UK, North Africa, Morocco, Tunisia, China, Russia and India. For many years, both marjoram and oregano were known as *O. majorana* L. and marjoram has often been mistaken for oregano in botanical descriptions (Tainter and Grenis, 1993). Today, only marjoram is identified as *O. majorana* L. Oregano is commonly referred to as *O. vulgare*.

According to Pruthi (1976), it is the dried leaves of marjoram or sweet marjoram with or without flowering tops in small proportions that constitute the spice of commerce. Sweet marjoram is an aromatic herb of the mint family and grows to a height of 30–60 cm. The herb develops a large number of leafy stalks with small leaves. The leaves are whole and the large ones are always fragmented. Leaves are light, greyish green and oblate to broadly elliptical, margin entire, reaching about 21 mm in length and 11 mm breadth (Parry, 1969). The flowers are small, white or pinkish or red. Essential oil is very strong and of very pleasant fragrance. The highest percentage is found in the leaves, whereas only traces are found in flowers and stalks. Long periods of blooming encourage the accumulation of oil in seeds (Guenther, 1974).

Sweet marjoram is characterized by a strong spicy and pleasant odour. The flavour is fragrant, spicy, slightly sharp, bitterish and camphoraceous. Though a perennial, it is treated as an annual under cultivation. The colour of the dried herb is light green with a slight greyish tint. The whole leaves are small with hairs on both sides of the leaf. When examined under the low power of a microscope, many dot-sized oil glands are seen on the leaf. They yield 3.5 % volatile oil (Pruthi, 1976). The colourless oil is obtained from the whole plant, including the square stem, the long leaves and the white labiated blossoms. The scent is reminiscent of a mixture of lemon and lavender.

Marjoram is known by the following names:

- English: marjoram
- French: marjolaine
- German: majoran
- Italian: maggiorana
- Spanish: mejorana

### 19.1.1 Different species of marjoram

#### *Wild marjoram*

Wild marjoram (*O. vulgare*) is a perennial herb native to Europe and West Asia and is commonly found in dry places and as hedge banks in England. It has been naturalized in the USA and is also known as oregano.

#### *Pot marjoram*

This is also a Mediterranean plant, growing to about 30 cm in height. Pot marjoram (*O. onites* L.) is cultivated for its aromatic leaves and is used for flavouring food. The plant is not very hardy and produces white flowers. Its taste is slightly bitter, and it can be used to some extent for the same purpose as sweet marjoram, especially in strong flavoured dishes as with onion, wine and garlic, where the delicate perfume of sweet marjoram would, in any case, be largely lost.



In Greece, there are no fewer than ten different species of *origanum* growing wild and they are commonly known as rigani. One of these, known as winter marjoram (*O. heracleoticum*) is sometimes cultivated in gardens. Other species are *O. smyrnaicum* and *O. paniflorum*. Rigani are used with grilled meats and other Greek dishes, but it is almost impossible at present to buy the authentic herb and reproduce exactly the flavour of such dishes outside that country. Another species of *origanum*, commonly known as Cretan dittany, is cultivated particularly on the island of Crete (it is known as *dictamo*, *ditamo*, *eronatus*, *stomatochorto* and *malliaro-chorto*) and is used mainly for medicinal purposes though also as a food flavouring.

## 19.2 Production, harvesting and post-harvest management

### 19.2.1 Production

In northern Europe, marjoram is usually replanted annually, as it does not survive the winter outdoors (Wisniewska *et al.*, 2006). Various aspects of the production process affect the yield of foliage and essential oil. Capecka *et al.* (2003) found that seed conditioning and treatment with gibberellic acid ( $GA_3$ ) had a beneficial effect on marjoram yield, but did not alter the oil composition. Studies of nutrient management by various groups have shown that marjoram plants positively respond in terms of yield to organic manure and a variety of chemical fertilizers (Edris *et al.*, 2003; Dzida and Jarosz, 2006). Bacterial inoculation and biostimulators also had a beneficial effect (Jelacic *et al.*, 2007; Banchio *et al.*, 2008; Gharib *et al.*, 2008; el Ghandour *et al.*, 2009). Organic cultivation has also been studied by Seidler-Lozykowska *et al.* (2008, 2009). The raw materials of herbs grown with composted manure contained more essential oils than those cultivated in a conventional fashion. Environmental conditions may also impact yield and essential oil content and composition, but studies have had conflicting results. Seidler-Lozykowska (2007) carried out an experiment for five years to investigate the influence of the weather on a marjoram cultivar in a specific location in Poland. The essential oil yield differed very significantly over the course of the study, but there was a significant positive correlation between essential oil content and the mean daily temperature and insolation. Galambosi *et al.* (2002) found higher marjoram yields at a site in the south of Finland compared to a site 500 km further north; however, essential oil content and composition were not strongly affected. The yield and chemical composition of harvested marjoram also varies according to plant density (Martyniak-Przybyszewska and Wojciechowski, 2003; Al-Kiyyam *et al.*, 2008; Zawislak, 2008).

### 19.2.2 Harvest

Marjoram grown in Egypt is traditionally harvested from March–July. French marjoram is harvested in September–November. Kybal and Kaplicka (1990) opined that the herb should be harvested and dried in early summer before flowering, since if the foliage is cut off about 6 cm above the ground the plant will put out new shoots and yield another crop in autumn. According to Prakash (1990), though, the first harvest of the leaves and tender tops of the herb is done as flowering commences. The plants are cut 5–8 cm above ground level and, with favourable conditions, a

second cutting may be made two months later. In Egypt, according to Edris *et al.* (2003), the most proper time for harvest is the start of flowering stage. According to Frąszczak (2004), it is also economical and recommended to harvest the herbs twice, the first harvest when the plants are flowering (late June–early July), and the second one in early October, without waiting for flower buds to appear. Omer *et al.* (1994) studied the effect of harvesting periods on the essential oil of marjoram in newly reclaimed lands of Egypt. It was observed that, although the best yields of herb, leaves and oil were obtained from the second and third harvest of each season, the oil content of leaves was lower in the second year. Edris *et al.* (2003) recommended that two harvests are taken from marjoram in the first year, while three harvests per year are made in the subsequent years. These authors also concluded that herb yield increases from first harvest to third harvest.

Mishra *et al.* (2004) found that the full bloom and pre-seed setting stages are the best time for harvesting for herbage and oil yields, respectively. Wierdak and Dzida (2009) also found that the essential oil content was significantly higher at the full flowering stage than at the bud formation stage. Zawislak and Dzida (2010) also concluded that a higher yield of essential oil was obtained from the second harvest (early September) than from the first (mid-July, when flowering commences). The influence of successive cuts on intrinsic quality characteristics and post-harvest behaviour are of special interest. Verma (2010) suggested that a good yield of high-quality marjoram essential oil can be obtained by harvesting the top 1/3 portion instead of harvesting the whole herb, which is the common practice of growers in most of the countries. Moreover, harvesting at this portion helps to cultivate it as a multi-harvest crop which will produce younger leafy herb rich in oxygenated monoterpenes. The upper and lower portions of the plant could be processed separately to get essential oils of two different qualities.

### 19.2.3 Post-harvest drying and storage

Aharoni *et al.* (1993) pointed out that harvested fresh green marjoram is a highly perishable produce due to senescence-accelerated metabolism accompanied by loss of freshness, chlorophyll and culinary quality. Hence, careful post-harvest management is necessary and temperature control plays a major role. Bottcher *et al.* (1999) observed that even 72–80 hours after harvest, at 10°C the plant material was maintained in a fresh green state, whereas at 20 and 30°C degradation was accelerated due to the onset of senescence. They observed that at 20°C, acceptable marjoram quality after harvest could be maintained for only 48 hours. A uniform deep green colour and an excellent anatomical appearance (without signs of wilt) were best maintained, as in dried material, when the herbs were kept at 10°C throughout the post-harvest period.

After the harvest, the leaves are dried, carefully cleaned and stored. Methods of drying depend on the size of the crop and climatic conditions in the producing countries. Cut plants may be tied as bunches in small quantities and dried in the open air or spread on wire trays in ventilated rooms and dried by regulated circulation of warm air. Sun drying may take 2–4 days for drying, and in the case of ventilating drying sheds it may take more than a week. Stems or stalks are separated from leaves by rubbing on hand sieves of 1–2 cm mesh. Chaff is removed by using

a fan, and extraneous sand, earth and dust are removed by shaking in wide-meshed sacks (Guenther, 1974).

More recently, in Egypt the harvested material has been pre-dried in the field and then in a solar drier to reduce the microbial load to less than 50 % in comparison with the traditionally dried material. Buckenhuskes *et al.* (1996) used a solar greenhouse drying system for marjoram in Egypt and found that the shoot essential oil content after drying was 98 % of the initial value. Microbial load could also be reduced considerably by this improved method. Singh *et al.* (1996) found that microwave blanching of marjoram gave the maximum retention of ascorbic acid (21.5 % which is 79.4 % of the composition of fresh herb). Blanching resulted in better retention of the original green colour of the fresh herb compared to direct drying of the herb. The herb had firmer texture when microwave blanched than when blanched by other methods and when fresh.

Paakkonen *et al.* (1990), while studying the effect of drying, packing and storage on quality of herbs, found that odour and taste of freeze-dried marjorams were sensitive to storage conditions. Freeze-dried marjoram exhibited a much more intensive colour than air-dried marjoram. After 9 months of storage in the light or raised temperature, the colour tone of the freeze-dried marjoram had changed only slightly. They could not notice any significant difference in odour and taste intensities for the frozen and air-dried products after 8 months of storage, whereas the taste of the freeze-dried marjoram differed from the marjoram stored frozen. The intensity of the odour and taste of the air-dried marjoram stored under vacuum was higher relative to the marjoram in glass jars or paper bags. An elevated storage temperature of 35°C was found to have a more detrimental effect on sensory quality than packaging. It was concluded that the intensity of odour and taste of dried herbs could be maintained for 2 years at 23°C in air-tight packaging. Malmsten *et al.* (1991) demonstrated that freeze drying was more effective than air drying as a means of preserving the herb and also for microbial decontamination. Raghavan *et al.* (1997) noticed that convection drying at about 45°C for 6 hours preserved the flavour quality of marjoram to a greater extent than microwave drying.

Bottcher *et al.* (1999) observed that freshly harvested marjoram, though having a slightly woody stem, was characterized by an unusually high respiration rate, which was maintained throughout a period post-harvest of 72–80 hours. The essential oil balance measured over the post-harvest period increased slightly (10 %). They have opined that respiration energy is involved actively in synthesis of essential oil in plant tissue and that the high respiration rate has to be considered in the development of future equipment and technologies for ventilating, cooling and drying during the post-harvest period.

#### **19.2.4 Irradiation processing**

Only limited information is available regarding irradiation of marjoram. Bachman and Gieszczyńska (1973), while studying various aspects of irradiation of different spices including marjoram, noticed that irradiation at 7.5–12.5 kGy produced a change in flavour of marjoram. Frag *et al.* (1995) evaluated the effect of irradiation on microbial loads of herbal spices and found that irradiation at 10, 20 and 30 kGy caused complete elimination of microorganisms whereas 5 kGy was less effective.

They observed a noticeable reduction in the amounts of terpenes present in irradiated marjoram, which were converted to monoterpenes alcohols. The results proved that 10 kGy was a sufficiently high dose to eliminate the microorganisms, causing only slight changes in the flavouring materials.

### 19.3 Marjoram essential oil

Sweet marjoram essential oil, known in the trade as 'Oil of sweet Marjoram', is obtained by steam distillation of the dried leaves and the flowering tops of the herb, yielding 0.3–0.4 % oil from fresh and 0.7–3.5 % from dry herb. It is a pale yellow or pale amber coloured rather mobile liquid (Shankaracharya and Natarajan, 1971; Farrell, 1985). Dayal and Purohit (1971) obtained 0.8 % essential oil from marjoram seeds, which was also a pale yellow mobile liquid with a characteristic smell. Considerable variations in the compositional pattern are observed depending on the origin of the herb, climatic and drying conditions, production procedure of the oil and many other factors (Tabanca *et al.*, 2004; Dzida and Jarosz, 2006; Seidler-Lozykowska *et al.*, 2008; Zawislak, 2008). In general, plants that grow more densely packed together and are harvested at full flowering stage appear to be more abundant in oils. The aroma and taste are spicy, fragrant, warm, aromatic, penetrating and resemble that of lavender. There is a slightly bitter aftertaste.

#### 19.3.1 Composition

Many investigators have made studies on the composition of essential oil of marjoram, and the important findings have been compiled by Lawrence (1981, 1983, 1984, 1989, 1997) and Prakash (1990). Other more recent studies include those by Novak *et al.* (2000), Tabanca *et al.* (2004), Seidler-Lozykowska (2007) and Zawislak (2008). Verghese (2000) has reported the following types of compounds in sweet marjoram.

- **Monoterpenes:** terpinolene,  $\beta$ -phellandrene,  $\alpha$ -terpinene,  $\gamma$ -terpinene limonene, sabinene,  $\alpha$ -thujene,  $\alpha$ -pinene,  $\beta$ -pinene, camphene, myrcene, ocimene.
- **Monoterpene alcohols:** linalool, geraniol,  $\alpha$ -terpineol, terpinene-4-ol, *cis*- and *trans*-2-*p*-menthen-1-ol, *cis*- and *trans*-sabinene hydrate, *cis*- and *trans*-piperitol, borneol, *p*-cymene-8-ol.
- **Monoterpene carbonyls:** carvone,  $\alpha$ -thujone, camphor.
- **Monoterpene esters:** neral acetate, geranyl acetate, linalyl acetate, and terpenyl-4-acetate.
- **Sesquiterpenes:**  $\beta$ -caryophyllene,  $\alpha$ -humulene,  $\alpha$ -copaene, farnesene, ledene,  $\gamma$ -elemene,  $\beta$ -bisabolene, bicyclogermacrene, allo-aromadendrane.
- **Terpinoid ether/oxides:** 1,8-cineol, arylphyllene epoxide.
- **Benzoid compounds:** *p*-cymene, eugenol, thymol, carvacrol, methyl chavicol, anethole.

#### 19.3.2 Chemotypes

Gora and Lis (2005) reported that marjoram oils are characterized by great variability in chemical composition, and cultivated varieties can be grouped according to

chemotype. Granger *et al.* (1975) reported that oil existed in two forms: one predominant in *cis*-sabinene hydrate (*cis*-thujanol) and the other in terpinen-4-ol. However, Gora and Lis (2005) reported three main chemotypes. In the first chemotype – monoterpene alcohols – *cis*-sabinene hydrate (also called *cis*-thujan-4-ol), *trans*-sabinene hydrate and the acetate of *cis*-sabinene hydrate are the main constituents of the volatile fraction. These unstable compounds rearranged during hydrodistillation to form terpinen-4-ol,  $\alpha$ -terpineol,  $\alpha$ - and  $\gamma$ -terpinene. Due to this, terpinen-4-ol alone or along with *cis*- and *trans*-sabinene hydrate,  $\alpha$ -terpineol,  $\alpha$ - and  $\gamma$ -terpinene were found to be the main constituents of the essential oils: in Lithuania – terpinen-4-ol (50 %); in Germany – terpinen-4-ol (20 %),  $\gamma$ -terpinene (18 %),  $\alpha$ -terpinene (11 %); in Egypt – terpinen-4-ol (20–40 %), *cis*-sabinene hydrate (12–27 %),  $\gamma$ -terpinene (10 %),  $\alpha$ -terpineol (11 %); Turkey – *cis*-sabinene hydrate (30–44 %) (Novak *et al.*, 2002; Edris *et al.*, 2003; El-Ghorab *et al.*, 2004; Tabanca *et al.*, 2004; Baranauskiene *et al.*, 2005). The second chemotype was rich in linalool or linalyl acetate: in Finland – linalool (30–40 %), *cis*-sabinene hydrate (6–20 %), sabinene (6–12 %); Morocco – linalool (33 %), terpinen-4-ol (32 %); Iran – linalyl acetate (26 %), sabinene (12 %) (Charai *et al.*, 1996; Barazandeh, 2001). The third chemotype was characterized by high concentration of carvacrol (78–80 %) and found in Turkey (Baser *et al.*, 1993).

Analysis of essential oil composition of marjoram in Cyprus by Novak *et al.* (2008) also revealed three chemotypes. Besides the standard ‘marjoramy’ composition (‘sabinyl chemotype’) with 74 % of oil compounds belonging to the bicyclic compounds sabinene, *trans*- and *cis*-sabinene hydrate and *cis*-sabinene hydrate acetate (‘sabinyl compounds’), two further chemotypes, namely a pure  $\alpha$ -terpineol chemotype (73 %  $\alpha$ -terpineol) and a mixed sabinyl/ $\alpha$ -terpineol chemotype (41 % sabinyl compounds, 40 %  $\alpha$ -terpineol) were present in the population. The chemotype frequencies found in this population by them were 56 % of the plants belonging to the sabinyl chemotype, 4 % to the pure  $\alpha$ -terpineol chemotype and 40 % to the mixed sabinyl/ $\alpha$ -terpineol chemotype.

#### *Selected studies of marjoram essential oil composition*

Many studies have investigated the composition of marjoram essential oil. A few are outlined below. Subramanian *et al.* (1972) studied the polyphenols of the leaves of marjoram and a new flavone designated majoranin, shown to be 4',5,7-trihydroxy-3,6,8-trimethoxyflavone and the 7-glucuronides of dinatin and diosmetin were isolated. Salehian and Netien (1973) compared major components of French, Hungarian and Egyptian marjoram essential oils and found that the ratio between the percentage composition of terpinen-4-ol to linalool and linalyl acetate decreased in the order Hungarian, French and Egyptian marjoram.

A study on marjoram by Taskinen (1974) from Bulgaria and Turkey by distillation with steam and by extraction and distillation with alcohol–water mixture disclosed 53 compounds. The amount of monoterpene alcohols in the alcoholic distillate was only around 20 % compared to about 60 % in the steam distillate. Karawya and Hifnawy (1976) reported the chemical composition of essential oil of marjoram from Egypt. Sarer *et al.* (1982) found that the oil of marjoram from Turkey contained monoterpene hydrocarbons, oxygenated monoterpenes and phenols. Ramachandraiah *et al.* (1984) reported the physicochemical characteristics of Indian sweet

marjoram oil from dried leaves. In a study by El-Keltawi and Khalil (1986) the highest percentage of essential oil in Egypt was recorded by applying 50 ppm of 2 styryl cyanine. Nykanen (1986) identified a total of 56 compounds, and the most prominent components were *cis*-sabinene hydrate and 4-terpineol. Later in 1987, Nykanen and Nykanen reported the composition of fresh and dried cultivated marjoram from Finland. Franz (1990) from Austria confirmed the importance of *cis*-sabinene hydrate and other compounds in giving the characteristic flavour of marjoram leaves. The essential oil composition of different lines of marjoram grown in Turkey was analysed by Kiryaman and Ceylan (1990), and it was found that linalool content ranged from 17.53–48.05 % and carvacrol content from 7.34–38.42 %. Yadava and Saini (1991b) reported 18 components of essential oil of marjoram and their percentages varied from 2.84 to 36.7. There was one unidentified compound whose concentration was 1.10 %. Karwowska and Kostrzewa (1991) noticed that essential oil of marjoram grown in Poland had no identical analogue, and the nearest in composition was essential oil from marjoram grown in Egypt.

Komatis *et al.* (1992) studied the composition of essential oil of marjoram in Greece. Gas chromatographic analysis revealed 65 compounds. The most prominent component was 4-terpineol (37 %) and, together with  $\alpha$ -terpineol and *cis*- and *trans*-sabinene hydrate, constituted 50 % of the essential oil. Three substances, *viz.* santalol, verbenone and carvacrol, were identified for the first time in marjoram. Pino *et al.* (1997) analysed the essential oil of marjoram grown in Cuba and found that among the 41 compounds identified, terpinen-4-ol (17.6 %), linalool (16.41 %) and thymol (11.55 %) were the main constituents.

Daferera *et al.* (2000) found that essential oil of Greek marjoram consisted of hydrocarbons (42.1 %), alcohols (24.3 %) and phenols. Analysis of oil of marjoram of Egyptian origin by Pavela (2005) produced 23 main constituents with the highest percentage for terpinen-4-ol followed by *cis*-sabinene hydrate and  $\alpha$ -terpineol. Helal *et al.* (2006) from their studies in Egypt observed that the oil of *origanum* contained a range of 17 compounds accounting for 78.97 % of total oil components. The seasonal variation in the essential oil composition of marjoram cultivated in Egypt was found to range from 2.5–3 % with the maximal value (3 %) in spring (Soliman *et al.*, 2009). Croteau (1977) studied the site of monoterpene biosynthesis in the leaves of marjoram. Excised epidermis of marjoram leaves incorporates label from (U-14C) sucrose into monoterpenes as efficiently as leaf discs, while mesophyll tissue has only a very limited capacity to synthesize monoterpenes from exogenous sucrose. These results strongly suggest that epidermal cells, presumably the epidermal oil glands, are the primary sites of monoterpene biosynthesis in marjoram.

Khanna *et al.* (1985) obtained 1.9 % of oil for marjoram grown in saline alkali soils (pH 9.0–10.5). The main constituents identified by them were  $\alpha$ -terpinene, *p*-cymene, geraniol, linalool,  $\alpha$ -terpineol, carvacrol and thymol. El-Bilay (1985) noticed that young leaves of marjoram contained high levels of terpinen-4-ol, whereas aged leaves contained high levels of *cis*-sabinene hydrate. The young leaves had an appreciably greater capacity to synthesize volatile oil than aged leaves. Although oil glands were not the centres of monoterpene biosynthesis, it was suggested that they are the main stores of terpenes in the plant. Fischer *et al.* (1987) demonstrated that *cis*-sabinene hydrate and its acetate represent the original flavour



compounds of the intact leaf. They opined that most of the monoterpenes described in the literature as being components of marjoram oil appear to be artefacts. Arnold *et al.* (1993) observed marjoram flowers to be richest in essential oil. They could identify 39 components, of which *cis*-sabinene hydrate (7.4–33.3 %) and terpinen-4-ol (16.6–21.6 %) were characteristic of *O. majorana* var. *tennifolium*, while the main compound in *O. dubium* was carvacrol (81 %). Carvacrol (69.4–81.6 %) was also the major constituent of *O. onites*. Omer *et al.* (1994) identified nine components from the essential oils and, although there were qualitative and quantitative differences, the main constituent of all oils was terpinen-4-ol (26.7–41.6 %).

While comparing the yield and other attributes of different forms of marjoram from various regions of USSR and abroad, Voronina (1988) found that the highest essential oil yield was 1.6 % from Crimea, followed by 1.56 % from Czechoslovakia. The oil yield was 1.52 % and 1.20 % for marjoram from Romania and Egypt, respectively, as reported by Refaat *et al.* (1990). Egyptian marjoram oil was richer in the main component, terpinen-4-ol (28.85 %), in the first cut than the Romanian sample (20.80 %).

Molina *et al.* (1999) from north west Argentina reported an essential oil content of 0.28–1.55 % on fw basis. Vera and Chane-Ming (1999) from Reunion Islands observed that the essential oil was rich in terpinen-4-ol, *cis*-sabinene hydrate, *p*-cymene and  $\gamma$ -terpinene, together with sabinene,  $\alpha$ -terpineol and  $\alpha$ -terpinene. They constituted about 80 % of the total essential oil. Ozguven and Tansi (1999), in Turkey, obtained the highest essential oil yield at the post-flowering period. The main components of the oil were  $\gamma$ -terpinene, *p*-cymol and terpineol.

Circella *et al.* (1995) found that *cis*-sabinene hydrate, the main component of essential oil of marjoram, was produced in larger quantities in plants grown under 16 hours light conditions than under conditions of 13 hours and 10 hours. However, terpinenes decreased with increasing day length. They opined that this effect on oil composition appeared as a reflection of the growth and development stage of the plants under the different photoperiods. Lower (older) leaves contained an essential oil with a relatively higher terpinene concentration. The essential oil composition of the inflorescences was found to be different from that of the leaf oils. The inflorescences were richer in sabinene and linalyl acetate, but poorer in *cis*-sabinene hydrate and  $\alpha$ -terpinene. As *cis*-sabinene hydrate is considered to be the key component for the typical marjoram flavour and fragrance (Fischer *et al.*, 1987), the photoperiod influences the quality of marjoram to some extent. This explains partly why marjoram grown in northern environments is sensorially preferred.

Studies by Raghavan *et al.* (1997) of hydrosteam distillation analysis revealed that fresh marjoram volatiles contained 95–97 % monoterpenes and their derivatives and 3–5 % sesquiterpenes. They observed that *cis*-sabinene hydrate, *trans*-sabinene hydrate and terpinen-4-ol were the major components responsible for the characteristic flavour of the herb. Indian marjoram contained more *cis*-sabinene hydrate (23.6 %) than any other sample referred to in the literature and this compound was retained in the convection and microwave (175 W) dried samples to a great extent. Rupasova *et al.* (1998) observed changes in biochemical composition of marjoram upon introduction in Belarus. Harvesting at the full flowering stage gave the highest yield of active compounds compared to the vegetative or budding phase.



### 19.3.3 Extraction

The steam-distillable essential oil gives the subtle and delicate flavour of marjoram (Raghavan *et al.*, 1997). Fischer *et al.* (1987) opined that carefully controlled extraction of flavour using supercritical CO<sub>2</sub> could maximize the content of *cis*-sabinene hydrate and minimize that of terpinen-4-ol. The formation of rearranged monoterpenes in essential oil of marjoram was due to two activated forms, *viz.* Z-sabinene hydrate pyrophosphate, Z-sabinene hydrate acetate along with Z-sabinene hydrate (Fischer *et al.*, 1988). Ikeda *et al.* (1962) used retention time data to characterize the hydrocarbons (36.4 % of the oil) found in sweet marjoram.

Jimenez-Carmona *et al.* (1999) made comparisons of marjoram oil by continuous subcritical water extraction (CSWE) of ground marjoram leaves (0.4 g) by subjecting them to dynamic extraction with water at 50 bar, 150°C and 2 ml/min. for 15 min and hydrodistillation of 140 g of marjoram leaves with 1000 ml of water for 3 hours. It was found that when CSWE was used, the compounds were removed from the aqueous extract by a single extraction with 5 ml of hexane, detected by gas chromatography–flame ionization detection (GC–FID) and identified by mass spectrometry, electronic impact. The CSWE method was quicker (15 min vs 3 hours), provided a more valuable essential oil (with higher amounts of oxygenated compounds and no significant presence of terpenes) and allowed substantial savings on cost, in terms of both energy and plant material. The efficiency, in terms of volume of essential oil/l g of plant material, of CSWE is 5.1 times higher than that provided by hydrodistillation.

### 19.3.4 Storage of the essential oil

Misharina *et al.* (2003) studied the changes in the composition of the essential oil of marjoram during storage. The organoleptic properties of the oil stored in the light for more than 3 months changed markedly and the oil lost the peculiar characteristics of marjoram and was unsuitable for use. However, marjoram essential oil was relatively stable during storage in the absence of light and air for 1 year.

## 19.4 Main uses of marjoram

Marjoram has pleasantly aromatic and distinctly mint-sweet flavour with slightly bitter undertones (Anon., 1989). This subtle aroma makes it an ideal addition to many herb mixtures as it helps give ‘body’ and depth to a variety of dishes. Marjoram is used in many foods where a well-rounded herb note is desired and, according to Kybal and Kaplicka (1990), it is more often used in western rather than eastern cooking. It is used in the whole and ground form and to a limited extent as an essential oil or oleoresin. Nowadays, marjoram is added to soups, salad dressings, sauces for stewed meats (mainly mutton) and stuffings. Hirasa and Takemasa (1998) mentioned use of marjoram in a ground herb blend which goes well with poultry flavoured foods. It is used in Italian herb blends and is often a component of pizza and spaghetti sauce mixes. Italian sauces often contain marjoram with other spices such as onion, oregano, basil, fennel, black pepper, red pepper if heat is desired, and possibly thyme. Greek cuisine uses oregano and marjoram frequently. Its widest use, however, is in seasoning sausages and salamis.

Marjoram has a delicate perfume, which can be lost easily while cooking. Hence, some consider it is at its best when added shortly before the end of cooking or used in dishes which are cooked very little, such as an omelette. It may also be used raw, and it is particularly delicious when finely chopped and with lemon juice. Pruthi (1976) reported that fresh leaves are employed as garnish and incorporated in salads. The dried leaves and floral tops are also superb for seasoning all meats, poultry, sea food and baked or grilled fish, egg and tomato dishes, soups such as chicken, mutton and turtle, green vegetables, stews and fruit salads, in flavouring vinegar and in formulation of liqueurs and vermouths. Sometimes it is used together with other fresh herbs in 'bouquet garni'. It has been used as a substitute for oregano when prices for that spice go up. Marjoram can also be added to practically any dish in which one would use thyme (Stobart, 1970).

Kybal and Kaplicka (1990) mentioned that marjoram was used in brewing beer before hops were known, and in France for making a wine called 'hippocras'. It was also added to water used to rinse the fingers at the table during banquets. Dried flowering tops are used for sachets and potpourri. Seed of sweet marjoram finds use in meat products and confectioneries, and French confitures make use of both the seeds and the oil. According to Chiej (1984), sweet marjoram oil is used for flavouring fats, oils, baked foods, coconut foods, meat products, processed vegetables, condiment relishes, soups, vinegars, snack food and gravies. It is also employed in perfumery to introduce a fresh slightly medicinal–aromatic warm note and in medicinal formulations.

Biacs and Wissgott (1997) noticed that addition of 0.2% (w/w) of marjoram and rosemary could reduce the pigment degradation in tomato products during storage. Dried marjoram gave one of the most efficient protections against light damage. During frozen storage, marjoram also gave protection against oxidation. The fresh marjoram protected the carotenoid pigment to a lesser extent than dried marjoram, which could be due to the high dry matter content of dried and the high water content of fresh marjoram. After 9 weeks of storage the total carotenoid content loss was least in the case of fresh and dried rosemary and dried marjoram (20–28%).

Shaaya *et al.* (1991) analysed the fumigant toxicity of 28 essential oils extracted from various spice and herb plants, and some of their major constituents were assessed against four major stored-product insects. The results showed that the compound linalool,  $\alpha$ -terpineol and carvacrol and the essential oils of oregano, basil, Syrian marjoram and thyme were most active against *Oryzaephilus surinamensis*.

#### 19.4.1 Other uses

Studies were carried out by Kraus (1990, 1992), Quedzuweit and Koeniger (1995) and Long *et al.* (1997) on the control of *Varroa jacobsoni* infestation in bees. Though Quedzuweit could not get any successful control under a simulated field condition, the other workers observed control of varroasis of honey bees (*Apis mellifera*) using formic acid and marjoram oil. Chiej (1984) reported the use of sweet marjoram for disinfecting beehives. Osmani *et al.* (1978) studied the effect of marjoram oil on metamorphosis of *Aedes aegypti* and found that, although oil of marjoram is a poor inhibitor of growth at early larval stages, the fourth stage larvae, when treated with 60 or 80 ppm, produced either malformed pupae or malformed adults which were

found to be dead within the pupal cases. The oil partially arrested the normal development of mosquito and is a good larvicide. El-Maksoud *et al.* (1999) observed that the highest weight gain of fingerlings of Nile tilapia (*Oreochromis niloticus*) was obtained when they were fed with 3 % marjoram leaves of the total diet. This also resulted in the best protein and energy utilizations apart from having a significant effect on body composition. Marjoram oil is also employed to a small extent in high-grade flavour preparations and perfumes and in the soap and liquor industries (Pruthi, 1976).

## 19.5 Functional properties

Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, notably antibacterial and antifungal as well as antioxidant properties (Tiziana and Dorman, 1998). With the increasing interest in the use of essential oil in both the food and the pharmaceutical industries, a systematic evaluation of plant extracts for these properties has become increasingly important. Bacterial and fungal infections pose greater threats to health and hence the use of natural antimicrobial compounds is important in the control of human and plant diseases of microbial origin.

### 19.5.1 Antimicrobial properties

Yadava and Saini (1991a) studied the antimicrobial effect of marjoram and found that fungi which were inhibited are *Aspergillus fumigatus* and *A. niger*. Studies by Ueda *et al.* (1982) revealed that the minimum inhibitory concentration (MIC) required for fungi such as *Saccharomyces cerevisiae*, *C. paracrusei*, *Candida krusei* and *A. oryzae* was <4.0 %. Tiziana and Dorman (1998) reported antifungal activity of marjoram oil against the common spoilage fungus *A. niger* (strain IMI 17454) even at concentration of 1 µl/ml broth. According to Daferea *et al.* (2000), marjoram essential oil totally inhibited the mycelia growth of *Penicillium digitatum* fungus at the concentration of 400 µl/ml (although in this study the essential oil belonged to the thymol/carvacrol chemotype). Daferera *et al.* (2003) also found that the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis* was completely inhibited by oregano essential oil at relatively low concentrations of 85–300 µg/ml.

Some studies, however, have shown resistance to the antifungal effects of marjoram, or that other herbs and spices were more effective. Hegazi and El-Kot (2010) evaluated the efficacy of essential oils as biocontrol agents against powdery mildew on *Zinnia elegans* L. The treatment of oil of marjoram used either at 25 or 50 % was found to be the least effective treatment during both the seasons compared to ginger, cinnamon and clove oils. De Oliveira *et al.* (2009) reported that five of the fungi (*Candida krusei* LM-09, *Trichophyton rubrum* ATCC28184, *Microsporum canis* LM-36, *Cladosporium herbarium* ATCC 26362 and *A. fumigatus* IPP-121) showed resistance to marjoram essential oil.

The solvent used to extract the essential oil may also be significant. Vagi *et al.* (2005a) observed that the supercritical fluid extracts (SFE) totally inhibited the

growth of filamentous fungi at the concentrations of 4–5 mg/ml, while ethanolic extracts, even at the concentration of 25 mg/ml, did not (or only slightly) inhibited the tested strains.

Huhtanen (1980) observed that the MIC of hexane extract of marjoram needed for control of bacteria such as *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, *Bacillus cereus* and *Campylobacter* was 10 % while Ueda *et al.* (1982) reported that only a lower concentration of 4 % was necessary for control of bacteria such as *B. subtilis*, *S. aureus* 209P, *E. coli*, *S. typhimurium*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *P. morganii* at pH levels of 5 or 7. *B. subtilis* and *S. aureus* were the most vulnerable, and at pH 5.0 the MIC for these two bacteria were 0.5 and 0.2 %, respectively. The antibacterial effect of marjoram oil was reported by many other workers (Galli *et al.*, 1985; Deans and Ritchie, 1987; Deans and Svoboda, 1990; Yadav and Saini, 1991a). Tiziana and Dorman (1998) noticed that marjoram oil was most active in inhibiting the growth of *Acinobacter calcoaceticus*, *Beuoeckea natriegens* and *S. aureus*. However, Deans and Svoboda (1990) noticed marjoram oil to be least effective against *S. aureus*, which is a food poisoning bacterium. More recently, Sipailiene *et al.* (2006) found from another study that marjoram oil contained only small amount of phenolic compounds and possessed weaker antimicrobial activity as compared to thyme. Marjoram oil at low concentration (3 %) showed antibacterial activity only against *S. typhimurium*, *E. coli* and *Enterobacter aerogenes*, while *S. aureus*, *Listeria monocytogenes* and *Enterococcus faecalis* were resistant against all concentrations of marjoram oil applied. However, all the microorganisms tested by Busatta *et al.* (2008) (six gram-negative and four gram-positive bacteria) were susceptible to the action of marjoram essential oil, with a range of MIC values from 0.069 mg/mL (*B. subtilis*) to 2.3 mg/mL (*E. faecalis*, *Serratia* sp. and *S. aureus*). The results of their study indicated the technical viability of using the marjoram essential oil to prolong the shelf-life of fresh sausage, as a relatively low concentration of oil was sufficient to reduce the number of natural occurring heterotrophic bacteria as well as *E. coli*. Other authors who have conducted studies on the use of marjoram as a bactericide include Ezzedine *et al.* (2001), Mejlholm and Dalgaard (2002), Daferera *et al.* (2003), Meza *et al.* (2007) and Klaus *et al.* (2008).

### 19.5.2 Insecticidal, miticidal and nematocidal properties

Jang *et al.* (2005) found that the insecticidal properties of marjoram oil and pulegone, ( $\pm$ )-camphor and verbenone against *Blattella germanica* L. females (German cockroach) were comparable to that of premethrin (0.05 mg/cm<sup>2</sup>), but more pronounced than that of propoxur (0.18 mg/cm<sup>2</sup>). Pavela (2005) tested 34 essential oils against larvae of *Spodoptera littoralis* and found that LC<sub>50</sub> value for marjoram was 19.6 ml/m<sup>3</sup>. The direct toxicity of marjoram essential oil to adult females of the predacious mites *Amblyseius zaheri* Yousef and El-Borolossy, *Amblyseius barkeri* (Hughes) and *Typhlodromus athiasae* Porath and Swirski was tested by Momen and Amer (2000). They noticed that sweet marjoram oil was relatively toxic to *T. athiasae* and slightly toxic to *A. barkeri*. The deterrent and toxic effect of essential oils of marjoram and rosemary on two tetranychid mites (*Tetranychus urticae* Koch and *Eutetranychus orientalis* Klein) was studied by Amer *et al.* (2003) and it was observed that both the materials used were more potent for *E. orientalis* than against *T. urticae*.

with a significant increase in repellency. Douda *et al.* (2010) studied the use of plant essences as alternative for root-knot nematode management in carrot and their results showed a negative correlation between gall numbers of the carrots and treatment concentrations, especially for clove and marjoram.

### 19.5.3 Antioxidant properties

The discovery of inhibition of lipid oxidation by some phenolic compounds during the late 1940s has contributed to the application of synthetic antioxidants in the food industry (Dapkevicius *et al.*, 1997). Widely used artificial antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Chan, 1987) are very effective in their role. However, their use in food products has been declining due to their instability, strict legislation on the use of synthetic food additives, a suspected action as promoters of carcinogenesis and growing consumer preference for 'clean label' foods (Namiki, 1990; Pokorny, 1991; Duh and Yen, 1997; Zheng and Wang, 2001; Ito *et al.*, 2005). The antioxidant properties of many herbs and spices are reported to be effective in retarding the process of lipid peroxidation in oils and fatty acids and have gained interest of many research groups (Shahidi *et al.*, 1992). Natural antioxidants can also protect the human body from free radicals and may be able to retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods (Arts and Hollman, 2005). For these reasons, there is a growing interest in the study of herbs and spices as potential antioxidants. Extensive studies have been conducted to assess the antioxidant capacity of marjoram (Nakatani, 2000; Zheng and Wang, 2001; Yanishlieva *et al.*, 2006; Hossain *et al.*, 2008; Misharina *et al.*, 2009).

Tsimidou and Boskou (1994) concluded that among the herbs and spices extensively studied, the plants obtained from the Lamiaceae (Labiatae) family possess a significant antioxidant activity. Lamiaceae plants in general are widely studied as natural antioxidant sources because of their high polyphenol content (Kosar *et al.*, 2003; Dorman *et al.*, 2004; Vagi *et al.*, 2005b). Zgorka and Glowniak (2001) reported that phenolic acids of marjoram were protocatechuic, *p*-hydroxybenzoic, gentisic, caffeic, vanillic, ferulic and rosmarinic acids. The high antioxidant property of marjoram was reported to be due to its high polyphenolic content in particular by various workers (Shan *et al.*, 2005; Hossain *et al.*, 2008). Vagi *et al.* (2005b) and Amarowicz *et al.* (2009) found that marjoram ethanolic extract contained considerable amounts of total phenolic compounds and had antioxidant activity and free radical scavenging capacity.

Tiziana and Dorman (1998) observed that in egg yolk assay, the antioxidant activity of marjoram was much higher than that of  $\alpha$ -tocopherol and comparable with that of BHT at all concentrations tested (100–1000 ppm). Saito *et al.* (1976) have obtained a higher antioxidant activity of marjoram at 0.02 % against lard than tocopherol. Similar reports of effectiveness of marjoram as an antioxidant are available from other workers (Dapkevicius *et al.*, 1997; Trombetta *et al.*, 1998). Biacs and Wissgott (1997) noticed that ground tomato seeds with rosemary and marjoram stabilized the carotenoid pigments by way of their antioxidizing power.

El-Alim *et al.* (1999) studied the effect of different dried spices and noticed that utilization of spices such as marjoram, wild marjoram and caraway and their

mixtures or extracts in semi-prepared meat products intended to be frozen for up to 6 months or more before consumption proved to be advantageous in regard to shelf-life of the food as well as for human health. Even though many workers noticed favourable antioxidant effect of marjoram as described above, Baardseth (1989) could not replace the antioxidant BHA in potato flakes production if stored for up to 24 months by others like TBHQ,  $\alpha$ -tocopherol, Prolong P (rosemary, thyme, marjoram mixture) or ascorbyl palmitate. Economou *et al.* (1991) found that marjoram extract was much less effective as an antioxidant than rosemary or oregano extract.

In terms of effects on human health, El-Ashmawy *et al.* (2007) studied the effects of administration of marjoram volatile oil or grape seed extract on oral administration of ethanol, simultaneously, daily for 10 weeks. Their results revealed a significant increase in lipid peroxidation and decrease in the level of glutathione in the testis, liver and brain in the ethanol-treated group. However, co-administration of the extracts resulted in minimizing the hazard effects of ethanol toxicity on male fertility, liver and brain tissues. They concluded that marjoram volatile oil and grape seed extract are useful for controlling oxidative damage.

#### 19.5.4 Control of platelet aggregation

Okazaki *et al.* (1998) investigated the inhibitory effect of methanol extracts of 20 herbal species on human platelet aggregation, a factor in conditions such as thrombosis. Allspice, basil, marjoram, tarragon and thyme strongly inhibited the platelet aggregation induced by collagen. Basil, marjoram and tarragon strongly inhibited platelet aggregation induced by adenosine diphosphate (ADP). They isolated an active compound, arbutin, from sweet marjoram as an inhibitor of platelet aggregation. Yazdanparast and Shahriyari (2008) reported that the methanol extract of marjoram ( $200 \mu\text{gml}^{-1}$ ) inhibited platelet adhesion to laminin coated wells by 40 % and decreased protein secretions by 30 %. They attributed the antiplatelet property of the methanol extract to the polyphenolic components of marjoram such as terpenoids, flavonoids, tannins, hydroquinone and phenolic glycosides.

#### 19.5.5 Effects on lipid profile

Ninfali *et al.* (2005) reported that marjoram extract improved lipid profiles by lowering plasma total cholesterol and triglycerides concentrations. Ahmed *et al.* (2009) made biochemical and histopathological studies on the water extracts of marjoram and chicory herbs and their mixtures on obese rats and found that oral administration of marjoram, chicory or their mixture water extracts at 5 and 10 % significantly lowered concentrations of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c), very low-density lipoprotein cholesterol (VLDL-c) and decreased activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes. Serum high-density lipoprotein cholesterol (HDL-c) levels were increased but not significantly by the administration of marjoram water extract at 5 or 10 %. Their study recommended that intake of water extract of a mixture of marjoram and chicory, especially at 10 %, may be useful for treating obesity accompanied by hyperlipidaemia. They attributed the hypocholesterolaemic effect of mar-



joram and chicory water extract to the presence of isoflavones in both herbs (49.21 mg/ml and 99.07 mg/ml of total isoflavones, respectively).

### 19.5.6 Carcinogenesis

Various studies have highlighted a potential role for marjoram in the prevention of cancer. In a study by Niture *et al.* (2006), aqueous and ethanolic extracts from marjoram played a role in preventing carcinogenesis and oncogenic mutations. These extracts caused alterations in methylguanine-DNA methyltransferase activity which is highly expressed in human cancers. Lin *et al.* (2002) found that marjoram exerted a low cytotoxicity on the hepatoma cell lines studied. Massih *et al.* (2010) revealed that marjoram ethanol extracts exhibit an antiproliferative and an apoptotic effect on human lymphoblastic leukaemia cell lines.

### 19.5.7 Effect on asthmatic patients

According to Leeja and Thoppil (2007), marjoram oil with its antimicrobial properties may reduce the production of white blood cells. An elevated level of eosinophils (a type of white blood cell) is an important indicator for occurrence of asthma (Zhao *et al.*, 2002). Marjoram oil may decrease the level of eosinophils and therefore improve health status of asthmatic patients. Mohamed *et al.* (2008) also suggested that administration of marjoram oil at a safe dose (two drops daily) was effective in improving the health and lung functions of adult patients with asthma. They suggested that marjoram oil may prevent the severity of inflammation through reducing the concentration of white blood cells responsible for inflammation.

### 19.5.8 Other therapeutic properties

Yamasaki (1995) studied the effect of both medicinal and edible herbs and plants of the Labaiatae family including marjoram against HIV. Although he found non-inhibitory action on HIV for 70 % ethanol extract of marjoram, aqueous extract at a concentration of 31 µg/ml showed effects of inhibition of HIV-1 on Molt 4 (MT-4) cells. Formation of giant cell was also found to be inhibited by concentration of marjoram extract at 125 µg/ml. He suggested that the mechanism of their anti-HIV activity is due mainly to their inhibitory action on cell-to-cell adsorption. Anderson *et al.* (2000) evaluated the effect of massage with essential oil on children with atopic eczema and found that aromatherapy massage is a good treatment for the control of atopic eczema. Acetylcholinesterase (AChE) inhibitors are used in the treatment of Alzheimer's disease. Chung *et al.* (2001) screened 139 herbal spices in search of AChE inhibitor from natural resources and found that ethanol extract from marjoram had the highest inhibitory effect on AChE *in vitro*. The active component was identified as ursolic acid (3 β-hydroxyurs-12-en-28-oic acid). El-Ashmawy *et al.* (2005) suggested that marjoram could be significant in ameliorating liver and kidney functions and genotoxicity induced by lead toxicity. Al-Howiriny *et al.* (2009) observed that marjoram at doses of 250 and 500 mg/kg of body weight significantly decreased the incidence of ulcers, basal gastric secretion and acid output.



### 19.5.9 Uses in traditional medicine

Herbal medicines are used by about 80 % of the world population for primary health care and particularly in the developing countries. These drugs are popular for their safety, efficacy, and cultural acceptability as well as fewer side-effects, and they ameliorate age-related diseases for which modern medicines have not been completely successful. Ghos (2000) estimated that the herbal medicine market in Europe and America is worth ten and four billion US\$ respectively.

Use of marjoram in medicinal preparations has been in vogue since many years back. Chopra *et al.* (1956) reported the use of marjoram oil in hot fomentations, for acute diarrhoea, and as an expectorant. Parry (1969) described marjoram as having the following properties: antiseptic, antispasmodic, carminative, stimulant, expectorant and nerve tonic. It functions as a cure for asthma, coughs, indigestion, rheumatism, toothache and heart conditions. According to Mabey (1988), marjoram contains tonic and astringent bitter principles, which rouse the appetite and hence it is helpful for invalids. The leaves and seeds of marjoram are considered as astringent and a remedy for colic (Dayal and Purohit, 1971). Chiej (1984) reported that the powder acts as a sternulatory (inducing sneezing) if inhaled and is, therefore, effective against head colds. Prakash (1990) mentioned the use of volatile oil as an aromatic stimulant in colic, dyspepsia, flatulence and dysmenorrhoea. Sweet marjoram appears to have a stronger effect on the nervous system than its wild cousin (Chevallier, 1996; Balz, 1999). Sweet marjoram is a good general tonic, helping to relieve anxiety, headaches and insomnia. The herb is also thought to reduce sexual desire (Mahindru, 1994). Junemann (1997) mentioned that marjoram oil relaxes and alleviates tension, both mental and physical, and is used in natural healing for many complaints accompanying or caused by tension. Guba Ron (2000) reported that essential oil of marjoram has menstrual-regulating or hormone-like effects.

## 19.6 Quality issues

### 19.6.1 Quality control and adulteration

As the vegetative parts of marjoram are used in the food and pharmaceutical industries, maintenance of quality of the produce is of utmost importance right from the harvesting stage onwards. All possible means should be taken to eliminate any chance of physical or microbial contamination.

El-Kady *et al.* (1995) highlighted the importance of quality control to prevent growth of fungi and bacteria in food products. They analysed 120 samples belonging to 24 kinds of spices collected from different places at Assuit Governorate (Egypt) for the natural occurrence of mycotoxins and reported for the first time the presence of aflatoxins (8–35 µg/kg) and sterigmatocystin (10–23 µg/kg) in different samples including marjoram. However, they could not detect the presence of ochratoxin and zearalenone.

Ravid *et al.* (1987) developed a method for detecting the adulteration of natural marjoram oil with racemic synthetics. They found that the determination of the enantiomeric composition of natural terpinen-4-ol, which is the major constituent in the essential oil of the fresh leaves and flowers from sweet marjoram, linalool

and linalyl acetate by hydrogen-nuclear magnetic resonance ( $^1\text{H-NMR}$ ) spectroscopy using a chiral lanthanide shift reagent is a fast and non-destructive method. With the help of the method developed, it should be possible to make quantitative detection of adulteration of essential oil with synthetic racemic alcohols and acetates. Tietz *et al.* (1991) standardized a flavour profile analysis method for the evaluation of  $\text{CO}_2$  extracts of marjoram. The method used for the development of a high-pressure extraction is quite suitable for the characterization of marjoram extracts as well as for the evaluation of commercial marjoram samples for any possible adulteration.

Pank *et al.* (1999) suggested that an intensive green colour was typical of high quality in marjoram. They also observed a positive correlation between taste and smell. From another study, Pank *et al.* (2000) opined that marjoram originated and processed in the northern region of the Harz Mountains and in the area of Madeburger Borde, Germany, has a high reputation in the world market due to its very high quality. They pointed out that the colour of the dried marjoram is of outstanding importance and consumers prefer a light green colour to a darker product.

### 19.6.2 Chemical and physical specifications

Marjoram should consist of the dried leaves, with or without a small portion of the flowering tops of *O. majorana* L., as specified. According to Heath (1978), the odour should be fresh and sweetly aromatic, slightly floral, penetrating but not irritating, cooling, pleasantly green, herbaceous with soft woody, fruity back tones, lingering and should not change much on airing from a smelling strip. The flavour should be warm, spicy, herbaceous but not particularly green, slightly bitter but not unpleasantly so. Farrell (1985) suggested that the leaves should possess a pleasant aromatic odour and have a warm, slightly bitter taste. The ground product should not contain more than 10 % of stems by weight; and the product must be uniformly ground to allow a minimum of 95 % by weight to pass through a US standard no. 30 sieve.

Various researchers have analysed the quality parameters of dry herb and the results are summarized in Table 19.1. Wide variations are noticed in the mineral content of Indian, French and German sweet marjoram as reported in Table 19.2. Table 19.3 outlines the typical chemical and physical specifications, including FDA

**Table 19.1** Analysis of dry herb

|              | Merory (1968) | Pruthi (1976)                  | Farrell (1985) |
|--------------|---------------|--------------------------------|----------------|
| Water        | –             | 7.0 %                          | 7.6 g          |
| Protein      | 14.5 %        | 14.31 %                        | 12.7–14.3 g    |
| Fixed oil    | 5.6 %         | 5.60 %                         | –              |
| Volatile oil | –             | 1.72 % in tops, 0.05 % in stem | –              |
| Pentosans    | 7.68 %        | 7.68 %                         | 7.68 %         |
| Fibre        | –             | 22.06 %                        | 18–22 g        |
| Ash          | –             | 9.69 %                         | 12–13 g        |
| Fat          | –             | –                              | 5.6–7.0 g      |

**Table 19.2** Mineral composition of marjoram from different sources (all values in per cent except for those given separately)

|               | Pruthi (1976) | Mahindru (1994) | Farrell (1985) | Winton and Winton (1939) |        |
|---------------|---------------|-----------------|----------------|--------------------------|--------|
|               |               |                 |                | German                   | French |
| Total ash     | 6.3–24        | –               | –              | –                        | –      |
| Sand          | 0.66–14       | –               | –              | –                        | –      |
| Sand-free ash | 5.4–14.3      | –               | –              | –                        | –      |
| Potash        | 18.3–20.2     | –               | –              | 20.18                    | 18.34  |
| Sodium        | 0.65–0.68     | 0.65            | 77 mg          | 0.68                     | 0.65   |
| Iron          | 6.1–7.3       | 67000 ppm       | 83 mg          | 7.30                     | 6.06   |
| Calcium       | 17.6–24.8     | 21.2            | 1990 mg        | 16.70                    | 24.80  |
| Silica        | 19.4–26.5     | –               | –              | –                        | –      |
| Phosphorus    | 8.9–9.1       | 9.1             | 306 mg         | 8.88                     | 9.10   |
| Magnesium     | 4.8–6.7       | 57500 ppm       | 346 mg         | 4.76                     | 6.74   |
| Manganese     | trace–1.05    | –               | –              | –                        | –      |
| Chlorine      | 1.51–2.05     | –               | –              | 2.05                     | 1.51   |

**Table 19.3** Whole marjoram: chemical and physical specifications

| Specification                                                                 | Suggested limits            |
|-------------------------------------------------------------------------------|-----------------------------|
| ASTA cleanliness specifications                                               |                             |
| Whole dead insects, by count                                                  | 3                           |
| Mammalian excreta, by mg/lb                                                   | 1                           |
| Other excreta, by mg/lb                                                       | 10.0                        |
| Mould, % by weight                                                            | 1.00                        |
| Insect defiled/infested % by weight                                           | 1.00                        |
| Extraneous, % by weight                                                       | 1.00                        |
| FDA DALs (6 subsamples)                                                       |                             |
| Insect infested and/or mouldy pieces by weight (unprocessed)                  | Av. of 5 %                  |
| Mammalian excreta per lb. identified as to source when possible (unprocessed) | Av. of 1 mg                 |
| Insect fragments and/or rodent hairs (processed)                              | Av. of 250                  |
| per 10 g subsample (processed)                                                | Av. of 2                    |
| Volatile oil                                                                  | 1.0 % min                   |
| Moisture <sup>1</sup>                                                         | 10.0 % max                  |
| Ash                                                                           | 15.0 % max                  |
| Acid insoluble ash                                                            | 5.0 % max                   |
| Federal specification<br>(EE-S-6311, 1981)                                    | <10 % of stems<br>by weight |
| Average bulk index (mg/100 g)                                                 | 660                         |

<sup>1</sup> ASTA suggested maximum moisture level.

**Table 19.4** ESA specifications of quality minima (marjoram – whole form) – ISO

|                                |    |
|--------------------------------|----|
| Ash (% w/w max)                | 10 |
| Acid insoluble ash (% w/w max) | 2  |
| Water (% w/w max)              | 12 |
| Volatile oil (% v/w min)       | 1  |

**Table 19.5** Ground marjoram: chemical and physical specifications

| Specification                             | Suggested limits               |
|-------------------------------------------|--------------------------------|
| FDA DALs (6 subsamples)                   |                                |
| Insect fragments                          | Av. of 1175 or more/10 g       |
| Rodent hairs                              | Av. of 8 or more/10 g          |
| Volatile oil                              | 0.8 %                          |
| Moisture                                  | 10.0 % max                     |
| Total ash                                 | 15.0 % max                     |
| Acid insoluble ash                        | 5.0 % max                      |
| Military specifications (EE-S-631J, 1981) |                                |
| Volatile oil (ml/100 g)                   | 0.6 min                        |
| Moisture                                  | 10.0 % max                     |
| Total ash                                 | 15.0 % max                     |
| Acid insoluble ash                        | 4.0 % max                      |
| Non-volatile ether extract                | 20–35                          |
| Granulation                               | 95 % min. through a U.S.S # 30 |
| Bulk index <sup>1</sup> (ml/100 g)        | 270                            |

<sup>1</sup> Average bulk index. Granulation will affect number.

**Table 19.6** Nutritional composition of marjoram per 100 g

| Composition             | USDA Handbook 8-2 <sup>1</sup> | ASTA <sup>2</sup> |
|-------------------------|--------------------------------|-------------------|
| Water (g)               | 7.64                           | 6.5               |
| Food energy (kcal)      | 271                            | 365               |
| Protein (g)             | 12.66                          | 12.5              |
| Fat (g)                 | 7.04                           | 6.8               |
| Carbohydrate (g)        | 60.56                          | 64.4              |
| Ash (g)                 | 12.10                          | 9.7               |
| Calcium (g)             | 1.990                          | 2.5               |
| Phosphorus (mg)         | 306                            | 230               |
| Sodium (mg)             | 77                             | 110               |
| Potassium (mg)          | 1522                           | 1400              |
| Iron (mg)               | 82.71                          | 72.7              |
| Thiamine (mg)           | 0.289                          | 0.290             |
| Riboflavin (mg)         | 0.316                          | 0.320             |
| Niacin (mg)             | 4.120                          | 4.10              |
| Ascorbic acid (mg)      | 51.43                          | 51                |
| Vitamin A activity (RE) | 807                            | 807               |

<sup>1</sup> Composition of Foods: Spices and Herbs. USDA Agricultural Handbook 8-2, January 1977.

<sup>2</sup> The Nutritional Composition of Spices. ASTA Research Committee, February 1977.

deficit action levels (DALs). Table 19.4 gives the ESA specifications of quality minima of marjoram in whole form. Table 19.5 details the chemical and physical specifications of ground marjoram and the nutritional information is given in Table 19.6. Different workers have reported the physicochemical properties of marjoram oil, which are presented in Table 19.7.

**Table 19.7** Typical physicochemical properties for the oil

| Reference                                         | Specific gravity         | Optical rotation   | Refractive index (220°) | Acid number | Ester number | Solubility                             |
|---------------------------------------------------|--------------------------|--------------------|-------------------------|-------------|--------------|----------------------------------------|
| Guenther (1974)                                   | 0.892 to 0.901 (15°/15°) | + 14°2' to +19°40' | 1.4707 to 1.4738        | 1.4 to 2.8  | 81.2         | Soluble in 1 to 2 vol. of 80 % alcohol |
| Gildemeister and Hoffmann (1961)                  | 0.894 to 0.910 (15°)     | +15°0' to +250'    | 1.470 to 1.476          | Up to 1.5   | 86.8*        | Soluble in 1 to 2 vol. of 80 % alcohol |
| EOA Book of Standards, USA                        | 0.890 to 0.906 (15°/15°) | +14° to +24°       | 1.4700 to 1.4750        | >2.5        | 41 to 78*    | Soluble in 2 vol. of 80 % alcohol      |
| Fenaroli's Hand Book of Flavor Ingredients (1985) | 0.886 to 0.902 (20°)     | +13° to +24°       | 1.4000 to 1.4760        | <1.4        | 68 to 86     | 1:1 to 1:2.2 vol. of 80 % alcohol      |

\* after acetylation.

## 19.7 References

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## Mint

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**Abstract:** The chapter discusses various aspects of mint, a popular herb with a long history of cultivation and usage. It is a perennial plant belonging to the genus *Mentha* and the family Lamiaceae. Mint oil and its main constituent l-menthol is extensively used in cuisines, foods, flavours and pharmaceuticals. Menthol crystals are obtained directly from mint oil and used in several commercial preparations. India is presently the largest producer and exporter of mint and its downstream products, with China, the USA and Brazil the other major producers. The chapter discusses mint cultivation, production, post-harvest management, marketing trends, quality parameters and commercial uses.

**Key words:** *Mentha* species, mint, essential oil, L-menthol, L-carvone.

### 20.1 Introduction

Mints (Fig. 20.1) are aromatic herbs belonging to the genus *Mentha* and the family Lamiaceae. Globally, the genus *Mentha* comprises more than 30 species distributed mainly in temperate and tropical/subtropical regions. The number of taxonomically valid species in the genus cannot be ascertained with certainty, as members freely cross amongst themselves, producing many intermediary forms. Most species are perennials with leafy runners, stolons or underground rootstocks. Almost all contain essential oils and half a dozen species are cultivated. The Indian subcontinent is one of the richest sources of the *Mentha* species, where at least ten species are known to grow. Several comprehensive reviews and books dealing with this important genus have been published (Lawrence, 2006).

#### 20.1.1 History of cultivation

Mint has a long history, possibly originating in Europe and the Mediterranean, where it was regarded as the symbol of hospitality, and proliferated from there to most other parts of the world. The name is believed to have been derived from a Greek legend of the nymph Minthe, who attracted the attention of Hades. Hades' wife, the jealous Persephone, attacked Minthe and was in the process of trampling her to death, when Hades turned her into the herb. The Romans brought mint and mint sauce to Britain; they strewed mints in feasts and banquets as a token of welcome to their guests. Mint was then taken to the USA by the pilgrims aboard



**Fig. 20.1** Mint leaves.

the Mayflower. In Asia, the Japanese had distilled peppermint oil for several centuries and the oil was used to produce menthol (Simonsen, 1947). Early descriptions and analyses of the menthol in mint were carried out in the nineteenth century (Oppenheim, 1862; Beckett and Alder Wright 1876; Moriya, 1881; Atkinson and Yoshida, 1882).

*Mentha* is an industrial crop that is widely cultivated for its essential oil, the major constituent of which is l-menthol, a monocyclic monoterpenic alcohol (Husain *et al.*, 1988). The essential oil, menthol and other chemical constituents of *Mentha* are used for a variety of purposes in the food, perfumery and pharmaceutical industries. Japan started commercial production of mint around 1870 AD. During that time, the product was called Japanese mint and Japan was the only commercial producer. After the Second World War, Brazil started producing mint commercially as it was found in the country's forests. Later on, the production of mint spread to other South American countries. The cultivation of mint also began in other countries such as China and India in around 1960. Initially, India was an importer of menthol but, after the green revolution in 1986, mint took off as an agricultural commodity.

Later developments and improvements have made the cultivation of mint more economical. There are several varieties of mints which include different species, hybrids and special selections that are grown all over the world. While the species that make up the *Mentha* genus are widely distributed and can be found in many environments, most *Mentha* species grow best in tropical/subtropical conditions and in moist soils. Due to their tendency to spread unchecked, mints are considered invasive.

### 20.1.2 Classification and species

**Kingdom**–Plantae; **Order**–Lamiales; **Family**–Lamiaceae; **Tribe**–Mentheae

The list below includes the taxa that have been recognized as species in recent works on *Mentha*; no study has recognized all of them. The classification is especially difficult because of frequent hybridizations occurring both in wild and cultivated populations (Saric-Kundalik *et al.*, 2009). As with all biological classifications of plants, this list may also swiftly become outdated (Kothari *et al.*, 1987a). Common names and synonyms are also given.

- *Mentha aquatica* L. – Water mint or Marsh mint
- *Mentha arvensis* L. – Corn mint, Wild mint, Japanese peppermint, Field mint, pudina
- *Mentha asiatica* Boriss. – Asian mint
- *Mentha australis* R. Br. – Australian mint
- *Mentha canadensis* L.
- *Mentha cervina* L. – Hart's pennyroyal
- *Mentha citrata* (Ehrh.) – Bergamot mint
- *Mentha crispata* L. – Wrinkled-leaf mint
- *Mentha dahurica* Fisch. Ex. Benth. – Dahurian thyme
- *Mentha diemenica* Spreng. – Slender mint
- *Mentha gattefossei* Maire.
- *Mentha japonica* (Miq.) Makino
- *Mentha kopetdaghensis* Boriss.
- *Mentha laxiflora* Benth. – Forest mint
- *Mentha longifolia* (L.) L.
- *Mentha sylvestris* L., Horse mint
- *Mentha pulegium* L. – Pennyroyal
- *Mentha requieni* Benth. – Corsican mint
- *Mentha sachalinensis* Briq. – Garden mint
- *Mentha satuireioides* R. Br. – Native pennyroyal
- *Mentha spicata* L. – *M. viridis*, *M. cordifolia*, Spearmint, Curly mint
- *Mentha suaveolens* Ehrh. – Apple mint, Pineapple mint (a variegated cultivar of apple mint)
- *Mentha vagans* Boriss. – Gray mint
- *Mentha grandiflora* Benth.

The most commonly available and commercially used varieties of mints are:

1. *Mentha arvensis* (Peppermint/Cornmint): Sweet, strong mint flavour. It is extensively used in food and flavourings.
2. *Mentha spicata* (Spearmint): Flavour stronger and less sweet than peppermint. It has been used to make traditional mint sauce for lamb.
3. *Mentha piperita* (Peppermint): A hybrid mint with strong mint flavour. Widely used in the flavouring industry in candies, chewing gums, toothpastes, etc.
4. *Mentha pulegium* (Pennyroyal): Strong spearmint-like flavour when crushed. Historically used in cooking and now used in aromatherapy.
5. *Mentha requieni* (Corsican mint): Fragrance similar to that of crème de menthe. It often used to flavour liqueurs along with peppermint.

### 20.1.3 Morphology of mint

Mint species are characterized by wide-spreading stolons that grow both under and above ground and have erect, square, branched stems. The leaves are oblong to lanceolate in shape, arranged in opposite, have serrated edges and are often downy.

There may be a variety of colours, including dark green, grey-green, purple-blue and occasionally pale yellow. The white or purple flowers are produced in false whorls known as verticillasters. The corolla has two lips and four sub-equal lobes, with the upper lobe usually the largest. The fruit is a small, dry capsule containing one to four seeds.

The morphology of some of the main *Mentha* species is given below:

1. ***Mentha arvensis*** (Fig. 20.2): An erect plant with a single stem, which is glabrous at the bottom with a few hairs near the top to a few branches. The leaves, which are arranged in opposite pairs are serrate, lanceolate to elliptical in shape and shiny dark green in colour, occasionally with a purple tinge. They are quite large in size at approximately 9.5 cm long and 2.5 cm wide, and have short hairs on the underside with sparse hairs above. The flowers are mauve in colour, approximately 5 mm in length and arranged in whorls in leaf axils. The plants have a minty aroma (Dutta, 1971).
2. ***Mentha piperita*** (Fig. 20.3): A herbaceous rhizomatous perennial plant growing 30–90 cm tall with smooth stems. The leaves are 4–9 cm long and 1.5–4 cm broad, dark green in colour with reddish veins, an acute apex and coarsely toothed margins. The leaves and stems are usually slightly hairy. The flowers are purple, 6–8 mm long with a four-lobed corolla about 5 mm in diameter. They are produced in verticillasters around the stem, forming thick blunt spikes. Flowering occurs generally from mid to late summer.



**Fig. 20.2** *Mentha arvensis*.



**Fig. 20.3** *Mentha piperita*.



**Fig. 20.4** *Mentha spicata*.

3. ***Mentha spicata*** (Fig. 20.4): A herbaceous rhizomatous perennial plant growing 30–100 cm long with a wide-spreading fleshy underground rhizome. Stems and foliage may be hairless or hairy. The leaves are serrate 5–9 cm long and 1.5–3 cm wide. The flowers are produced on slender spikes, each flower pink or white and 2.5–3 mm in diameter (Ghosh and Chatterjee, 1976).
4. ***Mentha longifolia*** (Fig. 20.5): Also known as wild mint or horse mint found in Africa, in tropical and temperate regions of Asia and Europe. In South Africa, three different sub-species are recognized (Codd, 1983). It is a fast-growing perennial herb that is generally 0.5–1 m high, which can reach up to 1.5 m. The leaves are lanceolate, coarsely hairy and sparsely toothed at the edges. They are 45–100 mm long and 7–20 mm wide and their colour varies from light and dark



**Fig. 20.5** *Mentha longifolia*.

green to grey. The small flowers are crowded into spikes at the tip of the stems, and vary from white to mauve throughout the winter months.

5. ***Mentha citrata***: A smooth, dark green plant dotted with yellow glands. The leaves are finely toothed and tinged with purple at the edges. There are very conspicuous lines of yellow glands on the purple calyx (Rao *et al.*, 1983).
6. ***Mentha pulegium***: An extremely resilient variety that can grow even in very poor soil. It is a groundcover plant with small leaves and mauve flowers.
7. ***Mentha requienii***: A mat-forming groundcover plant. The leaves are small, moss-like and bright green in colour. The plant grows best in light shade.

#### 20.1.4 Chemical composition of *Mentha* species

Mint oil comprises more than 200 constituents depending upon the species, cultivar, climatic conditions, geographic location of the plant and the method of extraction. Reviews of the constituents, chemistry, bioactivities and medicinal significance of mint species have been published in the past (Baslas, 1983a,b). Some of the more common constituents of mints include menthol, neo-menthol, menthyl acetate, neo-menthyl acetate, limonene, carvone, menthone, isomenthone, octan-3-ol, piperitenone, *p*-cymene, decanol, piperitenone oxide, linalool, linalyl acetate, pulegone, menthofuran, *cis*-3-hexenyl acetate and *cis*-3-hexenol. The following are chemical markers/major chemical constituents of the various *Mentha* species:

- ***Mentha arvensis***: l-Menthol (75–80 %), l-menthone (6–10 %)
- ***Mentha piperita***: l-Menthol (45–55 %), l-methyl acetate (20 %), l-menthone (10 %)
- ***Mentha spicata***: l-Carvone (60–70 %), l-limonene (15–25 %)
- ***Mentha longifolia***: l-Carvone (65–73 %), l-limonene (15–25 %)
- ***Mentha citrate***: Linalool (45–50 %), linalyl acetate (35–40 %)

Typical chemical compositions of three major *Mentha* species are listed in Table 20.1 and Fig. 20.6 illustrates the important markers/constituents of mint oils.



**Table 20.1** Typical chemical compositions of various *Mentha* species (bold indicates major constituents)

| <i>Mentha longifolia</i> <sup>1</sup><br>Anant Carvomint |              | <i>Mentha arvensis</i> <sup>1</sup><br>(Shivalik) |              | <i>Mentha piperita</i> <sup>2</sup> |             |
|----------------------------------------------------------|--------------|---------------------------------------------------|--------------|-------------------------------------|-------------|
| Compound                                                 | %            | Compound                                          | %            | Compound                            | %           |
| $\alpha$ -Pinene                                         | 0.71         | $\alpha$ -Pinene                                  | 0.25         | $\beta$ -Pinene                     | 0.1         |
| $\beta$ -Pinene                                          | 1.37         | $\beta$ -Pinene                                   | 0.29         | Limonene                            | 0.3         |
| Mycrene                                                  | 1.52         | Sabinene                                          | 0.10         | 1,8-Cineole                         | 1.3         |
| l-Limonene                                               | 22.02        | Mycrene                                           | 0.06         | <i>cis-p</i> -Cymene                | Tr          |
| <i>cis</i> -Ocimene                                      | 1.42         | Limonene                                          | 0.35         | Isopentenyl isovalerate             | 0.2         |
| 1,8-Cineole                                              | 1.5          | Cineole-1,8                                       | 0.20         |                                     |             |
| Menthone                                                 | Tr           | <i>p</i> -Cymene                                  | 0.02         | <i>cis</i> -3-Hexenol               | Tr          |
| Isomenthone                                              | Tr           | Hex-3-en-1-ol                                     | 0.15         | 3-Octanol                           | 0.2         |
| Terpinen-4-ol                                            | Tr           | Octan-3-ol                                        | 0.45         | 1-Octanol                           | Tr          |
| <i>cis</i> -Dihydrocarvone                               | 0.10         | l-Menthone                                        | 8.74         | 1-Octen-3-ol                        | Tr          |
| <i>trans</i> -Dihydrocarveol                             | 0.10         | Isomenthone                                       | 3.92         | <i>cis-p</i> -Menth-2-en-1-ol       | 0.5         |
|                                                          |              | Camphor                                           | 0.12         |                                     |             |
| <i>trans</i> -Dihydrocarvone                             | 0.16         | 3-Methylbutanoate                                 | Tr           | Menthone                            | 4.5         |
|                                                          |              |                                                   |              | Menthofuran                         | Tr          |
| l-Carvone                                                | <b>67.76</b> | Neopulegol                                        | Tr           | Isomenthone                         | 1.5         |
| Unidentified                                             | 0.15         | Neomenthyl                                        | 1.19         | Neomenthyl acetate                  | 0.5         |
| $\alpha$ -Cubebene                                       | 0.52         | acetate                                           |              |                                     |             |
| Unidentified                                             | 0.04         | Isopulegone                                       |              | <i>trans-p</i> -Menth-2-en-1-ol     | 0.2         |
| Unidentified                                             | 1.18         | Terpinene-4-ol                                    | 0.36         |                                     |             |
| $\beta$ -Caryophyllene                                   | 0.10         | Neomenthol                                        | 2.03         | Menthyl acetate                     | 9.2         |
| Germacrene-D                                             | 0.11         | Pulegone                                          | 0.16         | Isomenthyl acetate                  | 0.4         |
| $\beta$ -Caryophyllene oxide                             | 0.15         | Neomenthol                                        | 0.03         |                                     |             |
|                                                          |              | 1-Menthol                                         | <b>77.67</b> | Neomenthol                          | 4.7         |
|                                                          |              | Piperitone                                        | 0.44         | Terpinen-4-ol                       | 0.3         |
|                                                          |              |                                                   | 1.96         | $\beta$ -Caryophyllene              | 0.1         |
|                                                          |              | Carvone                                           |              | 1-Menthol                           | <b>64.0</b> |
|                                                          |              |                                                   |              | Isomenthol                          | 0.5         |
|                                                          |              |                                                   |              | Viridiorol                          | 0.1         |
|                                                          |              |                                                   |              | Spathulenol                         | 0.1         |
|                                                          |              |                                                   |              | Thymol                              | Tr          |

<sup>1</sup> Unpublished GLC/MS data generated at IIIM, Jammu.<sup>2</sup> Chemical composition of *Mentha piperita* (Shahi *et al.*, 1999).

## 20.2 Production, cultivation and harvesting

There are many different species, hybrids and special selections of mints that are grown all over the world, most of which will thrive in cool, moist locations with some shade. They can generally tolerate a wide range of conditions including direct sunlight. Mints grow quickly, extending a network of runners above and below the ground; consequently, one mint plant should provide enough mint for one household. This section provides details on the best practices for production and harvest of *Mentha* crops.



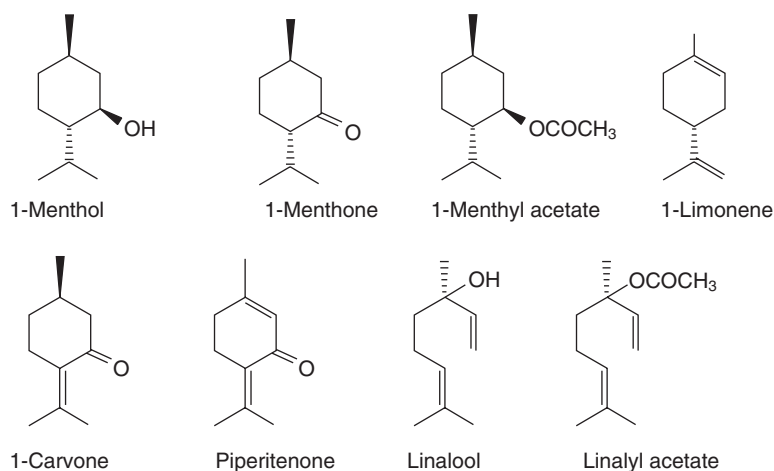


**Fig. 20.6** Important markers/constituents of mint oils.

### 20.2.1 Producing regions and production data

India is currently the world's largest consumer, producer and exporter of menthol and related products. Other major producers of mint oil are China, Japan, Brazil and the USA. In the USA, the major mint-growing regions are Eastern Oregon, Idaho and Michigan, while minor growing regions include Montana, California and South Dakota. A small amount of mint oil is also produced in Canada.

According to figures from the Multi-Commodity Exchange (MCX) of India, the total world production of *Mentha* oil in 2007–8 was nearly 32 000 tonnes, covering about 0.29 million hectares of land, with average productivity of about 110 kg/ha. The carryover stock was 3000–4000 tonnes which took the overall availability to nearly 35 000–36 000 tonnes for that specific year.

As India is the world leader in production and consumption, any change in trends in this country directly affects the international prices of mint oil and related products. Some of the mint products in high demand include *Mentha arvensis* oil, de-terpenated oil, l-menthol crystals, menthol flakes, menthol powder, neo-menthol, iso-menthol, peppermint oil, liquid-menthol, l-menthone, *cis*-3-hexenol, *cis*-3-hexenyl acetate, menthyl acetate, mint terpenes, 3-octanol, 3-octanyl acetate and l-limonene.

According to the MCX, India contributes 75–80 % of total production, with the rest contributed by China (9 %) followed by Brazil and the USA. In recent years, the global output has increased, and the output from India alone was estimated to be close to 35 000 tonnes in 2009–10. However, the oil production in 2010 fell from the expected 35 000 tonnes to 25 000 tonnes due to lower acreage and unfavourable weather conditions. In 2011, oil production was again expected to reach 31 250 tonnes, up by 20–25 % from the previous year's production. Due to rising overseas demand, exports of mint products from India have been increasing in recent years. India exports around 6000–10 000 tonnes of mint oil every year with the remainder consumed domestically. The total consumption in the domestic and international market usually stays at close to 25 000 tonnes/year. China has been the biggest importer of mint oil from India since 2007–8, accounting for about 31 % of the total

export, followed by the USA (19 %) and Germany (12 %). The other main importers are Argentina, Brazil, France, Japan, the UK, Indonesia and Belgium.

### 20.2.2 Mint cultivation in India

Mints have been popularly cultivated in India for more than 50 years. Of the commercially cultivated mint species in India, viz. *M. arvensis*, *M. piperita*, *M. spicata*, *M. longifolia* and *M. citrata*, the first is predominant, with India accounting for more than 73 % of the total global production. As it is the natural source of l-menthol, the essential oil of *M. arvensis* is used in a wide range of cosmetic and pharmaceutical preparations. Japanese mint or l-menthol mint was introduced to India by Col. Sir, R. N. Chopra of RRL, Jammu (presently renamed as IIIM) in 1953. The root suckers were introduced in Jammu and Kashmir State and from there its cultivation began to spread to other regions with suitable climatic conditions. The current areas of mint cultivation in India, predominantly in the fertile South Himalayan region are given below (Chopra *et al.*, 1958):

- **Uttar Pradesh (UP):** UP is the main *Mentha* producing state, accounting for around 90 % of total production in India. The sowing area is increasing year by year: as it is a cash crop, farmers give it preference over other crops. The main cultivating areas are currently Chandausi, Barabanki, Rampur, Badaun, Jallou and Coche.
- **Bihar:** *Mentha* cultivation was first started in Begusarai, Bihar. It is currently cultivated in Bhagalpur, Lakhi Sarai, Khagaria, Samastipur, Darbhanga and Patna. The total production of these places is around 2000–3000 tonnes.
- **Other producing areas:** Other producing areas are Jammu and Kashmir, Punjab, Haryana, Madhya Pradesh and Himachal Pradesh.

Pioneering work in the development of new varieties of *Mentha* has been carried out in India, particularly in CSIR laboratories such as CIMAP, Lucknow and IIIM (RRL), Jammu. For example, the CIMAP has developed two varieties of *M. arvensis*, ‘Himalaya’ and ‘Kosi’, which are early-maturing, disease- and pest-resistant with a high yield and a higher menthol content than conventional varieties. The ‘Himalaya’ variety was released for cultivation in 1997 and ‘Kosi’ in 1998. The Himalaya variety is resistant to rust, blight and leaf spot diseases, all of which can lower the yield of mint leaves drastically. IIIM, on the other hand, developed ‘Anant carvomint’ with a high l-carvone content; this was released for cultivation in 2007. Some of the important *Mentha* species developed and popularized in India include the following:

- *Mentha arvensis* L: MAS-I, Kalka, Gomti, Shivalik, Himalaya, Kosi, Saksham
- *Mentha piperita* L: Kukrail, Pranjali, Tushar, Pratik, Cim Indus, Cim Adurus
- *Mentha spicata* L: MSS-I, MSS-5, Arka and Neera
- *Mentha longifolia* L. var *incana*: Anant carvomint
- *Mentha citrata* (Ehrh): Kiran.

### 20.2.3 Climate

The climate plays a vital role in the successful cultivation and production of mints. Japanese mint prefers subtropical and tropical climates whereas peppermint, spear-

mint and bergamot mint are better suited to subtemperate and temperate climates. However, all the mint species are successfully cultivated in India's subtropical climate. Mints require ample sunshine and low rainfall during the harvesting period. Areas with average rainfall of 95–105 cm and an average temperature of 15–30°C are considered suitable for the cultivation (Dutta, 1971); if rainfall levels are too high, the soil may become too moist, resulting in root and tuber rot in *M. arvensis*.

#### 20.2.4 Soil

Mints require a high level of nutrients to thrive. Therefore, soils rich in organic matter with high nutrient status are most suited for its cultivation. Loam to sandy loam soils, with a pH in the range of 6.5–8.0 are ideal for the cultivation of mints. Crop rotation and intercropping is also useful for improving the quality and the biomass. Thus *Mentha* can be easily intercropped with potato, sugarcane, paddy, sweet pea, maize, mustards, lahi, arhar and so on.

#### 20.2.5 Land preparation

The land is ploughed three to four times and planked twice to make the tilth fine and weed free before the crop is planted. After levelling, it is divided into beds of suitable size with proper irrigation channels.

#### 20.2.6 Propagation

Since mints do not come true from seed, they are propagated by rhizome cuttings in early spring, by dividing in autumn or by softwood cuttings in summer. They grow by putting out creeping roots (rhizomes). Figures 20.7, 20.8 and 20.9 show suckers of three *Mentha* species. The first step in propagation is to prepare containers or an area of soil where the mint is to be replanted. Mint is a very strong-growing plant with creeping roots, and can easily kill other nearby plants. Therefore, when planting



**Fig. 20.7** *Mentha longifolia* suckers.



**Fig. 20.8** *Mentha piperata* suckers.

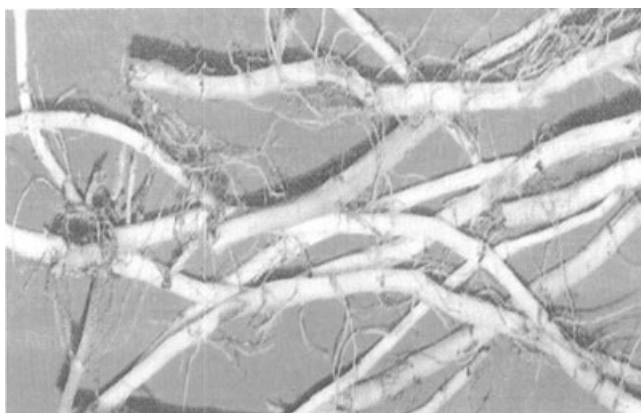


**Fig. 20.9** *Mentha arvensis* suckers.

mint outside near other plants, it is advisable to sink a bottomless container, such as an old bucket, in the ground and plant the mint in this area. Another alternative is to dig a hole to a depth of 30 cm and line it with black plastic (pierced with small holes for drainage). The soil is then replaced in the black plastic and the mint planted in that area.

Wherever the mint is planted, the soil should first be watered well and allowed to drain. Healthy roots of average thickness should be selected and cut into 1–3 inch sections that have at least one growth bud. It is advisable to cut the crown straight across and the root tip at an angle so that it is clear which end is which. The plant should then be inserted into the medium vertically with the bud towards the top. Once planted, the cutting should be kept in a bright place at 10°C or above, and watered and fertilized only when growth is observed. Mint can grow quite substantially before establishing roots, so it is important to ensure that the cutting has set roots before transplanting. In cold climates, cuttings should be hardened off by placing rooted cuttings outside in the day and in a cool greenhouse at night.



**Fig. 20.10** *Mentha* transplantation.

### 20.2.7 Planting

The last week of January–early February for Japanese mint and the second fortnight of December–early January for the rest of the species is considered the optimum period for planting; delay in planting causes significant reduction in yield (Katoch *et al.*, 1979). Two different methods are adopted for planting mints:

1. **Direct transplanting** (Fig. 20.10): For direct planting, 400–500 kg suckers/ha are required. Sucker pieces (about 5.0–7.5 cm in length) are placed in furrows, end to end and opened 45 cm apart. The furrows are covered with soil immediately after planting and the field is irrigated on the same day. Before transplanting, the suckers should be treated with a fungicide mixture for 5–10 minutes.
2. **Tissue culture raising:** In this new and productive method the seedlings are raised under *in vitro* conditions. The plants are raised from good-quality roots or apical buds under laboratory conditions. This allows the maximum numbers of plants raised through a small numbers of suckers.

### 20.2.8 Manures and fertilizers

Mints place a heavy nutrient demand on the soil. Hence, the crop requires liberal application of fertilizers for optimum growth and yield. Ten tonnes of well-decomposed FYM (farmyard manure) should be applied before the final land preparations and planting. Depending upon the soil fertility and the preceding crop, the application of 100–250 kg nitrogen, 80 kg  $P_2O_5$  and 40 kg  $K_2O$ /ha is also recommended. However, in soils of medium fertility, the application of 120–150 kg nitrogen, 60 kg  $P_2O_5$  and 60 kg  $K_2O$ /ha is optimum. The total amount of  $P_2O_5$  and  $K_2O$  is applied as basal before planting, along with one quarter of the nitrogen; the remaining nitrogen is applied before flowering, split into three equal doses (Rao *et al.*, 1983; Kothari *et al.*, 1987b,c). Nitrogen and phosphate fertilizers are sometimes applied to the leaves with a concentration between 0.1 and 0.3 %. This practice may help in reducing the cost of cultivation. The application of micronutrients such as iron increases the yield of *Mentha* by 20–55 %.



**Fig. 20.11** *Mentha* irrigation.

### 20.2.9 Irrigation

As mints are water-loving plants, the crop requires frequent irrigation for optimum growth (Fig. 20.11). Moisture stress even for a short period may affect the productivity. Normally 10–12 irrigations are required for successful crop production, applied at intervals of 12–15 days in the early growth period (February–March) and at intervals of 10 days during the peak summer months (April–June). In light-textured soil, weekly irrigations are required (Clark and Menary, 1981).

### 20.2.10 Disease, pest and weed management

Weed management is a key element of mint production. The periods between 30 and 75 days after planting and 15 and 45 days after first harvest are the most critical times. Manual weeding is the most common method of weed control in India, consequently, smallholders with family labour at their disposal are most successful in mint production. Herbicides such as oxylofen and pendimethalin 3 kg/ha in 300 l of water may also be used (Singh and Balyan, 1975; Duhan *et al.*, 1977).

### 20.2.11 Harvesting

Harvesting (Fig. 20.12) is an important operation affecting both the production and quality of the oil. It is therefore important to harvest the crop at the correct stage of plant growth. Early harvesting results in lower recovery and poor quality of oil, whereas delay in harvesting results in low production of oil due to the fall of leaves (mainly lower leaves) where the oil content is highest. Harvesting of Japanese mint should be carried out 100–110 days after planting and 65–70 days after first harvest, and a similar schedule is applicable for bergamot mint. For peppermint and spearmint, the first harvest should be taken 90–95 days after planting and the second 50–60 days later. Yellowing of the lower leaves is generally an indication that the plant should be cut and two to three cuttings are taken during the season. The harvest should take place on sunny days and irrigation should be withdrawn a week before





**Fig. 20.12** *Mentha* harvesting.

harvesting. Rain at the time of harvest or excess soil moisture in the field has adverse effect on oil accumulation in the plant, resulting in low recovery of oil on distillation. The freshly cut herb is left in the field to dry for 2–4 hours during sunny weather, and then further dried in small bundles that are hung over wires in the shade until the crop is reduced to  $\frac{1}{3}$  or  $\frac{1}{4}$  of its original weight, taking care that the leaves do not become crisp. The crop should not be heaped for drying in the sun, as this method is reported to result in a 20–25 % reduction in oil (Chandra *et al.*, 1983; Bhardwaj and Srivastava, 1984).

### 20.3 Production of mint essential oil and menthol crystals

The essential oil from mentha species, particularly from *M. arvensis*, is obtained through the steam distillation process. The oil content of the plant is at its highest at the flowering stage. Approximately 50–55 % of the frozen oil yields menthol in crystalline form. Of the remainder, 20 % (dementholized oil or DMO) used as peppermint oil and 20–25 % is used for other value-added products.

Mint oil is the main source of natural menthol crystals. The crystals may be obtained in large, medium or small sizes, with the size affecting the physical nature of the crystal. Menthol powder and flakes are also available according to the market requirements. After the mint oil has been collected from farmers/brokers, it should be filtered prior to cooling, as it usually contains some water and mucilaginous impurities which prevent ready formation of the menthol crystals. Figure 20.13 explains the processing of the crude distillate obtained after steam distillation.

The following steps are required to produce menthol crystals from mint oil (Timeis, 2006):



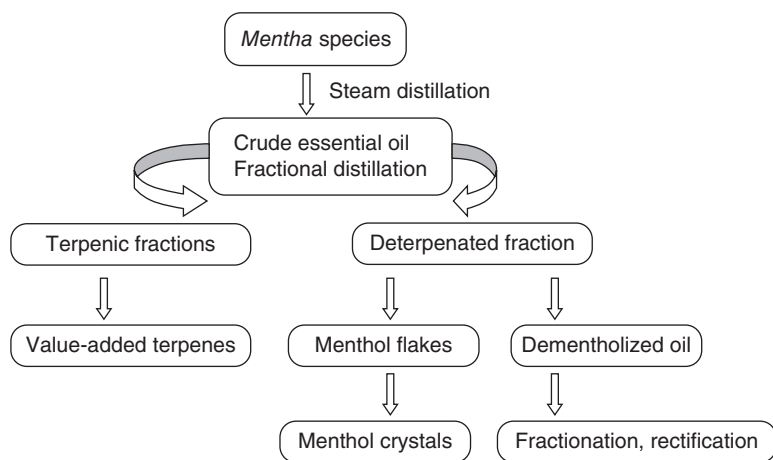


**Fig. 20.13** Flow sheet diagram of *Mentha* oil processing.

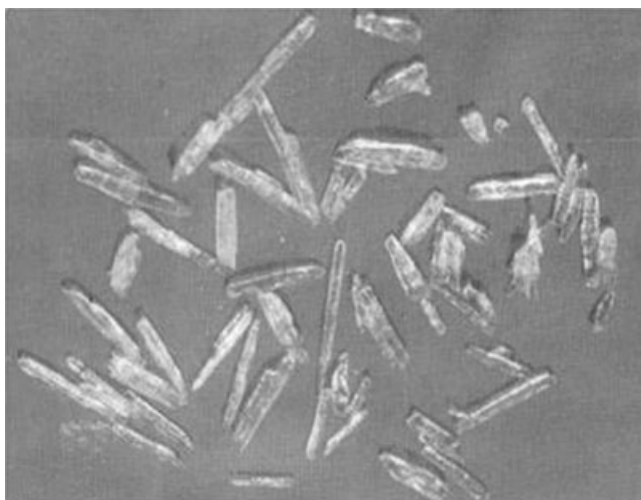


**Fig. 20.14** Bold menthol crystals.

1. Freezing of the mint oil;
2. Centrifugation/filtration to remove the oil crystals from the residual oil;
3. Drying of the crystals.

### 20.3.1 Crystallization

The purified oil is slowly and gradually cooled in order to crystallize menthol, allowing the formation of large and more regular crystals (Fig. 20.14, Alvi *et al.*, 2001). There are usually three steps involved in the freezing process: (i) cooling at 14 °C; (ii) cooling at 10 °C; and (iii) freezing at –5 °C for several hours. The whole process can sometimes take up to 48 hours and temperatures as low as –20 °C may be required. Some manufacturers use a large refrigerator compartment, while larger companies have regular freezing rooms.

### 20.3.2 Separation of menthol crystals from dementholized oil (DMO)

Menthol crystals are separated from the DMO by decanting the remaining liquid oil from the crystals and centrifuging at approximately 1200 rpm. During centrifugation, some producers also wash the crystals with small amount of water (Lawrence, 1981).

### 20.3.3 Drying of crystals

After centrifuging, the crystals are spread out on trays in large compartments or in special rooms, and dried for approximately 36 hours in a slow current of air at a temperature of about 26°C. Once dried, the crystals are ready to be packed and sold.

### 20.3.4 Quality control and standards

The essential oil-based industry is beset with problems related to the quality of the oil, the increasing use of pesticides/chemicals, resource depletion and variations in the cost of production. Since most of these products are used in drugs and pharmaceuticals and are ultimately intended for human consumption, it is imperative that good agricultural practices (GAP) including post-harvest managements are strictly followed to ensure economical and sustainable production. Important factors in GAP for *Mentha* include climate, soil, quality plant material and propagation, water management, nutritional requirements, weed management, insect, pest and disease management, harvesting and storage (Singh *et al.*, 2003).

The last of these is particularly important, since the essential oil of mint species undergoes considerable changes in its chemical composition during storage. If the oil is stored for a long period, polymerization takes place, resulting in a decrease of carvone and limonene content, thus adversely affecting the oil quality. The presence of moisture in the oil may cause rancidity. To avoid this type of loss, the essential oil should be completely dried by using anhydrous sodium sulphate or any other dehydrating agent before storage. Peppermint oil is stored in coloured bottles, air-tight aluminium or galvanized containers. Since it is acidic in nature, it should not be stored in tin containers. The containers should be air-tight and filled to the brim and preferably kept in dry, cold and dark conditions.

Essential oil should meet the following specifications:

- **Mint oil (*Mentha arvensis*):**
  - flash point: 65°C (149°F)
  - vapour pressure (50°C): 9 KpA
  - specific gravity (20°C): 0.880 to 0.910
  - optical rotation (20°C): –15° to –42°
  - refractive index (20°C): 1.458 to 1.470.
- **Carvomint (*Mentha longifolia*) oil:**
  - optical rotation (25°C): –52.36° to –53.95°
  - refractive index (25°C): 1.4858
  - specific gravity (25°C): 0.9331

**Table 20.2** Specifications of menthol crystals

| Specifications          | IP            | BP            | USP        | JP         | FCC-III       |
|-------------------------|---------------|---------------|------------|------------|---------------|
| Melting point (°C)      | 41 to 44      | 41 to 44      | 41 to 44   | 42 to 44   | 41 to 43      |
| Optical rotation (°)    | −49 to −50    | −49 to −50    | −49 to −50 | −49 to −50 | −49 to −50    |
| Non-volatile matter (%) | 0.05          | 0.05          | 0.05       | 0.05       | 0.05          |
| Menthol (NLT) (%)       | Not specified | Not specified | 97 %       | 98 %       | Not specified |

IP = Indian Pharmacopeia; BP = British Pharmacopeia; USP = US Pharmacopeia; JP = Japanese Pharmacopeia; FCC-III = Food Codex; NLT = Not less than.

The menthol crystals obtained must conform to Indian standard specification IS: 3134-1965, which states that the crystals should be colourless, acicular or prismatic, and should have a peppermint-like odour. Specifications for menthol crystals according to various pharmacopeiae are given in Table 20.2.

## 20.4 Main uses of mint

*Mentha* oil and menthol are important ingredients in a wide range of cosmetic and pharmaceutical preparations (Chadha and Gupta, 1995), mainly for their refreshing properties. The essential oil is used as flavouring component in a wide range of household and oral products. The dementholized oil is used in confectionery and peppermints.

### 20.4.1 Culinary uses

The leaf is the culinary source of mint flavour; fresh or dried leaves may be used, although fresh mint is usually preferred. The leaves have a pleasant warm, fresh, aromatic, sweet flavour with a cool aftertaste, and are used in beverages, jellies, syrups, candies, ice creams and as a flavouring for teas and yoghurts, particularly in Arab cuisine. They are used in mint sauces and jellies in British and American cuisine, and are commonly used to flavour lamb dishes.

Mint may be used to flavour alcoholic drinks, such as the mint julep and the mojito, and in the liqueur crème de menthe. Spearmint, peppermint and apple mint sprigs can be added to drinks and fruit dishes as a garnish.

The essential oil and menthol are also widely used to flavour breath fresheners, drinks, antiseptic mouth rinses, toothpastes, chewing gums, desserts, pan (betel) masalas, candies and mint chocolates.

### 20.4.2 Medicinal and cosmetic uses

Menthol and mint essential oil are used as ingredients in a variety of cosmetics, including shampoo products and perfumes, and have uses in medicine and in aromatherapy. The menthol in peppermint soothes the lining of the digestive tract and stimulates the production of bile, which is an essential digestive fluid. Mint was

originally used as a medicinal herb to treat stomach ache and chest pains. During the middle ages, powdered mint leaves were used to whiten teeth.

Mint is also commonly used to relieve itching, especially that caused by insect bites (often along with camphor). Menthol has an analgesic effect and can be combined with other products to make gels or creams for the relief of minor aches and pains, muscle cramps and sprains. Menthol is added to cigarettes to block the bitter taste of tobacco and to soothe the throat, and may also be used in the treatment of the common cold, thanks to its mild decongestant properties. It is extensively used in oral hygiene products, mouth fresheners, toothpastes, tongue sprays, chewing gums and candies.

The insecticidal activities of *Mentha* oil against various stored grain pests and vectors, including growth inhibition and repellent properties have been well studied in the past. A recent review provides a detailed description and an account of the probable mechanism of action of the insecticidal properties of the oil (Kumar *et al.*, 2011). The extracts and essential oils of *Mentha* species have also displayed moderate cytotoxic effects on various cancer cell lines used for screening (Rahimifard *et al.*, 2010).

### 20.4.3 Mint tea

Peppermint tea is often used to settle the stomach and aid digestion, and may help in the treatment of stomach ailments and digestive problems such as irritable bowel syndrome and constipation. Mint tea is also used to relieve stress and aid sleep. Peppermint in particular helps to relieve stress-related symptoms such as migraine, headaches and stomach upsets. Peppermint tea has a number of other benefits, including curing nausea, relieving menstrual cramps and other muscle spasms, soothing skin rashes, boosting alertness, and even controlling herpes simplex. It also acts as a strong diuretic (Hussain *et al.*, 1988). The antioxidant properties of water extracts of various *Mentha* species are also of interest. It was observed that the antioxidant properties were directly related to the phenolic contents of the extract, with *M. piperita* extract displaying the strongest effect (Dorman *et al.*, 2003).

### 20.4.4 Safety aspects

Peppermint oil is considered to be generally non-toxic and non-irritant in low concentrations; however, sensitization may be a problem due to the menthol content. It may cause irritation to the skin and mucous membranes and should be kept well away from the eyes. It should be avoided during pregnancy and should not be used on children. There is evidence that menthol can enhance penetration of other agents. Formulators are cautioned that this enhanced penetration can affect the use of other ingredients whose safety assessment is based on their lack of absorption. With the limitation that the concentration of pulegone in these ingredients should not exceed 1 %, it was concluded that *M. piperita* (peppermint) oil and related preparations are safe as used in cosmetic formulations (Nair, 2001).

Symptoms of peppermint oil overdose are slow or rapid breathing, abdominal pain, diarrhoea, nausea, vomiting and blood in urine, no urine production,

convulsions, depression, dizziness, twitching, unconsciousness, uncoordinated movement and flushing (Alankar, 2009).

#### 20.4.5 Uses in organic chemistry

Menthol is also commonly used in organic chemistry as a chiral auxiliary in organic synthesis. Some of the derivatives of menthol are used for the transfer of its chirality. The sulphinate esters are used in the preparation of sulphoxides. It is also used in the chiral resolution of racemic carboxylic acids via diastereomeric ester formation.

#### 20.4.6 Value-added products

In addition to l-menthol and related molecules, mint oil also contains other useful minor components which are frequently used in food and flavours. Some of these products which add value to the natural oil are commercially available in the market.

##### *Cis-3-Hexenol*

The natural *cis*-3-hexenol (Fig. 20.15) and its esters such as acetate and butyrate are by-products obtained from *M. arvensis* during fractional distillation of the essential oil. Natural *cis*-3-hexenol is a colourless to pale yellow liquid also known as 'leaf alcohol'. It is widely used in various foods, fragrances and flavourings including tobacco. It is used to impart a fresh, strong green grass odour (threshold: 70 ppb). It is used as a refreshing top note in delicate floral fragrances such as Muguet and Lilac. *Cis*-3-hexenol is widely used in 'green' flavours, for instance in mint and various fruit complexes. Its ester, *cis*-3-hexenyl acetate is also a natural product obtained during fractional distillation of rectified mint terpenic fraction and imparts a characteristic green floral fragrance.

##### *3-Octanol*

3-Octanol (Fig. 20.16) is another natural alcohol which is obtained from fractional distillation of mint oil such as *M. arvensis* and is used in food and flavours. 3-Octanol is a colourless liquid with citrus-like odour. Its natural esters, including 3-octanyl acetate and 3-octanyl butyrate are also used for imparting fresh flavour in cosmetic and perfumery products.



**Fig. 20.15** *Cis*-3-hexenol.

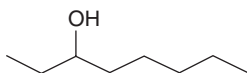


**Fig. 20.16** 3-octanol.

*L-limonene*

As a value-added product, l-limonene may be derived from fractional distillation of mint oil such as *M. arvensis* oil, although it is also an important ingredient of several other natural essential oils, e.g. orange/lemon peel oil. L-Limonene is a hydrocarbon, classified as a monocyclic terpene and is widely used in flavours and fragrances. It is used as a precursor in chemical synthesis of natural products and other useful chemicals and as a solvent in cleaning products.

*Dementholized oil*

Dementholized mint oil is derived from *M. arvensis* oil after the removal of menthol crystals. It is widely used in flavours, chewing gums, candies, pain relief balms, tooth-pastes, mouth washes, drugs, ointments, etc.

## 20.5 Improvements in quality and the impact of biotechnology

Due to their diversity in morphology and the great degree of variations in essential oil compositions, the number of species in the genus *Mentha* has been a source of confusion for a long time. Biotechnological tools such as amplified fragment length polymorphism (AFLP) analysis have been used to study the genetic variations at interspecies level to assess the genetic relationships among ten accessions of *Mentha* representing seven taxa and one interspecific hybrid (Shasani *et al.*, 2005).

Recent developments in the area of biotechnology and genetic engineering may also influence the production of mint oil; it is likely that mint will soon join other commercial plants in being produced largely from genetically modified plants. Scientists in developed countries feel that this is the only way for mint growers in those regions to compete with producers in China and India, where labour and land costs are much lower. The biochemistry, organization and regulation of essential oil metabolism in the epidermal oil glands of peppermint have been defined and most of the genes encoding enzymes of the eight-step pathway to the principal component (–)-menthol have been isolated (Bhat *et al.*, 2002). Using the tools for pathway engineering, two genes and two expression strategies have been employed to create transgenic peppermint plants with improved oil composition and yield (Mark and Rodney, 2005). Biotechnological advances could lead to the introduction of a plant which could be called ‘super mint’, yielding twice as much oil as the current commercial varieties; lines for this plant may become available for growers in the near future. Debates are also ongoing regarding the incorporation of a *Verticillium*-resistant gene into the mint. *Verticillium* fungi are extremely destructive to mint plants, almost impossible to control and can survive in soil without a host for 10 years. Plant transformation technologies have also played an important role in introducing insecticidal genes into the relevant mint crops and in improving the cultivars, as well as in the study of gene functions in plants (Bhat *et al.*, 2002).

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# 21

## Mustard

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**Abstract:** Mustard is among the oldest recorded spices dating back to around 3000 BC. Three varieties are in popular use – *Brassica alba*, *Brassica juncea* and *Brassica nigra*. This chapter looks first at the history of mustard, before going on to detail the botany of the different varieties. The chemical composition of mustard is described, including the constituents sinalbin and sinigrin, together with the nutritional value. Production and cultivation methods are discussed before the chapter proceeds to look at the main uses of mustard – culinary, therapeutic, industrial and as a fumigant and animal fodder. Finally, functional properties and quality specifications are presented.

**Key words:** mustard, *Brassica*, sinalbin, sinigrin, functional properties.

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### 21.1 Introduction

Mustard is among the oldest recorded spices as seen in Sanskrit records dating back to about 3000 BC (Mehra, 1968) and was one of the first domesticated crops. Originally it was the condiment that was known as mustard and the word was derived from the Latin *mustum*. *Must*, the expressed juice of grapes or other fruits mixed with ground mustard seeds to form *mustum ardens* (‘hot or burning *must*’) was a Roman speciality condiment. Romans’ love for mustard carried the same throughout Europe where it became popular for seasoning meat and fish. Apart from its use as a condiment, its medicinal value also was recognized early, as it was mentioned by Pythagoras in 530 BC as a remedy for scorpion bites. Mustard seeds were used for entombing their kings in Egypt. Some say that mustard was used for flavouring food to disguise the taste of degraded perishables.

Most mustard was prepared in the early days by pounding the seeds in a mortar and moistening them with vinegar. Dijon in France produced the famous mustard by using ‘*verjus*’, a unique grape juice of the Bourgogne region. The modern era of mustard, however, began in 1720 when Mrs Clements of Durham, England, found a way of milling the heart of the seed to fine flour. Other entrepreneurs experimented with combining various types of mustard seeds to create superb mustard powder.

Today there are countless mustard varieties available throughout the world, each reflecting local, regional and national cuisine.

Three types of mustard seeds are popularly used as condiments: pale yellow or white mustard (*Sinapsis alba* syn. *Brassica hirta* Moench or *Brassica alba*); brown or oriental mustard (*Brassica juncea*); and black or dark brown mustard (*Brassica nigra*). Apart from their use as a spice, mustards are widely used as green vegetables, as a salad crop, as an important oil seed crop (particularly in India where rape seed-mustard is the largest vegetable oil next to groundnut), green manure or as fodder crop and for industrial oil purposes.

### 21.1.1 Botany

Mustards are members of the Cruciferae or Brassicaceae family. The genus *Brassica* consists of 150 species of annuals or biennial herbs, several of which are cultivated as oilseed crops or as vegetables or as fodder. Seeds of *Sinapsis alba* (syn. *Brassica hirta*), *Brassica juncea* and *Brassica nigra* have only condiment value.

#### *Sinapis alba* (L.) Syn. *Brassica alba* (L.) *B. hirta* Moench

This is commonly called yellow mustard. Yellow/white mustard seeds are also known as *sufed rai* (Hindi), *moutarde blache* (French), *senape bianca* (Italian), *biji sawi* (Malaysian, Indonesian) or *mostaza blanca/mostaza silvestre* (Spanish). Yellow or white mustard is indigenous to southern Europe. Presently it is widely cultivated in Australia, China, Chile, Denmark, Italy, Japan, the UK, Netherlands, North Africa, Canada and the USA. (Farrell, 1985). *Brassica alba* is an annual herbaceous plant. Leaves are alternate, long, bristly branched, irregularly toothed, petiolate, hairy on both sides. Flowers are small, yellow with four petals, cruciform; stamens tetradynamous; pistil bicarpellate. The fruit is a bristly silique, round, ribbed, swollen at the seeds, and with a long ensiform beak, pods spreading in the raceme. Seeds are globular and yellowish. They are about 1.5–3 mm, minutely pitted, seed coat is thin, endosperm meagre and invisible to the naked eye; embryo large, yellowish, with curved hypocotyls, radicle partially surrounded by two folded cotyledons. Yellow mustard seed does not have any odour when crushed in water (Parry, 1969).

#### *Brassica juncea* (L.) Czern and Coss

Brown mustard was originally introduced from China into northern India from where it has extended to Afghanistan via Punjab (Sambamurthy and Subramanyam, 2000). It is popularly known as *rai* or Indian mustard, *moutarde de Chine* (French), *Indischer senf* (German), *senape Indiana* (Italian) and *mostaza India* (Spanish). This species originated from the hybridization of *Brassica nigra* with *Brassica campestris* and this probably happened in southwestern Asia and India where the natural distribution of the two species overlaps (Saucer, 1993). Brown mustard comprises two varieties, viz. 'Oriental', which is mostly used by Chinese, and the other darker and stronger 'brown' variety that is used by Indians. It is an annual herbaceous erect and much branched plant and is the main source for pungency among the cultivated mustards (Fig. 21.1). Flowers are small and bright yellow in colour. Seeds are small and contain 35 % oil. In the USA, prior to the 1940s, *B. juncea* was considered



**Fig. 21.1** *Brassica juncea*.

inferior to *B. nigra*. However, with the introduction of a new yellow-seeded variety of *B. juncea* from China, it became highly popular since the crop is amenable to combine harvesting.

#### *Brassica nigra* (L.) Koch

Black mustard seeds are called true mustard. They are also known as *senafich* (Amharic), *zwarte mosterd* (Dutch), *moutarde noir* (French), *rai* (Hindi), *senape near* (Italian), *biji sawi hitam* (Malaysian, Indonesian), *mostarda preta* (Portuguese), *abba* (Singalese) and *mostaza negra* (Spanish).

Black mustard is probably endemic in the southern Mediterranean region. *Brassica nigra* is of importance not only as a crop plant but it also contributed to the evolution of several species in the genus *Brassica*. It is an annual herbaceous plant which grows to a height of about 1 m. Leaves are petiolate, alternate and dark green hairy. Lower leaves are large, rough, irregularly sinuate – dentate, pinnate with terminal lobe large and small lower lobes. Upper leaves are smooth and moderately lobed. The flowers are small bright yellow, cruciform with four petals, stamens tetradynamus, pistil bicarpellate. The fruit is silique, quadrangular, smooth with a short slender beak. Seeds are small, red-brown to black in colour and minutely pitted. The seeds differ in outward appearance from those of brown mustard seeds.

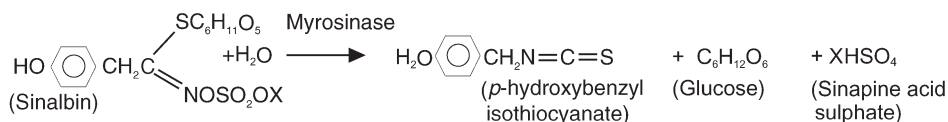
They are about 2 mm or less in size but tend to be a little more oblong than spherical, varying in colour from dark-reddish brown to black, more or less covered with white pellicle, smaller and much more pungent than the white. Black mustard is not as popular in the USA or Europe because of difficulties in harvesting (Uhl, 2000).

## 21.2 Chemical composition

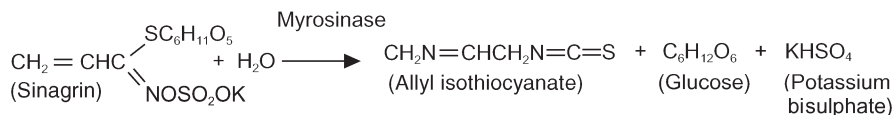
White or yellow mustard (*S. alba*) contains the glucosinolate **sinalbin** which on hydrolysis by enzyme (myrosin or glucosinolases) yields *p*-hydroxy benzyl-isothiocyanate, *p*-hydroxy benzylamine known as the 'white principles' and other similar compounds (protein, fixed oils, mucilage, etc.) as brown mustard.

The most important constituent in brown mustard is a glucosinolate, **sinigrin** (potassium myronate), and the enzyme myrosin (myrosinase), sinapic acid; sinapine (sinapic acid choline ester); fixed oils (25–37 %), consisting mainly of glycerides of erucic, eicosenoic, arachidic, nonadecanoic, behenic, oleic and palmitic acids (Leung and Foster, 1996). Sinigrin on hydrolysis by myrosin (myrosinase) yields allyl isothiocyanate, glucose and potassium bisulphate. Allyl isothiocyanate is volatile, its yield from *B. juncea* is 0.25–1.4 %. Minor volatile components that are also set free by enzymatic hydrolysis include methyl, isopropyl, *sec*-butyl, butyl, 3-butenyl, 4-pentenyl, phenyl, 3-methylthiopropyl, benzyl and  $\beta$ -phenylethyl isothiocyanates.

Black mustard (*B. nigra*) contains similar constituents as *B. juncea*, predominantly 2-propenyl (allyl) glucosinate (**sinigrin**), which on hydrolysis yields allyl isothiocyanate known colloquially as volatile oil. The difference between the mustard types is the components responsible for the reaction and the end products produced. The reactions are well illustrated by Tainter and Grenis (2001) and are shown in Fig. 21.2.



(a) Yellow mustard (*Sinapsis alba*)



(b) Brown mustard (*Brassica juncea*)

**Fig. 21.2** Chemical reaction of mustard. (a) Sinalbin in the presence of water reacts with myrosinase to form *p*-hydroxybenzyl isothiocyanate (sharp taste without pungent aroma). (b) Sinigrin in the presence of water reacts with myrosinase to form allyl isothiocyanate (pungent irritating odour). (Source: Tainter and Grenis 2001.)

**Table 21.1** Nutritional composition of mustard seed per 100 g

| Composition             | USDA Handbook 8.2" (Yellow)* | ASTA (powder)** |
|-------------------------|------------------------------|-----------------|
| Water (g)               | 6.86                         | 3.0             |
| Food energy (kcal)      | 469                          | 580             |
| Protein (g)             | 24.94                        | 32              |
| Fat (g)                 | 28.76                        | 42.6            |
| Carbohydrates (g)       | 34.94                        | 18.5            |
| Ash (g)                 | 4.51                         | 4.0             |
| Calcium (g)             | 0.521                        | 0.3             |
| Phosphorus (mg)         | 841                          | 790             |
| Potassium (mg)          | 682                          | 700             |
| Sodium (mg)             | 5                            | 10              |
| Iron (mg)               | 9.98                         | 8.3             |
| Thiamine (mg)           | 0.543                        | 0.65            |
| Riboflavin (mg)         | 0.381                        | 0.45            |
| Niacin (mg)             | 7.890                        | 8.5             |
| Ascorbic acid (mg)      |                              | 22              |
| Vitamin A activity (RF) | 6                            | 6               |

\* Composition of foods spices & herbs, USDA Agricultural Handbook 8–2, January 1977.

\*\* The nutritional composition of spices, ASTA Research Committee, February 1977.

### 21.2.1 Nutritional value

The nutritional data of the mustard seed are presented in Table 21.1. The moisture levels recommended by ASTA are a maximum of 11 %.

Brown mustard seeds, have a caloric value of 541, a little less than that of groundnut, which is 561. Mustard oil contains many fatty acids of which eruvic and lenoleic acid are very important. In *Brassica juncea* (L.) Czern and coss, oil content is usually 30–38 % but in certain types, viz. *Lahi* and *Lahta* cultivated in Uttar Pradesh of India, possess 42–43 %. The volatile oil content of *Brassica juncea* seeds is reported to 2.9 %. The characteristics of the Indian mustard volatile oil are as follows: specific gravity 0.995; refractive index 1.5185; optical rotation 0°12'; but these characteristics differs in black mustard (*B. nigra*) volatile oil; specific gravity 1.015–1.025; refractive index 1.5267–1.5291. The volatile oil is optically inactive and consists almost entirely of allyl isothiocyanate (93–99 %). The specification for pharmaceutical oil are (BPC), specific gravity 1.014–1.025;  $n_{20}$  1.525–1.530 and allyl isothiocyanate content is not less than 92 %. Mustard oil is a harmful because of its high of allyl isothiocyanate content. Fresh seeds or mustard powder do not possess essential oil and hence preparations made from these do not contain allyl isothiocyanate.

## 21.3 Production and cultivation

Around the globe, during 2002, mustard was grown over an area of 663697 ha with a total seed production of 468725 Mt (FAO database). Of the total world mustard seed sale, about 60 % accounts for seeds of *S. alba* and the rest by *B. juncea*. In the USA the present consumption of mustard is more than any other spice except pepper.

The important mustard-growing countries in the world are Canada, Nepal, the USA, Russian Federation, Myanmar, Czech Republic, Romania, Slovakia, Germany and France. But the condiment manufacturing is concentrated mainly in the USA, France, Germany, Japan, Canada and the UK. In the recent past, the area under brown mustard is increasing to the cost of other *Brassicas*, owing to its enhanced production and tolerance to biotic and abiotic stresses.

Mustard is a cool season crop, well suited to a short growing season. Mustard is grown in drier regions because of the better seed quality obtained under these conditions (Rosengarten, 1969). It prefers well-aerated soils that do not become waterlogged and are drought tolerant. Poor aeration in the root zone permanently stunts their growth. Mustard performs best in soil with a near-neutral pH, but will tolerate alkaline and slightly saline soils. Yellow mustard varieties mature in 80–85 days while brown and oriental types require about 90–95 days.

Mustard seeds are small and must be planted in moist, firm and shallow seed bed to ensure rapid germination and emergence. A seed rate of 6 kg/ha for brown and 10–12 kg/ha for yellow mustard is usually followed. Five weeks after emergence, the plants will begin to bud and after 10 days the plant will flower. Good moisture supply favours a long blossoming period and a longer flowering period ensures better yields.

Harvesting is invariably by direct combining in Europe, but it is a common practice to swath the crop in Canada to promote the drying process. Yellow mustard can be straight combined if the crop has matured uniformly. Many growers prefer to straight combine while the crop is still tough (12–13 % moisture) and artificially dry. Brown and oriental mustards are generally more susceptible to shattering and are usually swathed. Swathing should begin when 75 % of the seeds have reached their mature colour. Yield realization under commercial farming is around 1000 kg/ha for yellow and around 1500 kg/ha for brown mustard. Seeds can be stored for long periods if the moisture content is less than 10 %. While drying the mustard it is important to ensure that the seed temperature never exceeds 52 °C or damage to endogenous enzymes may result, which on processing will impair hydrolysis of the glucosinolate to the isothiocyanate, the principle imparting the 'hot' characteristic.

## 21.4 Main uses of mustard

Mustards are a versatile group of plants, which were used historically in a variety of ways. The leaves, seeds and oil are useful.

### 21.4.1 As vegetables

#### *Mustard greens*

Tender green plant as well as the green pods can be eaten as vegetables or salads. Mustard leaves, called as mustard greens, have a radishy taste. Dried/dehydrated mustard greens are available in the market as vegetables (Pruthi, 2001).



### 21.4.2 As a flavouring

#### *As whole seed*

The English enjoy brown mustard with roast beef and ham. The Japanese use the oriental brown variety as a dip for raw fish. The Barbadians and other populations in the Caribbean use yellow or brown mustard with fruits and chilli peppers for great tasting sauces, marinades and stews. In Indian cooking, especially in vegetarian meals in the south, whole brown or black mustards seeds are 'popped' in heated *ghee* or oil to bring out their nuttiness, and are then added to sauces, chutneys, pickles, curries, *sambars* and *dals*. Black mustard is sometimes used to flavour *ghee* in south India.

#### *Ground mustard or flour*

Ground mustard seeds provide flavour and consistency in Bengali fish curries. Ground mustard and mustard flour are used in seafood cocktail sauces, barbecue sauces, cheese dishes, spice cakes and cookies, devilled eggs, baked beans, ham dishes, roast pork, meat loaf, ham salad, salad dressings, chowders and bisques, Chinese dish accompaniments, and on beets, cabbage and cucumbers. Sprouts from the mustard seeds are used in salads in many Asian recipes.

#### *Compounded mustard or mustard compound*

Compounded mustard generally consists of the flavour of mixture of seeds of *B. nigra*, *B. juncea* and *S. alba* and in addition it contains turmeric powder, starch or wheat flour (not exceeding 10 %) and spices, etc.

#### *Spice blends*

A variety of blends are available in the market, which find their way into kitchens of every nationality:

- *American ballpark-style* mustard is made from the white seeds and blended with sugar and vinegar and coloured with turmeric.
- *Bordeaux mustard* is made from black seeds blended with unfermented wine. The seeds are not husked, producing a strong, aromatic, dark brown mustard often flavoured with tarragon.
- *Dijon mustard* is made from the husked black seeds blended with wine, salt and spices. This is the mustard generally used in classic French mustard sauces, salad dressings and mayonnaise.
- *English mustard* is hot, made from white seeds and is sometimes mixed with wheat flour for bulk and turmeric for colour.
- *German mustard* is usually a smooth blend of vinegar and black mustard, varying in strength.
- *Meaux mustard* is the partly crushed, partly ground black seed mixed with vinegar, producing crunchy, hot mustard that perks up bland foods.

### 21.4.3 Therapeutics and folklore

The ancient Greeks knew that mustard could be used as an antidote to scorpion and snake bites. Mustard plasters were used to stimulate blood circulation and to warm cold feet, to relax stiff muscles and to treat arthritis and rheumatism. Mustard

treats skin diseases because of its high sulphur content. Mustard also stimulates the flow of salivary and gastric juices and promotes appetite. It has been used as a laxative, as a treatment for asthma and to induce vomiting or relieve coughs.

In *Ayurveda*, the Indian medicine, as well as *Yunani*, mustard and its oil are extensively used (Krishnamurthy, 1993). Seeds are useful in itching skin diseases and the diseases of viscera and worm infection. Mustard oil is stimulative, pungent and enhances digestion. Excess use of oil causes impotency in males. It also forms an ingredient in many *Ayurvedic* medicated oils used as liniment or massage in many paralytic diseases of the nervous system.

Mustard is considered as diuretic, emetic, rubefacient and stimulant. Mustard relieves congestion by drawing the blood to the surface as in head afflictions, neuralgia and spasms. Mustard plaster is used externally for many afflictions, such as arthritis and rheumatism.

#### 21.4.4 Industrial

Mustard is used for the manufacture of blown oil, which is an oxidized and viscous oil. Animal skins contain a certain amount of fat in their cells, which is removed during tanning, thus making the leather slightly hard. In order to make the leather soft or pliable, mustard oil is incorporated in the hides. Oil cake is used as cattle feed and manure. Mustard soil is used in soap making and as a lubricant and illuminant (Leung and Foster, 1996).

#### 21.4.5 Other uses

Glucosinolates found in *Brassica* sp. are of interest due to the potential for using their degradation products as fumigants; isothiocyanates and nitriles have been demonstrated to control fungi, bacteria and nematodes (Mojtahedi *et al.*, 1991). Bio-fumigation with mustard could be integrated to provide environmentally friendly and affordable control of soil-borne pests and diseases under integrated pest management systems.

The plants are used as green fodder for cattle. Mustards are very important honey crops in the Lompoc valley of California where the mustard is grown commercially. Honeybees forage on mustard plants during the peak flowering season and produce substantial quality of mild-flavoured light-coloured honey. Mustard is agriculturally used as a cover crop. Its oil is used as cat and dog repellents.

### 21.5 Functional properties of mustard

Brown mustard seed is spherical, medium in size and has a nutty, sweeter and mellow burning flavour. The whole mustard seed has no flavour, but can provide a pungent taste after chewing (Hirasa and Takemasa, 1998). The heat experienced in yellow mustard is on the tongue, whereas in brown and black mustard the heat is also felt in the nose and eyes. The severity of pungent aroma varies with different mustards. The white or yellow type has a less pungent aroma than brown mustard seeds, which have a very pungent aroma. Black mustard seeds have the highest pungency.

In ground mustard, aroma does not persist. However, flavour and pungency are experienced when enzymatic action is triggered in the presence of water, which releases mustard's flavour or pungency. It is due to a variety of isothiocyanate compounds that exist in mustard tissue as glycosides. The major pungent compound of black and brown mustard is allyl isothiocyanate. The release of sensation, especially in brown and black mustard, is delayed and begins at the back of the mouth, with a shooting sensation to the sinuses, owing to the activation of an enzyme, myrosinase. The enzyme myrosinase, in the presence of water, breaks down the glycoside (sinalbin) in yellow mustard or sinigrin in black or brown mustard to *para*-hydroxybenzyl isothiocyanate, which is responsible for the characteristic pungent aroma. The odours last until the enzyme activities ceases.

Yellow mustard flavour and pungency, like brown or black mustard, can be fully experienced only by triggering the enzyme myrosinase action, which releases them. The most effective enzymatic trigger is in the presence of water at room temperature, although other low-acid liquids such as milk and beer also work. Acidic liquids such as wine, vinegar and lemon juice are poor triggers of mustard's overall flavours, but are good subsequent preservatives of the flavour and they extend the penetrating odour. When water, vinegar, milk, wine or beer is added to mustard, mixed and left to stand for a few minutes, different degrees of flavour sensations are produced. With water a very sharp and hot taste is produced, while with vinegar milder flavour is induced. With milk a milder spicier and pungent flavour is created. With beer a very hot flavour is brought out (Uhl, 2000).

Mustard flour has preservative and antioxidant properties in addition to providing flavour and colour. In salad dressings, the most important property of the spice is its emulsifying function, binding water and oil phases as well providing viscosity. Mustard's fixed oil, which amounts to 30–35 % by weight, is extracted by the cold pressed method. The oil is used for cooking in India and other Asian countries, including China and Japan.

Mustard oil is extracted from the black mustard seeds, which have been macerated in warm water by steam or water distillation. Crude oil is dark brown in colour and contains a large proportion of free fatty acids. The refined oil is bland and light brown in colour. The characteristic odour of mustard oil is due to sulphur-containing essential oils produced by the hydrolysis of glucosides contained in the seeds. The quality of the mustard oil depends on the contents of the fatty acids and their percentage therein. Mustard oil is hazardous because of its high content of allyl isothiocyanate.

Essential oil is obtained by steam distillation of pressed cakes obtained after extraction of mustard oil (brown seed), after it has been hydrolysed by the enzyme myrosinase to release the allyl isothiocyanate from the glucoside. The essential oil of yellow mustard is obtained by solvent extraction of the press cake because it contains little or no volatile oil. Again, the press cake must first be hydrolysed to release the end products caused by the action of enzymes. Hydrolysis in either case is brought about by maceration of the press cake with warm water.

Oleoresin of mustard seed is usually obtained from a blend of the three different types of mustard to provide a balanced flavour. It is usually a yellow to light brown oily type of liquid with a volatile oil content of 5 ml per 100 g. Two kilograms is equivalent to 45.45 kg of the mustard spice.

Of the spices, condiments and herbs studied with respect to their effect on yeast fermentation in wines, etc., mustard flour was easily the most effective. It was found stronger than the two chemical preservative tried, viz. benzoic acid and sulphur dioxide (Pruthi, 1992). Mustard and its constituent allyl isocyanate have bacteriostatic and bactericidal properties (Charalambous, 1994).

## 21.6 Quality specifications

The quality of whole mustard seed is determined by the quantity of mature, undamaged seeds. Ground mustard quality is dependent on its final use. As per federal specifications, whole mustards are the seed of *Brassica hirta* (white mustard), *Brassica juncea* or varieties, or closely related varieties of the types of *Brassica nigra* and *Brassica juncea*.

*Brassica hirta*, or yellow-seeded varieties of *Brassica hirta*, shall be small, globular, yellow, clean-looking hard seeds and shall not possess any volatile oil or 'nose heat' when crushed and mixed with water. *Brassica juncea* shall be small, globular, and yellow to brown or brown hard seeds. Seeds, when ground and mixed with water on a one to three basis, must be capable of liberating a sharp, piercing, irritating odour and a very pungent taste.

If whole or ground mustard is purchased, it is generally designated as no. 1, no. 2 or no. 3 grade. This generally denotes the number of dark seeds present and other parameters. The International Standard states that seeds, when ground, must produce an odour free of mustiness and rancidity. The seed must have no more than 0.7 % extraneous material and no more than 2 % damaged or shrivelled seed. *Brassica nigra* and *B. juncea* must yield a minimum of 1.0 % and 0.7 % allyl isothiocyanate, respectively, and *S. alba* a minimum of 2.3 % 4-hydroxybenzyl isothiocyanate.

According to the federal specification the condiment mustard is classified into three types: whole, ground and flavour. Whole mustard shell contains 5 % total ash, 1 % acid insoluble ash and 10 % moisture. Ground mustard is the powder prepared by grounding whole yellow mustard seed. The finished product of this represents the seed ground in its whole form and without the outer hulls of husk removed and without removal of fixed oils. It shall be uniformly ground to allow for a minimum of 5 % by weight to pass through a US standard no. 15 sieve. It shall contain not more than 5 % total ash, 1 % acidic insoluble ash and 6 % moisture. The mustard flavour is a white yellow powder prepared from blend of powder derived from milling the endosperm or interior portion of seed of whole mustard and ground mustard. It shall contain not more than 5 % total ash, 5 % acidic insoluble ash, 9 % crude fibre, 10 % moisture and not less than 2.5 % non-volatile ether esters. The US Department of Agriculture limits the use of ground mustard in sausages, owing to its high protein content.

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## Nutmeg and mace

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**Abstract:** Nutmeg and mace are the two major primary products of *Myristica fragrans* and are commercially considered as spices. Besides nutmeg and mace, a number of other products of *M. fragrans* are commercially important. Oleoresins, nutmeg butter and essential oils are also derived from *M. fragrans* and they find varied uses in the food, medicine and perfume industries. The properties of nutmeg, nutmeg butter, mace, their oils and oleoresins are reviewed in this chapter. The functional properties and uses of nutmeg and mace are also discussed. The International Standards applicable for trade in spices such as nutmeg and mace are also described.

**Key words:** functional properties, mace, mace oleoresin, mace oil, medicine, nutmeg, nutmeg butter, nutmeg oil, nutmeg oleoresin, quality, standards, toxicity, uses.

### 22.1 Introduction

Nutmeg and mace are two different parts of the same fruit of the nutmeg tree, *Myristica fragrans* Houtt. (Myristicaceae). The nutmeg tree is indigenous to the Banda islands in the Moluccas. The species of the genus *Myristica* are distributed from India and South-East Asia to North Australia and the Pacific Islands. Sinclair (1958) listed a total of 72 species distributed in these areas. The major nutmeg-growing areas are Indonesia and Grenada (West Indies). It is also grown on a smaller scale in Sri Lanka, India, China, Malaysia, western Sumatra, Zanzibar, Mauritius and the Solomon Islands.

Nutmeg is a conical tree reaching a height of 4–10 m. The tree is dioecious with male and female flowers occurring on different trees. The fruits are pendulous, broadly pyriform, yellow, smooth, 7–10 cm long and fleshy. They split open into two halves when ripe, showing the ovoid 2–3 cm long dark brown shining seed with a hard seed coat, surrounded by a lanciate red aril attached to the base of the seed. The seed of nutmeg is large with ruminant endosperm and is considered as the most primitive among the flowering plants (Corner, 1976).

### 22.2 Production and chemical structure

Nutmeg and mace are the two major primary products of *M. fragrans* and are commercially considered as spices. Nutmeg is the dried kernel of the seed and mace is

the dried aril surrounding the seed. Both the spices have a similar flavour. However, nutmeg is reported to be slightly sweeter than mace and is more preferred in food. Besides nutmeg and mace, a number of other products are commercially important. Oleoresins, nutmeg butter and essential oils are also derived from *M. fragrans* and they find varied uses in the food, medicine and perfume industries.

Nutmeg is produced in the tropical areas of Indonesia and the West Indies. World production of nutmeg is about 12000 tons per year with an annual world demand of 9000 tons. Production of mace is about 2000 tons. Indonesia and Grenada dominate production and export both products with a world market share of 75 % and 20 %, respectively. Other producing countries include India, Malaysia, Papua New Guinea, Sri Lanka and a few Caribbean Islands.

The East Indian islands of Siau, Sangihe, Ternate, Ambon, Banda and Papua produce highly aromatic nutmeg, traded as East Indian nutmeg. Grenada produces the West Indian nutmeg which is milder in flavour and lighter in colour. International trade in nutmeg is either of the East Indian or the West Indian nutmeg, with a negligible quantity of wild 'Bombay' nutmeg imported by the USA. The principal import markets are the European Community, the USA, Japan and India. Singapore and the Netherlands are the major re-exporters. The USA is the biggest individual market for whole nutmegs. US importers prefer the East Indian type of deep brown, aromatic nutmeg and orange – red mace in their whole form. Indonesia has traditionally been the principal supplier of nutmeg and mace to the US market, accounting for an average 65 % of total US imports of nutmeg per year in terms of volume.

### 22.2.1 Nutmeg

Fruits are harvested when they split open on ripening. The split fruits are either plucked from the tree with a hook bill or are collected soon after they drop onto the ground. Nutmeg is dried in large trays by various procedures. The unshelled nutmegs are dried in the sun until the seeds inside rattle on shaking. Normally, nutmeg dries in about a week. The seed cover is removed by breaking the hard seed coat mechanically. Nutmeg is usually packed in double-layered linen, jute, sisal or polythene bags. If other packing materials are used, care must be taken to avoid materials which might lead to 'sweating' and mould development.

Packaging should be such that the maximum weight loss is 10 %. Spices must be dried thoroughly prior to shipment. They can then be transported in conventional vessels. Powdered nutmeg is prepared by grinding at ambient temperature. Since during traditional grinding, most of the volatile oil escapes and quality deteriorates, chill conditioning and cryogenic grinding are alternative methods currently in use (McKee and Harden, 1991). The myristicin fraction of the volatile oil together with elemicin is responsible for the hallucinogenic property of the seed. Two new phenolic compounds, (–)-1-(2,6-dihydroxyphenyl)-9-[4-hydroxy-3-(*p*-menth-1-en-8-oxy)-phenyl]-1-nonanone (1) and (7*R*,8*R*)-7,8-dihydro-7-(3,4-dihydroxyphenyl)-3'-methoxy-8-methyl-1'-(*E*-propenyl)benzofuran (2), were isolated from the fruits of nutmeg (Lin *et al.*, 2009). A fluorescent naphthaquinone derivative 2-methyl-1,4,4a,8a-tetrahydro-endo-1,4-methanonaphthalene-5,8- was obtained in sun-dried nutmeg. Absence of this compound in green nutmeg suggested its formation during sun drying (Chatterjee *et al.*, 2008).



### 22.2.2 Mace

Mace is carefully detached from the nut soon after harvest, washed, flattened by hand or between boards and then sun dried until they become brittle. Hot air ovens or solar a tunnel drier can be used for drying and the colour retention is much better than sun-dried mace. Mace dried in a solar tunnel drier reduced the drying time and also maintained the ASTA cleanliness specifications. Moisture content of the dried samples ranged between 8.4 and 9.5 % (Joy *et al.*, 2001). The dried mace is then graded and packed. The fixed oil content of mace ranges from 20–35 %. Gopalakrishnan (1992) reported that the moisture content, acidity, total reducing sugars, total sugars, crude fibre, total ash and pectins were highest in the rind, whereas the concentrations of volatile oil and non-volatile substances extracted by ether were highest in the kernel and starch and protein contents were highest in the mace. The general composition of nutmeg and mace are given in Table 22.1.

### 22.2.3 Nutmeg oil and mace oil

The essential oil from nutmeg is steam distilled, usually from sub-standard nutmeg, and nutmeg oil ranges from 5–15 % of the seed weight. The essential oil is highly sensitive to light and temperature and yields a colourless, pale yellow or pale green oil with a characteristic odour of nutmeg. The oil is soluble in alcohol and insoluble in water. The essential oils of East Indian nutmeg and West Indian nutmeg differ in their flavour and odour characteristics. The East Indian nutmeg oil is considered superior to the West Indian nutmeg oil, having a better aroma and a higher amount of phenyl propanoid ethers (Masada, 1976) and terpenes (Lewis, 1984). The physicochemical properties of the two oils are reported to be different (Table 22.2). East Indian nutmeg oil is also reported to have a higher concentration of myristicin (up

**Table 22.1** Composition of nutmeg and mace (%)

| Composition                | Nutmeg | Mace  |
|----------------------------|--------|-------|
| Moisture                   | 40.00  | 40.00 |
| Volatile oil               | 11.00  | 15.30 |
| Non-volatile ether extract | 33.60  | 21.98 |
| Starch                     | 30.20  | 44.05 |
| Sugars                     |        |       |
| Glucose                    | 0.10   | 0.17  |
| Fructose                   | 0.07   | 0.10  |
| Total reducing sugars      | 0.17   | 0.27  |
| Sucrose                    | 0.72   | 0.39  |
| Total sugars               | 0.89   | 0.65  |
| Protein                    | 7.16   | 9.91  |
| Crude fibre                | 11.70  | 3.93  |
| Total ash                  | 2.57   | 1.56  |
| Ash insoluble in HCl       | 0.20   | 0.15  |
| Polyphenols                |        |       |
| Total tannins              | 2.50   | –     |
| True tannins               | 1.00   | –     |

Source: Gopalakrishnan (1992).

**Table 22.2** Specifications of British Standards Institutions for nutmeg oil

| Specification                                     | East Indian Oil                         | West Indian Oil                               |
|---------------------------------------------------|-----------------------------------------|-----------------------------------------------|
| Colour                                            | BS 2999/37:1971<br>Colourless to yellow | BS 2999/38: 1971<br>Colourless to pale yellow |
| Apparent density (mass per ml) at 20°C            | 0.885 to 0.915                          | 0.860 to 0.880                                |
| Optical rotation at 20°C                          | 8.0° to 25.0°                           | 25.0° to 45.0°                                |
| Refractive index at 20°C                          | 1.4750 to 1.4880                        | 1.4720 to 1.4760                              |
| Solubility in ethanol (90 per cent (v/v) at 20°C) | 3.0 volumes                             | 4.0 volumes                                   |

Source: Purseglove *et al.* (1981).

**Table 22.3** Composition of nutmeg oils of different geographical origins (%)

| Source component               | Grenada | St. Vincent | Malay seedlings | Papua | Indonesia | Penang | Singapore |      |
|--------------------------------|---------|-------------|-----------------|-------|-----------|--------|-----------|------|
|                                |         |             |                 |       |           |        | (1)       | (2)  |
| $\alpha$ -pinene               | 10.6    | 12.6        | 12.8            | 21.3  | 18.0      | 19.9   | 21.2      | 19.2 |
| Camphene                       | 0.2     | 0.2         | 0.2             | 0.3   | 0.3       | 0.3    | 0.3       | 0.4  |
| $\beta$ -pinene                | 7.8     | 12.1        | 9.3             | 14.3  | 9.7       | 17.7   | 12.7      | 11.0 |
| Sabinene                       | 50.7    | 49.6        | 44.1            | 30.0  | 27.0      | 36.3   | 17.8      | 15.4 |
| Myrcene                        | 2.5     | 2.8         | 2.9             | 2.4   | 2.2       | 2.5    | 2.6       | 2.3  |
| $\alpha$ -phellandrene         | 0.4     | 0.6         | 0.6             | 0.5   | 0.5       | 0.4    | 1.0       | 0.7  |
| $\alpha$ -terpinene            | 0.8     | 1.9         | 1.8             | 1.1   | 2.0       | 0.8    | 4.0       | 2.5  |
| Limonene                       | 3.1     | 3.3         | 3.1             | 2.7   | 2.7       | 2.8    | 3.6       | 3.4  |
| 1,8-cineole                    | 2.5     | 2.3         | 2.1             | 1.9   | 1.8       | 1.5    | 3.2       | 2.7  |
| $\gamma$ -terpinene            | 1.9     | 3.1         | 2.8             | 1.9   | 3.3       | 1.3    | 6.8       | 4.1  |
| p-cymene                       | 3.2     | 0.7         | 0.8             | 0.5   | 0.7       | 0.3    | 1.8       | 2.7  |
| Terpinolene                    | 1.7     | 1.2         | 1.2             | 1.1   | 1.1       | 0.6    | 2.1       | 2.6  |
| <i>trans</i> -sabinene hydrate | 0.8     | 0.3         | 0.5             | 0.1   | 0.6       | 0.2    | 0.3       | 0.5  |
| Copaene                        | 0.3     | *           | *               | 0.2   | 0.3       | *      | 0.2       | 0.2  |
| Linalool                       | 0.9     | 0.4         | 0.2             | 1.0   | 0.3       | 0.2    | 0.8       | 0.9  |
| <i>cis</i> -sabinene hydrate   | 0.7     | 0.2         | 0.4             | 0.2   | 0.6       | 0.2    | 0.2       | 0.4  |
| <i>cis</i> -p-menth-2-en-ol    | 0.4     | 0.1         | 0.1             | 0.3   | 0.5       | 0.1    | 0.3       | 0.3  |
| Terpinen-4-ol                  | 6.1     | 3.5         | 6.0             | 3.9   | 7.3       | 2.0    | 9.3       | 10.9 |
| <i>cis</i> -piperitol          | 0.5     | 0.4         | 0.4             | 0.6   | 0.4       | 0.3    | 0.5       | 0.3  |
| Safrole                        | 0.2     | 0.1         | 0.8             | 1.5   | 2.1       | 0.6    | 1.9       | 3.2  |
| Methyl eugenol                 | 0.2     | 0.1         | 0.5             | 0.2   | 1.2       | 0.6    | 0.6       | *    |
| Eugenol                        | 0.2     | *           | 0.3             | 0.1   | 0.7       | 0.3    | *         | *    |
| Elemicin                       | 1.4     | 1.3         | 1.7             | 0.4   | 0.5       | 4.6    | 0.3       | 0.3  |
| Myristicin                     | 0.5     | 0.8         | 4.1             | 10.4  | 13.5      | 3.3    | 6.3       | 12.4 |

\* Traces detected.

Source: Lewis (1984).

to 13.5 %) compared to West Indian nutmeg oil (less than 1 %) (Table 22.3). Mace oil is obtained by steam distillation of dried aril and yields 4–17 % oil. It is a clear red or amber dark red liquid with characteristic odour and flavour. Mace oil is more expensive than nutmeg oil. Leaves also yield oil (0.34–0.65 %), chemically similar to nutmeg oil, but its flavour and odour are inferior to both mace and nutmeg oil.

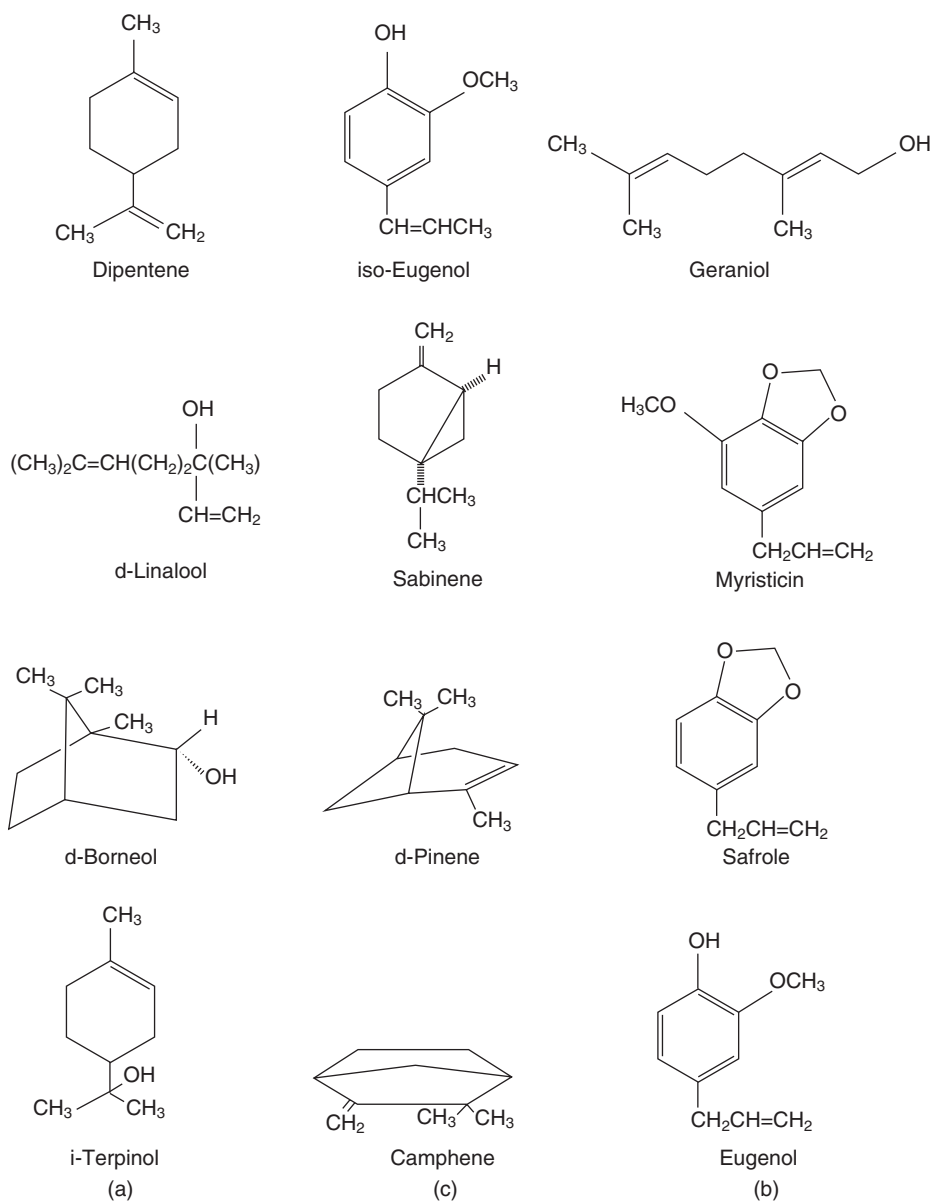
Extraction of essential oil can be carried out by different methods. However, mace oil extracted using liquid and dense CO<sub>2</sub> was superior in quality and flavour compared with the steam-distilled oil (Naik *et al.*, 1988). Essential oil contains several compounds, most of which are invaluable to industries. Because of its aroma, the essential oil is used as a natural flavouring extract in the cosmetics industry. In addition to its use in the cosmetics industry, nutmeg is also used in the pharmaceutical industry. The pharmacological properties of nutmeg are attributed to the compounds found in the essential oil.

The first reports on nutmeg constituents were by Frederick Power and Henry Salway (Power and Salway, 1907, 1908). Numerous compounds have been isolated from nutmeg and mace. The yield and quality of the oil depends on the geographical location (Table 22.3), grades and the distillation process involved. The major constituents of both nutmeg and mace oil are monoterpene hydrocarbons, together with smaller amounts of oxygenated monoterpenes and aromatic ethers (Purseglove *et al.*, 1981).

Major constituents of the monoterpene hydrocarbons are pinene and sabinene and the major aromatic ether constituent is myristicin. Aromatic ethers, myristicin, safrole and elemicin determine the flavour and medicinal properties to a great extent. Gas chromatography (GC) analysis of the oils of nutmeg and mace showed 33 constituents in nutmeg oil and 51 in mace oil. The two oils are qualitatively similar in composition, differing only in their quantity. Nutmeg oil consists of 76.8 % monoterpenes, 12.1 % oxygenated monoterpenes and 9.8 % phenyl propanoid ether whereas mace oil contains 51.2 % monoterpenes, 30.3 % oxygenated monoterpenes and 18.8 % phenyl propanoid ethers (Mallavarapu and Ramesh, 1998). The composition varies with the geographical location (Baldry *et al.*, 1976; Masada, 1976; Lawrence, 1981; Kumar *et al.*, 1985; Gopalakrishnan, 1992). The Indonesian nutmeg contained 2 % myristicin compared with 0.13 % in *M. argentea*. No myristicin was reported in *M. muelleri*. The safrole content, a suspected carcinogen, was 0.13, 0.51 and 0.245 % in *M. fragrans*, *M. argentea* and *M. muelleri*, respectively (Archer, 1988). The myristicin fraction, together with the elemicin, is responsible for the hallucinogenic properties of nutmeg seed. The composition of essential oil changes on prolonged storage. The structures of some of the compounds are given in Fig. 22.1. During storage and transportation, oils should be protected from light and stored in tightly-packed containers at a temperature not exceeding 25°C. Prolonged storage deteriorates the composition of the oil.

#### 22.2.4 Nutmeg oleoresin

Nutmeg oleoresin is obtained by solvent extraction of spices. Oleoresins contain saturated volatile oil, fatty oil and other extractives soluble in the particular solvent employed. Nutmeg oleoresins, obtained by solvent extraction from the dried spice of nutmeg, are used in colouring and flavouring in the food industry. The spice oleoresin can be used in place of the dried spice. The commercial products exhibit a range in their essential oil and fixed oil content depending on the method of extraction and solvents employed. Nutmeg extracted with benzene yields 31–37 % of oleoresins and that extracted with cold ethanol yields 18–26 %. A higher fatty oil is obtained by hydrocarbon solvents while polar solvents like alcohol and acetone

**Fig. 22.1** Chemical structure.

yield low fatty oils and resins. Commercial mace oleoresins are available with volatile oil content ranging from 10–55 %. When extracted with petroleum ether, it yields 27–32 % and contains 8.5–22 % volatile oils and, after chilling, the yield reduces to 10–13 % (Naves, 1974). Oleoresins extracted with non-polar solvents are preferred in flavouring processed foods since they are more stable to heat, whereas the perfume industry prefers polar solvents since they are soluble in most perfume materials and do not deposit any fatty materials in the bottles or containers (Purseglove *et al.*, 1981).

### 22.2.5 Nutmeg butter

The fixed oil of nutmeg is known as nutmeg butter. Nutmeg butter contains 25–40 % fixed oil, which is obtained by expressing the crushed nuts or by extracting with solvents. Fixed oil is a semi-solid or reddish brown fat with both the aroma and taste of nutmeg. It is completely soluble in hot alcohol and sparingly soluble in cold alcohol. The fixed oil is freely soluble in ether or chloroform and is composed of trimyristin (84 %), unsaponifiable constituents (9.8 %), oleic acid (3.5 %), resinous materials (2.3 %), linolenic acid (0.6 %) and formic, acetate and cerotic acids in traces. Trimyristin is a triglyceride of myristic acid. Reports say that the best nutmeg butter is imported from the East Indies. The fixed oils are also used in perfumes and in medicines for external application for sprains and rheumatism.

## 22.3 Main uses of nutmeg and mace

### 22.3.1 Uses in food

Nutmeg, mace, their oleoresins and essential oils are used in the food and beverage industries. Although whole nutmeg is available, ground nutmeg is more popular. The spice in the ground form is mainly used in the food processing industry. In South-East Asia, China and India, both the spices are used sparingly. Nutmeg is a standard seasoning in many Dutch dishes. Nutmeg and its oleoresin are used in the preparation of meat products, soups, sauces, baked foods, confectioneries, puddings, seasoning of meat and vegetables and to flavour milk dishes and punches. The fleshy outer cover of the fruit (pericarp) is utilized to produce nutmeg rind preserve, candy, pickle, chutney, jellies and powder. Joshi *et al.* (1996) studied the quality of these products prepared from the pericarp and found they were organoleptically acceptable even after three months of storage at ambient temperature. The preserve recorded the highest organoleptic score (8.06) and the powder recorded the lowest score (6.85).

Mace is sold either as whole or as ground spice and is used in savoury dishes. Mace is used to flavour milk-based sauces and processed meats like sausages. Soups, pickles, ketchup and chutneys are also seasoned with mace. Because of its aroma, the essential oil is used as a natural flavouring extract and is employed for flavouring food products and liquors. Nutmeg oil and mace oil are used mainly in flavouring soft drinks, canned foods and meat products.

### 22.3.2 Uses in traditional medicine

Both nutmeg and mace are now used in the pharmaceutical industry. They are more commonly used in oriental than in western medicine. though. Nutmeg, for example, has long been used in traditional Ayurvedic, Chinese and Thai medicine, as an aphrodisiac, antipyretic, abortifacient, anti-inflammatory and stomachic. It is also known for its deodorant properties and is useful for flatulence, nausea and vomiting. An ethnobotanical survey in the Maluku Province of Indonesia, and in Java and Bali, showed that nutmeg is used in the treatment of diarrhoea, mouth sores and insomnia. (Gils van and Cox 1994). Powdered nutmeg is rarely administered alone, but it enters into the composition of numerous medicines as aromatic adjuncts. The use

of essential oils through aromatherapy is gaining in importance. The main constituents of nutmeg and mace, myristicin, elemicin and isoelemicin, when presented in aroma form, act as stress relievers. In Japan, many companies diffuse such aromas through air ventilation systems to improve the work environment as well as the quality of air. Nutmeg butter is a mild external stimulant used in the form of ointments, hair lotions and plasters, against rheumatism, paralysis and sprains.

Mace is traditionally used to treat stomach problems, fever, asthma and bowel complaints. Oil of nutmeg is useful in the treatment of inflammation of the bladder and urinary tract, halitosis, dyspepsia, flatulence, impotence, insomnia and skin diseases. It is also used externally as a stimulant and the ointment as a counter-irritant. The essential oils of nutmeg and mace contain a variety of compounds, most of which are valuable in industry. Most of the pharmacological properties of nutmeg are attributed to the compounds found in the essential oil. Mace oil possesses almost identical physiological and organoleptic properties to nutmeg oil.

### **22.3.3 Uses in the perfume and cosmetics industries**

Nutmeg oil is used in cosmetics, men's perfume and toiletries due to its aromatic properties. Mace oil is also used to a limited extent in perfumes and soaps. Recently, research has indicated that mace lignan effectively inhibits melanin biosynthesis and thus could be employed as a skin-whitening agent (Yumi *et al.*, 2008).

### **22.3.4 Other uses**

Pinene of the essential oil of nutmeg is used to make camphor, solvents, plasticizers, perfume bases and synthetic pine oil. Dipentene is used in the manufacture of resins and is used as a wetting and dispersing agent. Myristic acid is used in the preparation of soaps, liquid detergents, shampoos, shaving creams, perfumes, plastics, in compounding rubber, paints and greases, in the synthesis of esters for flavours and perfumes and as a component of food grade additives.

## **22.4 Modern research into the functional properties of nutmeg and mace**

### **22.4.1 Antioxidant activity**

Nutmeg and mace possess antioxidant properties. The antioxidant property of nutmeg has been discussed by various authorities (Saito *et al.*, 1976; Kimura *et al.*, 1979; Dorman and Deans, 1995, 2000; Lagouri and Boskou, 1995; Madsen and Bertelsen, 1999). Nutmeg has strong 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Owen and Johns, 2002; Kong *et al.*, 2010). Antioxidative activity was exhibited by the phenylpropanoid compound present in a nutmeg seed extract (Maeda *et al.*, 2008). Repeated administration (100 mg/kg/day, p.o.) of myristicin and dehydrodiisoeugenol (mono- and dimeric phenylpropanoids from mace) suppressed lipid peroxidation and normalized decreased superoxide dismutase activities in FeCl<sub>2</sub>-ascorbic acid-ADP-treated livers of mice. The antioxidant activity of nutmeg extract was affected not only by the amounts of phenolic compounds but

also by the amount of monoterpenes present (Chyau *et al.*, 2009). Eugenol, present in nutmeg, inhibits the accumulation of lipid peroxidation products in red blood cells and maintains the activities of the antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase(s), glutathione reductase and glucose-6-phosphate dehydrogenase at normal levels (Kumara-velu *et al.*, 1996). Checker *et al.* (2008) reported that the lignans present in the aqueous extract of fresh mace also possess antioxidant properties. Acetone extract of mace-containing lignans inhibited lipid oxidation and prevented oxidative damage to cells (Chatterjee *et al.*, 2007). Mace lignan, showed a protective effect against tert-butylhydroperoxide (TBHP)-induced cytotoxicity in the human hepatoma cell line, HepG<sub>2</sub>. The lignin significantly reduced the cell growth inhibition and necrosis caused by TBHP. It also reduced intracellular reactive oxygen species (ROS) formation and the DNA damaging effect caused by TBHP (Sohn *et al.*, 2007).

#### **22.4.2 Anticarcinogenic and hepatoprotective activity**

Hussain and Rao (1991) reported that 3-methylcholanthrene (MCA)-induced carcinogenesis, in the uterine cervix of Swiss albino mice, could be prevented by oral administration of mace. The hepatoprotective activity of nutmeg was observed in rats with liver damage, when supplemented with nutmeg in their diets (Morita *et al.*, 2003). Banerjee *et al.* (1994) studied the influence of essential oil from nutmeg on the activities of hepatic carcinogen-metabolizing enzymes, namely, cytochrome P-450, aryl hydrocarbon hydroxylase, and glutathione-S-transferase and acid-soluble sulphhydryl level in albino mice and reported that the essential oil interferes with the activities of the host enzymes associated with activation and detoxication of xenobiotic compounds, including chemical carcinogens and mutagens.

#### **22.4.3 Hypolipidaemic effect**

Ram *et al.* (1996) reported that the levels of lipoprotein lipids (total cholesterol: 574 ± 61 versus 210 ± 27 mg/dl; low-density lipoprotein (LDL) cholesterol: 493 ± 57 versus 131 ± 25 mg/dl; and triglycerides 108 ± 14 versus 67 ± 9 mg/dl) were reduced significantly when hypolipidaemic rabbits were administered with 500 mg/kg of ethanolic extract of nutmeg kernels daily for a period of 60 days. The extract also exhibited platelet anti-aggregatory ability in platelet-rich rat plasma.

#### **22.4.4 Anti-inflammatory activity and metabolic syndrome**

The anti-inflammatory activity of nutmeg and its oil has been reported by several authors (Olajide *et al.*, 1999, 2000; Mueller *et al.*, 2010). Nutmeg oil exhibited pharmacological activities similar to those of non-steroidal anti-inflammatory drugs (Olajide *et al.*, 2000), but only the petroleum ether extracts showed anti-inflammatory activity (Shidore *et al.*, 1985). The total extract of nutmeg activated the AMPK enzyme (AMP-activated protein kinase) which is a potential therapeutic target for the treatment of metabolic syndrome including obesity and type-2 diabetes in differentiated C2C12 cells. As active constituents, seven 2,5-bis-aryl-3,4-dimethyltetrahydrofuran lignans, tetrahydrofuroguaiacin B (1), sauceretindiol



(2), verrucosin (3), nectandrin B (4), nectandrin A (5), fragransin C<sub>1</sub> (6), and galbacin (7) were isolated from this extract. Among the isolates, compounds 1, 4, and 5 at 5 µM produced strong AMPK stimulation in differentiated C2C12 cells. Nutmeg and its active constituents can be used not only for the development of agents to treat obesity and possibly type-2 diabetes but may also be beneficial for other metabolic disorders (Nguyen *et al.*, 2010).

#### 22.4.5 Antimicrobial and antiparasitic properties

##### *Antifungal properties*

Antifungal properties are exhibited by nutmeg and it is suggested that nutmeg could be an alternative to the use of synthetic fungicides to control some microorganisms. Nutmeg inhibited the mycelial growth of *Aspergillus niger* (Ascomycota), *Fusarium sambucinum* (Ascomycota), *Pythium sulcatum* (Oomycota) and *Rhizopus stolonifer* (Zygomycota) (Mvuemba *et al.*, 2009). Nutmeg oil is very effective in controlling the pathogen *Phomopsis azadirachtae* which causes die back in neem (Fathima *et al.*, 2009). Mace oil is inhibitory to the growth of *A. parasiticus* and *F. moniliforme* (Juglal *et al.*, 2002) and prevented the formation of aflatoxins. Ur-Rahman *et al.* (2000) reported the antifungal properties of essential oil from nutmeg. Phenyl propanoids and 8-O-4' neolignans isolated from mace exhibited antifungal properties (Zacchino *et al.*, 1997, 1998). Three lignans, namely, erythro-austrobailignan-6 (EA6), meso-dihydroguaiaietic acid (MDA) and nectandrin-B (NB), which were isolated from the methanol extract of nutmeg seed exhibited antifungal properties. The antifungal property of these lignins against *Alternaria alternate*, *Colletotrichum coccoodes*, *C. gloeosporioides*, *Magnaporthe grisea*, *Agrobacterium tumefaciens*, *Acidovorax konjaci* and *Burkholderia glumae* were studied *in vitro* and *in vivo*. The fungi were sensitive to the three lignins and showed varied antimicrobial activity. In *in vivo* studies it was reported that the various lignans suppressed the development of rice blast, wheat leaf rust, barley powdery mildew, tomato late blight and rice sheath blight (Cho *et al.*, 2007).

##### *Antibacterial properties*

Nutmeg can be used as a bactericide, and several reports are available on the antibactericidal activity of nutmeg and mace. Rath *et al.* (2002) reported that essential oil of nutmeg was effective against four gram-negative organisms, namely, *Escherichia coli*, *Salmonella typhimurium*, *Shigella boydii* and *Pseudomonas aeruginosa*, and a gram-positive organism *Bacillus cereus*. The growth of *B. stearothermophilus* was also strongly inhibited by the hot extracts of nutmeg (Madkour 1999), and *Listeria monocytogenes* was extremely sensitive to nutmeg oil: a concentration of <0.01 % was bacteriostatic and 0.05 % was bactericidal at 35 °C, but at 4 °C nutmeg was bacteriostatic at 0.5 % and bactericidal at >1 % (Smith-Palmer *et al.*, 1998). Minakshi De *et al.* (1998) reported that nutmeg exhibited strong antimicrobial properties against *B. subtilis*, *E. coli* and *Saccharomyces cerevisiae*, Pérez and Anesini (1994) demonstrated antibacterial activity against *S. aureus* and Dorman and Deans (2000, 2004) found that nutmeg oil showed strong antibacterial activity against 25 genera of bacteria. In addition, methanol extract of nutmeg seed, at minimum inhibitory concentration (MIC) of 12.5 µg/ml, inhibited *Helicobacter pylori*, the gram-

negative bacterium associated with the development of gastritis and peptic ulcer diseases (Mahady *et al.*, 2005).

Two resorcinols, malabaricone and malabaricone C, isolated from the aril of nutmeg exhibited strong antifungal and antibacterial properties against a range of microorganisms including *S. aureus* and *Candida albicans* (Orabi *et al.*, 1991). Dehydroxy-isoeugenol and 5'-methoxy dehydro-di-isoeugenol were the antibacterial principles isolated from mace against *Streptococcus mutans* (Hattori *et al.*, 1986). Chloroform extract of seeds showed potent antibacterial activity against gram-positive and gram-negative bacteria. Trimyristin and myristic acid isolated from the extract also showed good antibacterial activity (Narasimhan and Dhake, 2006). Nutmeg extract possessed strong antibacterial activity against non-pathogenic and pathogenic *E. coli*, but the strain O<sub>157</sub> showed more sensitivity to  $\beta$ -pinene than non-pathogenic *E. coli* strains (Takikawa *et al.*, 2002).

#### *Anti-amoebic activity*

Petroleum ether and water extracts of seeds showed antidiarrhoeal activity in rats (Shidore *et al.*, 1985). De Blasi *et al.* (1990) reported the anti-amoebic property of nutmeg essential oil against *Entamoeba histolytica*.

#### *Oral care*

Mace is used in Ayurvedic medicine as a constituent of preparations to prevent dental caries. The methanolic extract of aril showed antibacterial activity against cariogenic bacterium. Dehydrodiisoeugenol and 5'-methoxydehydrodiisoeugenol were identified as the major active principles (Hattori *et al.*, 1986). Macelignan is a potent natural antibiofilm agent against oral primary colonizers such as *S. mutans*, *S. sanguis* and *Actinomyces viscosus* (Yanti *et al.*, 2008). The specific activity and fast effectiveness of macelignan against oral bacteria strongly suggest that it could be used as a natural antibacterial agent in functional foods or oral care products. Chung *et al.* (2006) reported that the methanol extract of nutmeg possessed strong inhibitory activity against *S. mutans*, a pathogen associated with the occurrence of dental caries. The minimum inhibitory concentration of macelignan against *S. mutans* was 3.9  $\mu\text{g/ml}$ , which was much lower than that of other natural anticariogenic agents such as 15.6  $\mu\text{g/ml}$  of sanguinarine, 250  $\mu\text{g/ml}$  of eucalyptol, 500  $\mu\text{g/ml}$  of menthol and thymol and 1000  $\mu\text{g/ml}$  of methyl salicylate. A concentration of 20  $\mu\text{g/ml}$  of macelignan inactivated *S. mutans* completely in 1 min.

### **22.4.6 Pesticide**

#### *Insecticide*

Larvicidal properties have been reported in mace. The larvicidal principle this spice contains against second stage larvae of *Toxocara canis* was identified as diarylnonanoide, malabaricone C (Nakamura *et al.*, 1988). Nutmeg oil showed contact toxicity and strong antifeedant activity against stored product insects, namely, *Tribolium castaneum* and *Sitophilus zeamais*. The feeding deterrence index of *T. castaneum* was 7 %, whereas that of *S. zeamais* was 33 %. Nutmeg oil also prevented the hatching of *T. castaneum* eggs and the subsequent survival of the larvae. Adedire (2002) reported that oviposition and adult emergence in cowpea storage bruchid,

*Callosobruchus maculatus*, was inhibited by nutmeg oil. The essential oil could be used as an insecticide against larvae of *Lycoriella ingenua* and the mortality rate was reported to be >90 % (Park *et al.*, 2008).

#### *Nematicide*

The nematocidal properties of nutmeg and mace have been reported by several researchers. Antinematicidal activity against root-knot nematode, *Meloidogyne incognita*, was reported by Gotke *et al.* (1990) who observed that mace oil was more effective than nutmeg oil in controlling the nematode. The methanol extracts of nutmeg also exhibited moderate activity against pinewood nematode, *Bursaphelenchus xylophilus* (Mackeen *et al.*, 1997).

#### *Molluscicide*

Jaiswal and Singh (2009) evaluated the molluscicidal activity of powdered nutmeg and mace against the snail *Lymnaea acuminata* which is an intermediate host of the flukes *Fasciola hepatica* and *F. gigantica* which cause endemic fascioliasis in the northern part of India (Sharma *et al.*, 2009). The mace powder (96 h  $LC_{50}$  = 28.61 mg l<sup>-1</sup>) was more toxic than nutmeg powder (96 h  $LC_{50}$  = 36.95 mg l<sup>-1</sup>). The ethanolic extracts of nutmeg and mace were reported to be more toxic than their other organic solvent extracts.

### **22.4.7 Effects on reproductive functions**

Nutmeg oil is reported to have antifertility and recovery effect on male reproductive functions in rats. The effect of the oil of nutmeg at the dose levels of 0.2, 0.4 and 0.5 ml/kg body weight/day for 60 days was studied (Agarwal *et al.*, 2009). The oil reduced fertility to 0 % by 60 days of treatment at a dose level of 0.5 ml/kg body weight/day. The oil also caused significant reduction in the weight of testis, epididymis, seminal vesicle, ventral prostate and vas deferens. Sperm motility as well as sperm density were significantly reduced which resulted in the reduction of male fertility by 100 % at the highest dose level tested. A recovery of 80 % fertility was recorded after withdrawal over 120 days. Nutmeg has long been used in traditional Ayurvedic, Chinese and Thai medicine as an aphrodisiac and is used to treat male sexual disorders. Studies were undertaken in animal models to evaluate the aphrodisiac effect of 50 % ethanolic extract of nutmeg and it was observed that the sexual activity in animals increased which might be attributed to its nervous stimulating property (Tajuddin *et al.*, 2005).

## **22.5 Quality issues and toxicity**

Nutmeg and mace are classified by origin (East Indian nutmeg and West Indian nutmeg) and grade. Good quality has to be maintained for trade of nutmeg and mace. Whole nutmegs are grouped under three broad quality classifications:

- **Sound:** Nutmegs which are used mainly for grinding and, to a lesser extent, for oleoresin extraction.

- **Substandard:** Nutmegs which are used for grinding, oleoresin extraction and essential oil distillation.
- **Distilling:** Poor-quality nutmegs used for essential oil distillation.

In Indonesia, high-quality, sound, whole nutmeg are traded in grades which refer to their size in numbers of nutmeg per pound: 80s, 110s and 130s or 'ABCD' which is an assortment of various sizes. Substandard nutmegs are traded as 'sound, shrivelled', which in general have a higher volatile content than mature sound nutmegs and are used for grinding, oleoresin extraction and oil distillation, and 'BWP' (broken, wormy and punky) which are used mainly for grinding as volatile oil generally does not exceed 8 %. Distilling grades of nutmeg are of poor quality: 'BIA' or 'ETEZ' with a volatile oil content of 8–10 % and BSL or 'AZWI' which has less shell material and a volatile oil content of 12–13 %.

In Grenada, sound nutmegs are sold as sound unassorted which corresponds to the Indonesian grade 'ABCD'. Substandard nutmegs are classified as floats and as defective, the latter being similar to the Indonesian BWP grade but considered of high quality. Distilling grades of nutmegs are primarily exported to the USA and consist of floats.

Mace is classified as whole pale mace, No. 1 broken mace, selected, unassorted or siftings (Indonesia) and as whole, broken blades or siftings (Grenada).

The international standard applicable for trade in spices of nutmeg and mace is ISO 6577:2002. Although national standards are available for maintaining quality (see Table 22.2 and Tables 22.4–22.6), European traders prefer the ASTA cleanliness specifications (Table 22.4) as they are more strict than the national standards. The Quarantine System and Plant Protection Law and the Food Sanitation Act set the quality standard in Japan. Aflatoxin (the Netherlands, Japan) and salmonella (UK) are the common complaints regarding imparted nutmeg. The presence of insects is a major complaint for US importers.

Adulteration is common in the nutmeg trade. The essential oil has often been extracted before marketing; such nuts can be detected by their light weight and are more subject to insect attack. *M. fragrans* is adulterated with *M. argentea*, *M. malabarica* and *M. otaba* which can be identified by their poor quality. The mace from *M. argentea* is imported as Papuan nutmeg from Papua New Guinea, *M. malabarica* is traded as Bombay nutmeg from India and *M. otaba* as *Otaba* nutmeg. Trade in wild nutmegs exists, and these are marketed as long, female, Macassar, Papua, Guinea or Norse nutmeg. All these have been traced to *M. argentea* of New Guinea

**Table 22.4** American Spice Trade Association (ASTA) cleanliness specifications

| Product         | Insect<br>(by count) | Excreta<br>mammalian<br>(mg/lb) | Excreta<br>other<br>(% wt) | Mould<br>(% wt) | Insect<br>infested<br>(% wt) | Foreign<br>matter<br>(% wt) |
|-----------------|----------------------|---------------------------------|----------------------------|-----------------|------------------------------|-----------------------------|
| Nutmeg (broken) | 4                    | 5                               | 1.0                        | *               | *                            | 0.5                         |
| Nutmeg (whole)  | 4                    | 0                               | 0.0                        | *               | *                            | 0.0                         |
| Mace            | 4                    | 3                               | 1.0                        | 2.0             | 1.0                          | 0.5                         |

\* Not more than 5% by weight insect defiled and mould infected.

Source: Sivadasan and Kurup (1999).

**Table 22.5** Defect Action Levels prescribed by US Food and Drug Administration for spices

|                           |                                                                          |
|---------------------------|--------------------------------------------------------------------------|
| <i>Mace</i>               |                                                                          |
| Insect filth and/or mould | Average of 3% or more pieces by weight are insect infested and/or mouldy |
| Mammalian excreta         | Average of 3 mg or more of mammalian excreta per pound                   |
| <i>Nutmeg (whole)</i>     |                                                                          |
| Insect filth and/or mould | Average of 10% or more pieces by count are insect infested and/or mouldy |

Source: Sivadasan and Kurup (1999).

**Table 22.6** Dutch regulations for cleanliness specifications

| Product | Ash content<br>(max. %) | Sand content<br>(max. %) | Volatile oil<br>(min. %) | Others     |
|---------|-------------------------|--------------------------|--------------------------|------------|
| Nutmeg  | 3.5                     | 0.5                      | 4.0                      | NVEE * 5.0 |
| Mace    | 3.5                     | 0.5                      | 4.0                      | NVEE * 4.0 |

\* NVEE – Non-volatile ether extract.

Source: Sivadasan and Kurup (1999).

from where they enter into the market as Macassar nutmegs. *M. malabarica*, *M. otaba* and *M. argentea* are devoid of any aroma of *M. fragrans*.

### 22.5.1 Toxicity

Both nutmeg and mace contain the active ingredient myristicin which possesses narcotic properties. Nutmeg butter contains elemicin and myristicin which are also narcotic and cause psychotropic effects. Ingestion in large quantities produces narcosis, delirium, drowsiness, epileptic convulsions and even death. It also causes temporary constipation and difficulty in urination and increased fat deposition in liver. Powdered nutmeg is used occasionally as a hallucinogenic drug, but such use is dangerous as excessive dose of mace has a narcotic effect and symptoms of delirium and epileptic convulsions 1–6 hours after consumption.

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## Onion

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**Abstract:** Onion is a mass-cultivated vegetable consumed worldwide for 4000 years. The genus *Allium* is at its most diverse in the Mediterranean region, with China and India the largest onion-producing countries in the world. It is consumed in various forms and used in various cuisines. Onion is characterized by its distinct flavour and pungency which are due to various sulphur compounds. The chemical composition varies from variety to variety and with season of production and storage life. Dehydrated onion in the form of rings, powder, kibbles, etc. is in great demand. The processing industries require white onion varieties with high TSS. Various other products, viz. onion oil, onion juice, onion salt or onion pickles, can be prepared.

**Key words:** onion, biochemistry, nutraceuticals.

### 23.1 Introduction

Onion (*Allium cepa*) is a famous spice commodity grown all over the world and consumed in various forms. It has been in cultivation for more than 4000 years. The maximum diversity of *Allium* species is found in a belt from the Mediterranean basin to Iran and Afghanistan, i.e. Iran, north Iraq, Afghanistan, Soviet middle-Asia and west Pakistan,<sup>1</sup> indicating the primary centre of origin. The earliest record comes from Egypt where onions appear as carvings on pyramid walls and in tombs from the third and fourth dynasties (2700 BC).<sup>2</sup> It is thought that the Romans took the onion north of the Alps. The onion was among the first cultivated plants taken to the Americas from Europe. Europeans took the species to East Asia during the last century.

The distinctive flavour of alliums has established the plants as an essential part of the cuisine of the world. It is used as immature or mature bulbs as vegetable and spice as well as a food for poultry and non-milking cattle. In India and China, onion is the basis of many dishes. Nearly every Indian recipe starts with the same procedure. Fry chopped or sliced onion slowly, add spices (frequently fresh garlic and ginger and dried spices like cumin, turmeric, chillies) and fry until the onion turns golden. The mixture (wet masala) may afterwards be puréed, simmered with tomatoes or yogurt or just added to boiled or fresh vegetables, meat, chicken and fish to form curry. The onion rings can be fried and used as fast food. Onion pakodas made out of sliced onion and chickpea flour are an important snack item in Indian hotels.

Onion can be eaten raw or cooked; mild-flavoured or coloured bulbs are often chosen for salad.

Alliums are also used widely in food processing. However, handling of onions in bulk and storage of bulbs for 5–6 months is cumbersome. Losses of fresh onion in storage have been reported to be about 25–30%.<sup>3</sup> Value addition of raw onion in the form of processed products is the most practical solution. Whilst onion is traded in its raw form, the international market is increasingly focused on dehydrated products such as flakes, rings, granules, powder, etc., and processed onion as frozen or canned, or onions in vinegar and in brine.

*A. cepa* is cultivated mainly as a biennial, but some types are treated as perennials. It is propagated by seeds, bulbs or sets (small bulbs). Very wide variation in shape, size and colour is observed in this species. Intensive selection during domestication and natural hybridization might have created variability. There is a lot of controversy regarding the taxonomic position of *Allium* and related genera. The USDA PLANTS Database (<http://plants.usda.gov>) suggests the following hierarchy:

- class: Liliopsida – monocotyledons
- sub-class: Liliidae
- order: Liliales
- family: Liliaceae – lily family
- genus: *Allium* L. – onion
- species: *Allium cepa* L. – garden onion

Rhodes<sup>4</sup> gives a simple classification of genus *A. cepa* for the use of horticulturists as:

1. The common onion:
  - bulbs are formed as single plants
  - inflorescence does not form bulblets
  - biennial, often grown as an annual
  - most important commercially
  - propagated in the main from true seeds
  - sometimes planted as transplants
  - small bulbs (sets) are produced from thickly planted seed
  - when sets are replanted, these bulbs reach maturity rapidly
  - cultivars vary for bulb shapes, dry scale colour (white, yellow and red), pungency (ranging from mild and sweet to pungent), soluble solids, adaptation to different day lengths
  - when harvested early they produce spring or bunching onions
  - mature bulb onions are usually dried before marketing.
2. The aggregatum group:
  - with many lateral bulbs or shoots
  - inflorescences lacking bulblets
  - sterile seed production
  - propagation by vegetative means.

This group includes the potato onion or multiplier onion, ever-ready onions and shallots. Shallots are sometimes called scallions, a source of confusion because *A. fistulosum* is also called scallions

### 3. The proliferous (proliferum) group:

- ground bulbs are sometimes poorly developed
- inflorescence bears bulblets
- true seed is usually lacking
- reproduction is by vegetative inflorescence and bulblets
- not commercially cultivated
- almost exclusively for home gardening
- includes tree onions, top-set onions and Egyptian onions.

## 23.2 Chemical structure and influences on flavour

The importance of onion lies in the flavour that it imparts to various other dishes. A common onion contains the following nutrient components:<sup>5</sup>

|                |                               |
|----------------|-------------------------------|
| moisture:      | 88.6–92.8 %                   |
| protein:       | 0.9–1.6 %                     |
| fat:           | trace–0.2 %                   |
| carbohydrates: | 5.2–9.0 %                     |
| ash:           | 0.6 %                         |
| energy:        | 23–38 cal 100 g <sup>-1</sup> |

and elements (mg 100 g<sup>-1</sup> fresh weight):

|     |           |
|-----|-----------|
| Ca: | 190–540   |
| P:  | 200–430   |
| K:  | 80–110    |
| Na: | 31–50     |
| Mg: | 81–150    |
| Al: | 0.5–1     |
| Ba: | 0.1–1     |
| Fe: | 1.8–2.6   |
| Sr: | 0.8–7     |
| B:  | 0.6–1     |
| Cu: | 0.05–0.64 |
| Zn: | 1.5–2.8   |
| Mn: | 0.5–1.0   |
| S:  | 50–51     |

and vitamins (100 g<sup>-1</sup>):

- vitamin D: 0.3 mg
- riboflavin: 0.05 mg
- nicotinic acid: 0.2 m
- vitamin C 1: 0.0 mg
- folic acid: 16.0 µg
- biotin: 0.9 µg
- pantothenic acid: 0.14 mg

Among various free amino acids which vary greatly, glutamic acid and arginine are abundant in onion. The concentration of these amino acids is higher at the centre of the bulb and decreases towards the outer scales.

Onion is characterized by its distinctive flavour and pungency which is due to sulphur-containing compounds available in the scales of bulbs. The sulphur is in the form of various non-protein amino acids which include the precursors of volatile flavour compounds. These precursors are odourless, non-volatile amino acids of general name Salk(en)yl cysteine sulfoxides.<sup>6</sup> The precursors occur naturally in four types:

1.  $\text{CH}_3-\overset{\text{O}}{\underset{|}{\text{S}}}-\text{CH}_2-\text{CH}(\text{NH}_2) \text{COOH}\{(+)\text{-S-Methyl}\}$
2.  $\text{CH}_3-\text{CH}_2-\text{CH}_2-\overset{\text{O}}{\underset{|}{\text{S}}}-\text{CH}_2-\text{CH}(\text{NH}_2) \text{COOH}\{(+)\text{-S-Propyl}\}$
3.  $\text{CH}_3-\text{CH}=\text{CH}-\overset{\text{O}}{\underset{|}{\text{S}}}-\text{CH}_2-\text{CH}(\text{NH}_2) \text{COOH}\{\text{trans}(+)\text{-S-(1-propenyl)}\}$
4.  $\text{CH}_2=\text{CH}-\text{CH}_2-\overset{\text{O}}{\underset{|}{\text{S}}}-\text{CH}_2-\text{CH}(\text{NH}_2) \text{COOH}\{(+)\text{-S-(2-propenyl)}\}$

Intact onion cells have no odour, but when cells are disrupted the enzyme alliinase is released. This enzyme hydrolyses the S-alk(en)yl cysteine sulfoxides to produce pyruvate, ammonia and many volatile sulphur compounds associated with flavour and odour.<sup>7</sup> The enzyme is confined to cell vacuole, whereas the flavour precursors are confined to cytoplasm. The enzyme has access to precursors only when cells are disrupted. Boiled onion does not produce flavour as the enzyme is destroyed before having access to the flavour precursors. The tear-producing character of onion on cutting is known as the lacrimatory factor. Lacrimator is formed enzymatically during the hydrolysis of S-propenyl cysteine sulfoxide.<sup>8</sup> Thus alliums with S-propenyl cysteine sulfoxide have a tear-producing effect and alliums with S-allyl cysteine sulfoxide resemble the taste of garlic.

Besides the bulb, the flavour precursors are also available in leaf blades, base plate and roots of onion. However, they are absent in seeds.<sup>9</sup> The taste of onion differs from variety to variety and within varieties grown under different conditions and different growth stages. Flavour precursors are synthesized in leaf blades and transported to the scales of bulb where they are stored.<sup>10</sup> Younger blades are more productive than older ones. Precursor content increases during bulbing and then gradually decreases towards maturity. During storage of bulbs, the flavouring compounds increase; although the bulb is in a resting stage, it is still metabolically active. The increase in sulphur content is maximum until the sprouting of bulbs. The level of sulphur suddenly drops after sprouting. The reduction of flavour at the end of the storage period may be due to metabolism and translocation of the flavour precursors themselves for nutrients for developing roots.<sup>11</sup> Kopsell *et al.*<sup>12</sup> observed increase in *trans*-(+)-S-(1-propenyl)-1-cysteine sulfoxide in seven cultivars under study. There was decrease in S-methyl-L-cysteine sulfoxide.

As well as genotype and stage of growth, environmental factors exercise great effect on flavour strength. The bulbing response in onion is a function of tempera-

ture and photoperiod. Plants grown at higher temperature under optimum photoperiod bulb more rapidly than those grown at lower temperatures. A three-fold increase in volatile sulphur at higher temperature was observed by Platenius.<sup>13</sup> The varieties grown in India and Pakistan produce bulbs with higher pungency as they mature during temperatures ranging from 25–35 °C. Similarly, high amounts of watering produce bulbs with less flavour.<sup>13</sup> Soil fertility status and high amount of sulphur-containing fertilizers alter the flavour strength. More pungent bulbs were produced by Platenius on peat than on sandy soil.<sup>13</sup> Addition of sulphur in major nutrients was found to increase pungency in onion bulbs.<sup>14</sup>

### 23.3 Production and functional properties of onion

An FAO review of 2009 regarding area and production of onion shows that it is grown in 126 countries over an area of 3.6 million hectares producing 73.20 million tons of dry onion. Sixty-two per cent of the world's production is from Asiatic countries. Among them, China (28.7 %) and India (18.98 %) are the giants by area as well as by total production. The world's onion productivity is 20.00 tons/hectare.<sup>15</sup> About 90 % of onion is consumed within the countries of production. Total import of dry onion in the world is worth \$2216 million, while export is \$2184 million (<http://faostat.fao.org>).<sup>16</sup>

Onion cultivation and processing is at its most advanced stage in countries such as the USA. Systematic breeding programmes for the development of high TSS (total solute solids) white onion varieties launched by various research organizations and seed companies in the USA have been the backbone of the processing industry. Development of suitable varieties in short-day onion grown in Asiatic countries holds good promise with the available input.

#### 23.3.1 Dehydrated onion

Onion is dehydrated in the form of flakes, rings, kibbles and powder. A large part of dehydrated onion production is used as seasoning in production of catsup, chilli sauce and meat casseroles as well as cold cuts, sauces, soup, mayonnaise, salad dressing, sweet pickles, dog food, potato chips, crackers and other snack items. Food service outlets also use dehydrated onion because of its convenience in storage, preparation and use. The USA, India and China are leaders in onion dehydration and other processed products. Processing units require white onion varieties with important attributes:

- white onion with globose shape, free from greening;
- high total soluble solids (>20 %);
- high degree of pungency;
- high insoluble solids;
- low reducing to non-reducing sugars ratio to avoid caramelization;
- high yields;
- good storage quality.

Important cultivars for dehydration include White Creole, Southport White, Dehydrator No. 8, Dehydrator No. 14, V-12, etc. Indian varieties are of short-day



type and do not possess total soluble solids more than 15 %. However, due to high pungency, they make good-quality dehydrated flakes and granules. The coloured varieties like dark red, red and yellow are also used for dehydration purposes, but the quality of dehydrated produce is inferior to white varieties as they lose colour during storage.

Traditionally, onion dehydration is performed by sun drying in India, on a domestic scale. Various types of solar driers have also been designed for dehydration purposes.<sup>17</sup> However, controlled drying under optimum temperature and time gives good-quality product. Cabinet drying at 55–60 °C for 10–15 h gives a better quality dehydrated product than sun drying and drying in solar huts.<sup>18,19</sup> Commercial dehydration is achieved by forced hot air with the total process divided into three stages: drying at 75, 65 and 55–60 °C, the conditions of dehydration becoming milder as the moisture content falls.<sup>5</sup> Van Arsdell *et al.*<sup>20</sup> have given a schematic representation of the process for onion dehydration as shown in Fig. 23.1.

The process is completed by placing the dehydrated onion pieces in bins where the final moisture content (~4 %) is achieved via the circulation of warm air currents. The approximate composition (100 g<sup>-1</sup>) of dehydrated onion should be as shown in Table 23.1.<sup>21</sup>

### 23.3.2 Onion oils and other onion products

There are a number of other onion products:

- **Onion oils:** Concentrated oils extracted from onion can be used to impart the flavour of onion to processed food without the difficulties of handling a large bulk of fresh bulbs.<sup>6</sup> Onion oil is obtained by the distillation of minced onions which have been allowed to stand for a number of hours before distillation. The oil is a dark amber coloured liquid. The yield of oil varies from 0.002–0.03 %. One gram of oil is equivalent to 4.4 kg fresh onions or 500 g onion powder.<sup>21</sup> Use of onion oil is very safe from a microbiological contamination point of view, but there is the problem of flavour being lost. Onion oil is also used in non-alcoholic beverages, ice creams, confectionery, baked goods, condiments, meats and pickles.<sup>5</sup>
- **Onion juice:** Onion juice with low flavour component is another processed product. Massarated pulp of onion is flash heated (140–160 °C) and then cooled at 40 °C. The product is evaporated to 72–75 % solids to facilitate preservation. During processing, aromatic components may be removed so that the product has a low flavour profile.<sup>21</sup>
- **Onion salt:** Onion salt is prepared by mixing 19–20 % onion powder with 78 % free-flowing pulverized refined table salt and 1–2 % anticaking agent which prevents water absorption and caking, etc.<sup>22</sup>
- **Onion pickles:** Pickled onions are eaten in large quantities in many European countries. Onion pickles are prepared out of two types, namely (i) brown or dark red onion 28–45 mm diameter and (ii) white or silver skin (pearl or cocktail) onions between 10 and 28 mm in diameter.<sup>5</sup> These onions are produced by planting with high plant density. The onions are first peeled and allowed to ferment in 10 % brine solution for 24–96 hours. During fermentation, sugars from the

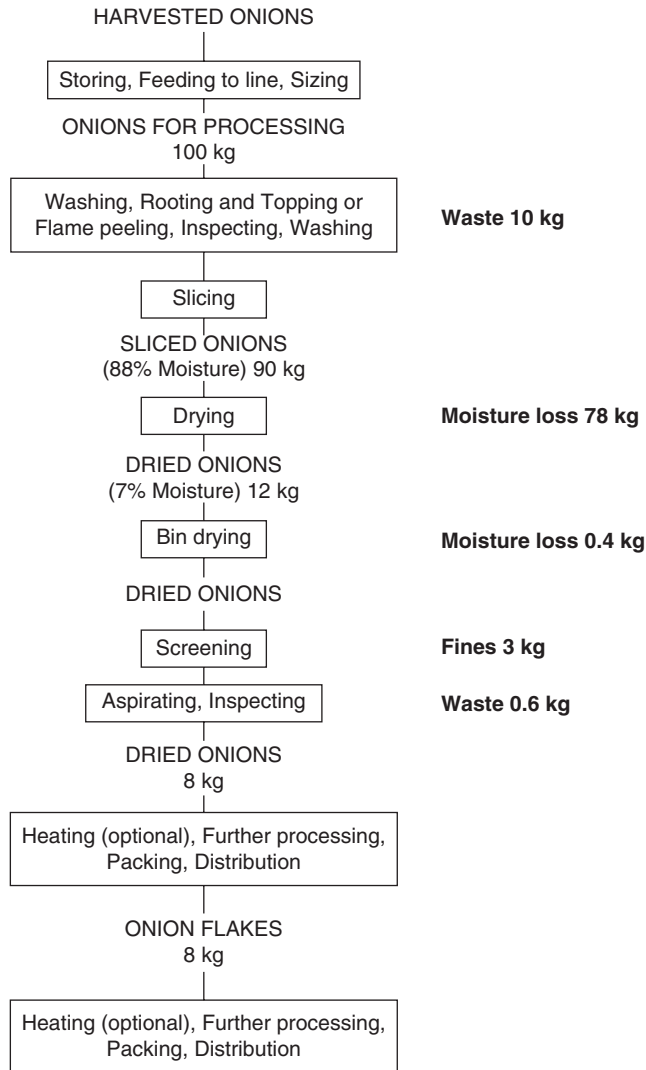


**Fig. 23.1** Onion dehydration process.

bulbs are converted to lactic acid and a small amount of acetic acid and alcohol. The fermentation is controlled by adding small quantities of lactic acid. The pickled bulbs are bottled in vinegar, possibly darkened with caramel and pasteurized at 80 °C.<sup>6</sup>

- **Vinegar from onion:** A new type of vinegar can be produced from onions that have been rejected for other conventional purposes because of low quality. Horiuchi *et al.*<sup>23</sup> tested several types of onion as raw material for vinegar production. Vinegar was produced successfully from juice of red onion cv. Kurenai by batch culture using yeast and *Acetobacter aceti*. The vinegar produced from onion had a higher potassium content, while sodium was lower than in conventional vinegars. The total amino acid and organic acid contents of onion vinegar

**Table 23.1** Composition of dehydrated onion<sup>21</sup>

|                          |          |
|--------------------------|----------|
| Water                    | 5.0 g    |
| Energy                   | 347 Kcal |
| Protein                  | 10.1 g   |
| Fat                      | 1.1 g    |
| Carbohydrates            | 80.7 g   |
| Fibre                    | 5.7 g    |
| Ash                      | 3.2 g    |
| Ca                       | 363 mg   |
| Fe                       | 3 mg     |
| Mg                       | 122 mg   |
| P                        | 340 mg   |
| K                        | 943 mg   |
| Na                       | 54 mg    |
| Zn                       | 2 mg     |
| Vitamins (ascorbic acid) | 15 mg    |

was much higher than in other kinds of vinegars. The commercial feasibility of this new type of product needs to be assessed. In a country like India, a colossal quantity of red onions go to waste during the lean season and these can best be utilized for value addition.

### 23.3.3 Functional properties

Besides use as a condiment and spice for flavouring and enriching various cuisines, onion has been known for its high medicinal properties for thousands of years. Chinese, Indians and Egyptians have known about its various medicinal properties since antiquity and these have been well documented. *Charak Samhita*, an ancient Indian medical treatise, describes many curative uses of onion. Augusti<sup>24</sup> listed various traditional uses of onion, including:

- It acts as stimulant, diuretic and expectorant and, mixed with vinegar, it is useful in the case of sore throat.
- Essential oil from onion contains a heart stimulant, increasing pulse volume and frequency of systolic pressure and coronary flow.
- Onion consumption lowers blood sugar, lipids and cholesterol.
- Fresh onion juice has antibacterial properties due to allicin, disulphide and cysteine compounds and their interactions.
- An antiplatelet aggregation effect in human and animal blood has been reported due to regular consumption of onion.<sup>25, 26</sup>

The antioxidant activity of onion (*A. cepa*) and onion scales has been studied in lipid oxidation models<sup>27–32</sup> and in radical scavenging assays.<sup>33, 34</sup> Both yellow and red onion were poor antioxidants towards oxidation on methyl linoleate<sup>32</sup> in contrast to their high antioxidant activity towards oxidation of low-density lipoprotein (LDL).<sup>34</sup> Onion had also a poor antioxidant score in the ORAC activity test while garlic (*A. sativum* L) scored four times higher.<sup>33</sup> Yin and Cheng<sup>35</sup> reported that the presence of garlic bulb, garlic greens, Chinese leek, scallion, onion bulb

and shallot bulb significantly delayed lipid oxidation of phosphatidylcholine liposomes. While allicin **4** is responsible for the antioxidant activity of garlic bulb,<sup>36</sup> compounds other than allicin are involved in determining the antioxidant effect of other *Allium* members. According to Velioğlu *et al.*<sup>37</sup> anthocyanin-rich vegetables including red onion scales generally showed very strong activities towards oxidation of  $\beta$ -carotene linoleic acid model systems. Similarly, green onion tops were reported twice as active as green onions with quercetin **5** included in the antioxidant substances.<sup>27, 28</sup>

More recently, Yang *et al.*<sup>38</sup> characterized the varietal differences in the phenolic content, antioxidant and antiproliferative properties of onions. Both total phenolic and flavonoid contents, which varied six-fold and eleven-fold between the varieties, respectively, were strongly correlated with total antioxidant activity. Nutilla *et al.*<sup>39</sup> compared the antioxidant activities of onion and garlic extracts. This group found that radical scavenging extracts were positively correlated with the total phenolics in the extracts and that onion extracts had higher radical scavenging activities than garlic extracts. Another topic of study has been the antimicrobial properties of onions, as well as other *Allium* species.<sup>40</sup>

## 23.4 Quality issues

### 23.4.1 Dry onions

For export from India, the following specifications have been defined by the Agricultural Processed Products Export Development Authority (APEDA).<sup>41</sup>

#### A. General big onions:

1. 4–6 cm bulb diameter, light red to dark red colour, globular, pungent – onions are suitable for Asian markets and Arab countries.
2. 3–4 cm bulb diameter, globular, pungent and light red colour – onions are suitable for Bangladesh market.
3. 7–8 cm bulb diameter, globular and oval round shape, yellow or brown colour – onions are suitable for European and Japanese markets.

#### B. Small onions:

2–3 cm bulb diameter, dark red to violet red, globular shape – onions are suitable for Malaysia and Singapore markets.

#### C. Multiplier onions:

2.5–3.5 cm bulb diameter, dark red colour, bigger size bulblets – onions are suitable for Malaysia, Singapore and Sri Lanka markets.

As per international quality standards,<sup>42</sup> dry onions should be intact, sound, clean, sufficiently dry for intended use, free from abnormal external moisture, free from off-odours and the stem must be twisted or clean cut. Shape and colour should be typical to the variety. The size should be uniform with minimum variation in the group. The size of group can be 10–20 mm, 15–25 mm, 20–40 mm, 40–70 mm and 70 mm plus. They should be free from abnormal swelling, doubles, sprouting and saprophytic fungus. The bulbs should be packed in sacks of jute or nylon nets with appropriate capacity varying from 25–40 kg. Consumer

prepacks of 1–2 kg capacity, such as nets, plastic film bags or stretch-wrapped trays can be used.

### 23.4.2 Dehydrated products

The American Dehydrated Onion and Garlic Association have standardized quality and grade specifications for dehydrated onion products.<sup>43</sup> Based on particle size the products are classified as shown in Table 23.2.

In all products, moisture should be 5.0 %. The products should be free from black or dark brown pieces, seed stems, sediment or sediment attached to onion, extraneous vegetable matter (such as tops, rootlets and other harmless vegetable matter), outer roots, metallic particles, hair, etc. The material should be packed in moisture barrier material like multi-walled polythene bags, fibre drums and stored under cool and dry conditions. Exposure to high temperature and light reduces the colour quality of dehydrated products.

Freshly harvested as well as stored bulbs are used for dehydration. During harvesting, handling and storage, the bulbs carry a heavy load of harmful bacteria, fungi, yeast and mould. There is every possibility of passing these microbes to the final product. The count of various microbes should be at tolerable levels as follows:

aerobic plate count: < 500.000/g

yeast and mould: < 5000/g

coliforms: < 200/g

salmonella: absent/25 g

*E. coli*: Absent/g

**Table 23.2** American Dehydrated Onion and Garlic Association quality standards and grade specifications

| Products              | Colour (optical index) | Bulk index (ml/100 g) |
|-----------------------|------------------------|-----------------------|
| Sliced                | 90                     | 400                   |
| Large chopped         | 90                     | 300                   |
| Minced                | 150                    | 180                   |
| Granulated            | 150                    | 140                   |
| Agglomerated – coarse | 150                    | 140                   |
| Agglomerated – fine   | 150                    | 140                   |
| Powdered products     | 150                    | 140                   |

**Table 23.3** Chemical composition of onions in brine and acetic acid<sup>41</sup>

| Composition            | Onions in brine | Onions in acetic acid |
|------------------------|-----------------|-----------------------|
| Salinity as NaCl       | 16 % ± 0.5 %    | 5 % ± 0.5 %           |
| Acidity as acetic acid | 0.3 % ± 0.5 %   | 4 % ± 0.2 %           |
| CaCl <sub>2</sub>      | 0.5 %           | 0.5 %                 |
| SO <sub>2</sub>        | 250 ppm max     | 250 ppm max           |
| pH                     | below 3.5       | below 3.5             |

### 23.4.3 Other onion products

Onion is processed in the form of pickled onion, as onion in brine and onion in acetic acid. For processing, bright white onions with globose shape, fully cured, free from rots, mould, fungus, external damage, sprouting and greening are used. Bulbs of 16–25, 25–45 and 45–70 mm diameter grade are used for processing. Smaller grades fetch better prices. The chemical composition of onions in brine and acetic acid should be as shown in Table 23.3.<sup>44</sup> The processed onions are packed in food grade HMHDPE barrels of 220–240 l capacity.

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## Parsley

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**Abstract:** Parsley is an annual culinary herb widely grown in Europe and Western Asia. This chapter describes the cultivation, parts of the parsley plant, its general uses and functional properties. The active constituents of the seed, root and leaves of parsley are discussed in detail together with the composition and physicochemical properties, and quality standards of essential oils. The chapter highlights the use of parsley in different recipes around the world. Finally, the functional properties and adverse effects of parsley are discussed in great detail. It has extensive information on the diuretic, carminative, antipyretic, antiviral, antibacterial, anti-inflammatory, anticancer and antioxidant properties of parsley.

**Key words:** cultivation, organic farming, flavouring, antioxidants, essential oils, flavonoids, apigenin, apiole, myristicin,  $\alpha$ -pinene,  $\beta$ -pinene, functional properties.

### 24.1 Introduction and description

Parsley (*Petroselinum crispum* (Mill.) Nymen ex A. W. Hill) belongs to the family (Umbelliferae) Apiaceae. Other scientific names are: *P. hortense* Hoffm., *P. sativum* Hoff., *Carum petroselinum* (L.) Benth. and Hook. F. Parsley (Fig. 24.1) has the following common names: English–parsley, Chinese–heung choi, Danish–persille, Dutch–peterselie, French–persil, German–petersilie, petersil, peterwurz (root), Greek–maintanos, makedonisi, petroselino, Hindi–ajmood, Italian–prezzemolo, Polish–pietruszka zwyczajna, Portuguese–salsa, Russian–petrushka, Spanish–perejil, Swedish–persilja. Also included in this family are parsnip, celery, dill, carrot, lovage, and a number of other well-known herbs and vegetables.

Parsley is an upright, much branched plant, reaching heights of 0.8 m (2'6") with green leaves and yellow greenish flowers growing in clusters extending from the main stem. Parsley has thin, spindle-shaped roots, with erect, grooved, glabrous, angular stems. The upper leaves are dark green and divided pinnately into featherlike-sections. The lower leaves are bi- or triterately divided. The small greenish yellow flowers have five petals on compound umbels. The dried leaves mostly consist of leaf fragments with the stem pieces removed. The epidermis on both leaf surfaces is undulate-sinuate, only straight-walled above the nerves, axially stretched with distinctly striated cuticula. The lower epidermis has anomocytic stomata. Trichomes are absent. The fresh roots are yellowish, carrot-shaped, with yellowish-white to



(a)



(b)

**Fig. 24.1** Fresh parsley.

reddish-yellow cross-section. The cut and dried root consists of yellowish-white to reddish-yellow pieces with roughly wrinkled surface; in the bark, dark brown and shiny oil cells are present, the xylem is lemon yellow on the outside and white on the inside. In the bark, dark brown, shiny oil ducts are visible and the xylem especially is radially striate with brown medullary rays. The seeds are roundish, ovoid to pear-shaped, strongly laterally compressed, greenish grey to greyish brown double achenes readily split along the commissural surface to give the somewhat sickle-shaped mericarps which are up to 2 mm (0.08 in) long and 1–2 mm (0.04–0.08 in) broad.

Parsley is mostly cultivated as an annual culinary herb and is widely grown in Europe and Western Asia. Parsley is to the Western world what *cilantro* (sometimes called Chinese parsley or coriander) is to the Eastern world. Most parts of the plant are used – the leaves, the above-ground herb and the seeds. The essential or volatile oils can be obtained through distillation. The volatile oil obtained from the above-

ground herb has the aroma of the fresh herb and is marketed as herb oil. The volatile oil obtained from the seed has a unique distinctive flavour and is marketed as seed oil.

It is interesting to note that the name 'parsley' originates from the name *Petroselinum* ascribed by Dioscorides. Later on, in the Middle Ages it became *petrocilium* and later expressed in the English language as *petersylinge*, *persele*, *persley* and finally *parsley* as it is known today. The name *petroselinum* is derived from the Greek word *petros* which means stone, referring to the plant's habit of growing in rocky places. *Selinon* was the Greek word for parsley in ancient history.

Parsley has been known in the Mediterranean region for about 2000 years, where it was cultivated as a medicinal plant. Hippocrates (466–375 BCE) and his students recommended it as a diuretic. According to Linnaeus, parsley originated in Sardinia and belongs to the family Apiaceae (Umbelliferae). Parsley was known in England around 1548, though some, such as Bentham and De Candolle, believe that the plant was a native of Eastern Mediterranean regions and also of Turkey, Algeria and Lebanon. During the sixteenth century parsley was naturalized in England. The Greeks used parsley for ritual purposes and it is mentioned in Greek mythology. It was planted near graves and anyone near death was said 'to be in need of parsley'. In Greece, parsley had long been associated with death. According to legend, the fertility king, Archemorus, was the one from whose blood the plant sprouted. For centuries, the association of parsley with death continued and every generation connected parsley with the 'gods' of the day. The Greeks used parsley for funerals and placed wreaths of parsley on tombs. Parsley is also said to have been used as a decorative garland for the head of Hercules, signifying his victory as a pillar of strength. Parsley garlands were given to the winners of athletic competitions. In Roman culture, parsley was often used as a deodorant to mask the smell after consuming garlic. Both the Romans and Greeks fed parsley to their chariot horses for stamina.

Parsley, being associated with death, later on became associated with evil or Satan. It was powerful with all its devilish powers and, for those who undermine its powers, there were negative consequences. Virgins could not plant parsley without losing their virginity to the devil. The safest day to plant parsley was Good Friday, and it was usually done by a male head of the household to ward off any evil effects the devil might generate. Since parsley seeds have a slow rate of germination, the popular belief was that the seeds had to travel to hell and back two, three, seven or even nine times, before they could germinate.

Parsley is also used in the Hebrew celebration of Passover as a reminder of the grief and sorrow of Hebrews, which came to an end that day. Parsley is also associated with Catherine de Medici and Charlemagne as one of the plants in their garden. It is said that Medici was responsible for the popularity of parsley when she brought it to France from Italy. In ancient times, parsley was used in medicinal concoctions for cure-alls, general tonics, poison antidotes, antirheumatics and formulations to relieve kidney and bladder stones.

## 24.2 Production and cultivation

As with most herbs, parsley grows best in a sunny area that receives direct light for 6–8 hours a day, although it can tolerate some shade. Plants will be more productive

if grown in well-drained soil that is fairly rich in organic matter, with a pH range of 6–7. The US Food and Drug Administration (FDA) has set regulations and directives that address microbiological hazards and appropriate control measures for such hazards (FDA, 2008). The FDA has also set good manufacturing practices (cGMPs) for food production and packaging (FDA, 2002). The guidance covers fresh-cut fruits and vegetables that have been minimally processed and altered in form, by peeling, slicing, chopping, shredding, coring or trimming with or without washing or other treatment, prior to being packaged for use by the consumer or a retail establishment.

### 24.2.1 Cultivars

In the USA, three distinctive types of parsley are commonly grown, although there are several types of parsley cultivated in different parts of the world. The curly-leaf or common parsley var. *crispum* is widely cultivated in the USA, Germany, France, Hungary and Belgium. Common parsley types are Moss curled, Dark moss, Banquet, Colored, Market gardener, Decorator, Deep green, Improved, Sherwood. These types are used primarily as dried or dehydrated in food products, but mostly used fresh as garnish. These curly types are quite versatile, typically 20–35 cm (8–14 in) tall, forming dense clumps, which are great for borders or interplanting in the garden beds, not to mention growing in containers. The Italian parsley var. *neopolitanum* Danert is flat with crisped leaves and is also known as flat-leaf or plain, its types are known as plain, plain Italian dark green. Italian parsley is used to flavour sauces, soups and stews. This can grow quite tall, up to 1 m (2–3 ft) and is more gangly in habit. The flat serrated leaves have a much stronger and sweeter flavour than the other varieties, making it desirable for cooking. The turnip rooted or Hamburg parsley var. *tuberosum* Bernh is used in specialized markets for its edible roots. Tall, fern-like leaves make up the foliage. Japanese parsley, *Cryptotaenia japonica*, is not commonly grown, and resembles the Italian parsley. It is mostly used in Oriental cooking and has a bitter taste.

### 24.2.2 Germination

Parsley seed numbers approximately 134 000/kg (296 500/lb). Since the germination rate of the smooth ribbed and ovate parsley seeds is very low and erratic, which is common to the Apiaceae family (Simon and Overly, 1986), a pre-treatment soaking is recommended to increase the usual slow rate of germination which is 3–6 weeks. The seeds have a very slow rate of germination in wet soils characteristic of early spring. Propagation is by seed grown from early spring to early summer and in autumn or by seed grown in spring (*P. crispum* var. *tuberosum*). The commercially produced parsley seeds are mericarps. The Italian variety is very slow to germinate.

### 24.2.3 Field preparation

Since parsley plants need a rich, moist soil and a good drainage, a pH of 5.3–7.3 is preferred (Simon *et al.*, 1984). Like other small-seeded crops, parsley needs a fine

seedbed and the soil should be finished after ploughing and disc harrowing with rototillers and bed shapers (Simon *et al.*, 1984). For proper germination, the seeds should be covered about 0.5 cm (1/4 in) but, in case of a heavy soil rich in minerals, the seeds should be covered with leaf mould or sand so that no crust is formed (Simon *et al.*, 1984).

#### 24.2.4 Sowing

Sowing rates may differ based on environmental and soil conditions from 5–9 kg (12–20 lb) to as high as 18–27 kg (40–60 lb) in some areas (Simon *et al.*, 1984). Usually to avoid freezing conditions in the north, plants can be grown indoors and then transplanted to open prepared fields. In spring, as soon as the soil is ready, the parsley seeds are sown into 150 cm (60 in) raised beds with three or four rows 45–55 cm (18–22 in) apart. Transplants could be spaced 10–20 cm (4–8 in) apart on 90 cm (36 in) rows. The highest yields can be obtained with very high plant populations (Simon *et al.*, 1984).

#### 24.2.5 Fertilization

The soil type and prior cropping history determine the suggested fertilization NPK ratio of 1–1–1 or 3–1–2. One application of N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O at a rate of 22–22–22 kg/ha (120–120–120 lb per acre) for heavy textured soils should be sufficient. Usually, one-third of the fertilizer is applied, which is followed by two side-dressings of NPK fertilizer and supplemented with N according to crop needs and cropping (Simon *et al.*, 1984).

#### 24.2.6 Irrigation

Parsley, like other leafy green vegetables, needs overhead sprinklers or drip irrigation (Simon *et al.*, 1984).

#### 24.2.7 Weed control

For weed control, most states in the USA use Stoddard solvent on parsley. When the seedlings are 5 cm (2 in) tall and have three true leaves, the herbicide is applied at the rate of 90 l/ha (60 gallons/acre) (Simon *et al.*, 1984).

#### 24.2.8 Insect and disease control

The parsley crop has many pests, which cause viral diseases such as Aster yellows. For pests such as aphids and cabbage looper, phosdium and methomyl are recommended, but the local extension service should be consulted before using the insecticides. Other pests such as carrot weevil, corn earworm, flea beetles and leafhoppers can be treated with different pesticides. '*Septoria apiicola*' is one of the most important foliage diseases, it is seedborne or splash disseminated (Simon *et al.*, 1984).

### 24.2.9 Harvesting

Depending on the crop quality, multiple harvests of parsley are possible by machine or hand. For multiple harvests, parsley should be cut at least 3 cm (1.25 in) above the crown. The machine-harvested fields are mechanically chipped 2.5–7.5 cm (1–3 in) above the crown and transported to dehydrators. Hand labour is the preferred method for harvesting parsley and is intensive because there must be minimal crop damage for parsley best suited for fresh market use. Usually the workers bunch up a group of plants manually, slice the stalks with a knife and tie them up in bunches by slipping a rubber band around the stalks. Sometimes the bunching is done later. In southern areas, late summer and autumn sowings are harvested in winter. In the north, harvesting can be continuous from April–December. Summer sowing can be harvested in autumn; autumn sowing can be harvested during winter and spring; or early spring sowing can be harvested late spring or throughout summer. The highest price and quality are obtained from the earliest harvested spring-seeded crop. Hamburg parsley can grow in moderate freezing conditions. Prior to marketing, roots should be washed.

### 24.2.10 Storage

Parsley should be shipped and packed in ice or coolers to maintain crispness and fresh appearance. Relative humidity of 95 % and temperatures from 0–2°C (32–36°F) are recommended for storage and handling (Simon *et al.*, 1984). Leaves can be dried by spreading them on a screen or hanging them upside down in bunches in a warm, well-ventilated room out of direct light. For faster drying, keep the leaves in an oven at 38–43°C (100–110°F) for just a few minutes. Store the dried leaves ground or whole in an air-tight container away from heat sources or bright light.

## 24.3 Organic farming

Farmers, consumers and policy-makers have shown a renewed interest in organic farming as the objective of today's common agricultural policy – the sustainability of both agriculture and the environment without compromising food production and conservation of finite resources and protecting the environment so that the needs of people are met today and for generations to come.

The organic industry is growing worldwide. Certified organic acreage in the USA reached 1.95 million hectares (4.8 million acres) in 2008 (ERS/USDA, 2010). California leads with the most certified organic cropland with over 174 014 ha (430 000 acres), largely for fruit and vegetable production. The world totalled 35 million hectares (86.5 million acres) of acreage managed organically in 2008 by almost 1.4 million producers in 154 countries (Willer and Kitcher, 2010). The regions with the largest area of organically managed land are Oceania (12.1 million hectares) (30 million acres) in Australia, New Zealand and surrounding island states, Europe (8.2 million hectares) (20 million acres) and Latin America (8.1 million hectares) (20 million acres). The sales of organic food and beverages in the USA have grown from \$1 billion in 1990 to \$24.8 billion in 2009. The total US organic sales, including food and non-food products, were \$26.6 billion



in 2009, up 5.3 % from 2008. The global organic sales reached \$50.9 billion in 2008, double the \$25 billion reported in 2003 (Organic Trade Association (OTA), 2010; Willer and Kitcher, 2010). Organic foods can be found at natural food stores and major supermarkets, as well as through growers' direct marketing such as CSAs (community-supported agriculture) and farmers' markets. Many restaurant chefs across the country are using organic produce because they desire its superior quality and taste.

Some of the essential characteristics of organic farming include: design and implementation of an 'organic system plan' – a detailed record-keeping system that tracks all products from the field to point of sale, and also the maintenance of buffer zones to prevent inadvertent contamination from adjacent conventional fields. Organic farming is an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity. The primary goal of organic agriculture is to optimize the health and productivity of interdependent communities of soil life, plants, animals and people. The holistic vision includes the maintenance of valuable relationships between soil, water, air, plants, animals and people. Organic farmers build healthy soils by nourishing the living component of the soil, the microbial inhabitants that release, transform and transfer nutrients. Soil organic matter contributes to good soil structure and water-holding capacity. Organic farmers feed biota and build soil organic matter with cover crops, compost and biologically-based soil amendments. These produce healthy plants that are better able to resist disease and insect predation.

The key principles used by organic farming systems are not to use chemo-synthetic mineral fertilizers and to minimize the use of permitted external fertility inputs, crop protection products and energy use – 'external' meaning that they are not produced on the farm as a group of collaborating farm businesses. Chemo-synthetic mineral fertilizers such as nitrogen and phosphorus fertilizers are not permitted and this prohibition has resulted from a range of considerations. Most importantly, such fertilizers are thought to substitute for natural mechanisms of nutrient acquisition by plants. Clover and other legumes have traditionally been used to enrich agricultural soils with nitrogen.

Legume plants have the unique ability to form symbiotic relationships with a specific group of soil bacteria called rhizobium. The symbiotic relationship between the legume plant and the rhizobium bacterium is extremely close in that the bacterium is taken up by the plant and 'housed' in a separate plant organ in the plant root (called the root nodule). Rhizobium growth and nitrogen fixation activity in the nodule is fuelled by transfer of carbohydrate from the plant to the root nodule. The bacterium focuses all the energy on transforming or fixing atmospheric nitrogen into ammonia. Application of mineral fertilizers to soil reduces soil microbial activity and, in particular, the activity of bacterial nitrogen fixation.

In soil, most phosphorus is usually present in non-water-soluble forms and therefore is not readily available for plants. In nature, most plants (this includes most crop plants) have developed symbiotic relationships with a specific group of soil fungi (called mycorrhizal fungi), which can access and make available non-water-soluble forms of phosphorus (phosphorus the plant could not access on its own) to the plant. The fungus colonizes the plant's roots and expands a web of 'hyphae' (microscopic fungal tubes) from the plant root into the soil. This web of hyphae

greatly extends the area of soil the plant can reach in 'collaboration' with the fungus. Fungal enzymes and acids then allow the fungus to take up soil phosphorus in a water-soluble form. The phosphorus is then transported via the fungal hyphae to the root and taken up by the plant tissue, which has been colonized by the fungus. As with the rhizobium bacteria, the plant supplies energy to the fungus in return for the phosphorus the fungus supplies to the plant.

In many respects, the symbiotic relationship between plants and mycorrhizal fungi is even closer than that between legumes and the nitrogen-fixing rhizobium. For example, the mycorrhizal fungus cannot grow and multiply in soil without plant roots being present. The addition of chemo-synthetic, water-soluble phosphorus fertilizers (e.g. superphosphate) inhibits the development of the symbiotic relationship between mycorrhizal fungi and plant roots.

The use of chemo-synthetic nitrogen and phosphorus fertilizers increases the risk of environmental pollution, since they are highly water soluble. They can therefore cause pollution and environmental problems such as algal blooms when they are: (i) leached into ground water (especially nitrate) or (ii) transported by run-off into the terrestrial and marine aquatic ecosystems. Organic farmers mostly use compost or manure to replenish the soil with minerals, as it is rich in beneficial soil microorganisms which, in turn, slowly and steadily make minerals available to plants. Using cover crops, practising low-till farming and leaving portions fallow conserves soil quality and prevents soil erosion.

Organic farming uses a variety of methods to control fungus and harmful insects. Farmers often use the method of intercropping. This is done by planting different crops in alternating rows, thereby interrupting the movement of disease-causing organisms through a field. Sometimes crops are sprayed with bacteria, which in turn destroy the larvae of harmful insects. Organic farmers also use pesticides derived from chemically unaltered plant, animal or mineral substances in which the active ingredient becomes non-toxic after being applied to the crops. For example, pyrethrum extracted from chrysanthemums and oil extracted from neem trees are widely used.

In organic farming, weed control is mainly done through mulching to smother weeds and by planting cover crops such as cereal rye and oat, which either inhibit weed seed germination or deprive them of the nutrients they need to grow. Sometimes tractor-drawn equipment is also used to uproot weeds.

Organic farming represents long-term savings and also maintains ecological balance and harmony. The crops are free from synthetic toxic chemicals and pesticides, being pure and natural and often believed by organic farmers to be more nutritious. Organic farming also preserves topsoil so more crops can be grown in future without polluting the environment, and savings on fuels, pesticides and fertilizers make it more attractive than conventional farming. 'Certified Organic' refers to agricultural products that have been grown and processed according to strict uniform standards, verified annually by independent state or private organizations accredited by the US Department of Agriculture (USDA). Certification includes inspection of farm fields and processing facilities. Farm practices inspected include long-term soil management, buffering between organic farms and any neighbouring conventional farms, product labelling and record keeping. Processing inspections include review of the facility's cleaning and pest

control methods, ingredient transportation and storage, and record-keeping and audit control.

The phrase 'certified organic' needs to be understood from the point of origin; these are products using organic agriculture. In organics, the focus is not so much on pushing the resources to produce tremendous yields and profits. Instead, organics foster the ecological processes that produce resources and add value to the resulting products: the added value of the designation, and the assurance that the products consumers are buying are not just chemical-free products but are good for consumers and are good for the environment as well. Organic products help minimize the adverse effects that agriculture can have on soil, water and air.

Organic food is as safe to consume as any other kind of food. Just as with any kind of produce, consumers should wash food before consuming it to ensure maximum cleanliness. Organic produce contains significantly lower levels of pesticide residues than conventional produce. It is a common misconception that organic food could be at greater risk of *Escherichia coli* contamination because of raw manure application, although conventional farmers commonly apply tonnes of raw manure as well with no regulation whatsoever. Organic standards set strict guidelines on manure use in organic farming: either it must be first composted or it must be applied at least 90 days before harvest, which allows ample time for microbial breakdown of any pathogens.

The cost of organic food is higher than that of conventional food because the organic price tag more closely reflects the true cost of growing the food: substituting labour and intensive management for chemicals, the health and environment costs of which are borne by society. These costs include clean-up of polluted water and remediation of pesticide contamination. Prices for organic food include costs of growing, harvesting, transportation and storage. In the case of processed foods, processing and packaging costs are also included. Organically produced foods must meet stricter regulations governing all these steps than conventional foods. The intensive management and labour used in organic production are frequently (though not always) more expensive than the chemicals routinely used on conventional farms. There is mounting evidence that, if all the indirect costs of conventional food production were factored into the price of food, organic foods would cost the same or, more likely, be cheaper than conventional food.

Organic growing of crops is a challenging, more detailed type of agriculture that requires the farmer to take responsibility for, and make a commitment to, the land even while pursuing the traditional business goal of making a profit. This commitment is embodied by the organic farming practices of weed, pest and disease control.

## 24.4 Environmental management systems

The International Organization for Standardization (ISO) developed an international standard, ISO 14001, for the design of an effective environmental management system (EMS) (ISO, 2004), originating in 1996 in Amsterdam. The fundamental principle and overall goal of ISO 14001 is the control and continuous improvement of an organization's environmental performance. This standard is a tool that enables

an organization to identify and control the environmental impact of its activities, products and services; to continuously improve its environmental performance; and to implement a systematic approach to set and achieve environmental objectives and targets. The standard's framework sets a holistic and strategic approach to the organizations' environmental policy, plans and actions.

ISO 14001 is based on a Plan–Do–Check–Act methodology. The first Plan phase is developing the environmental policy and planning the EMS. In this phase, an initial review or gap analysis of an organization's processes and products is recommended, to assist in identifying all elements of the current operation and, if possible, future operations. The second Do phase is implementing the EMS. In this phase, the organization should identify the resources required and work out those members of the organization responsible for the implementation and control of the EMS. This includes the documentation of all procedures and processes, including operational and documentation control, the establishment of emergency procedures and responses and the education of employees, to ensure they can competently implement the necessary processes and record results. The third Check phase is checking and reviewing the system. In this phase, internal audits are regularly done to ascertain whether the processes and procedures are being properly maintained and monitored and whether the EMS is being properly implemented. The fourth Act phase is the continuous improvement of the EMS. In this phase, a regular planned management review is conducted to make sure the planning and objectives of the EMS are being maintained, in order to make recommendations for further improvement. The organization gradually moves from operational measures to a more strategic approach to deal with environmental challenges.

Within this standard there are 17 elements that are required to be met by organizations seeking formal recognition for their EMS. These 17 elements are listed in the standard and could be grouped into phases that relate to the Plan–Do–Check–Act; The Environmental Policy, Planning Policy, Implementation and Operation, Checking and, finally, the Management Review by senior management. Several steps are outlined in this standard for the successful development and implementation of an effective EMS. Once a business successfully develops and implements their ISO 14001-compliant EMS, they can apply for certification. Organizations that implement an effective ISO 14001 EMS can reap benefits including higher conformance with legislative and regulatory requirements. Some of the other benefits include lowering business costs through reduced resource consumption and waste production; committing to social responsibilities; increased demand for their services and products through a marketing advantage; promoting positive image with stakeholders, customers and employees; and meeting environmental legal requirements. Up to the end of December 2009, at least 223 149 ISO 14001:2004 certificates had been issued in 159 countries and economies.

## 24.5 Chemical composition of parsley

The essential oils of parsley leaves and seeds have been studied extensively (Garnero and Chretien-Bessiere, 1968; Kasting *et al.*, 1972; Franz and Glasl, 1974; Freeman

*et al.*, 1975; Clark and Menary, 1983; Berger *et al.*, 1985; MacLeod *et al.*, 1985; Heide *et al.*, 1986; Srinivas, 1986; Shaath *et al.*, 1988; Simon and Quinn, 1988; Gbolade and Lockwood, 1989; Porter, 1989; Nitz *et al.*, 1989; Kim *et al.*, 1990; Lamarti *et al.*, 1991, 1993; Spraul, 1991; Baritau *et al.*, 1992; Jung *et al.*, 1992; Perineau *et al.*, 1992; Zheng *et al.*, 1992a,b; Molli *et al.*, 1993; Beier *et al.*, 1994; Vernin *et al.*, 1994; Pino *et al.*, 1997; Valterova *et al.*, 1997; El-Nikeety *et al.*, 1998; Masanetz and Grosch, 1998; Lopez *et al.*, 1999; Shatar and Altantseteg, 2000; Broda *et al.*, 2001; Diaz-Maroto *et al.*, 2002; Kubeczka and Formacek, 2002; Stankovic *et al.*, 2005; Kurowska and Galazka, 2006; Zhang *et al.*, 2006; Wei and Shibamoto, 2007b; Nickavar, 2008). Parsley seed contains 2–8 % essential oil with  $\alpha$ -pinene,  $\beta$ -pinene, apiole, myristicin and tetramethoxyallylbenzene as the major constituents (Leung, 1980; Lamarti *et al.*, 1991; Spraul, 1991). It also contains 13–22 % fixed oil consisting mainly of petroselinic acid, and smaller amounts of linoleic, myristic, myristolic, oleic, palmitic, stearic and 7-octadecenoic acids (Leung, 1980). Kubeczka and Formacek (2002) reported  $\alpha$ -pinene +  $\alpha$ -thujene (30.18 %),  $\beta$ -pinene (17.21 %), myristicin (8.31 %) and apiole (39.69 %) as the major constituents of a commercial sample of parsley seed oil. Kurowska and Galazks (2006) analysed the parsley seed oils from three Polish cultivars and found  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -phellandrene, myristicin, 1-allyl-2,3,4,5-tetramethoxybenzene and apiole as the major constituents. Nickavar (2008) found apiole (19.8 %), myristicin (27.4 %) and  $\beta$ -phellandrene (10.4 %) as the major constituents of parsley fruit oil from Iran.

Parsley leaf oil contains 0.04–0.4 % essential oil with  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, limonene,  $\alpha$ -phellandrene, *p*-cymene,  $\alpha$ -terpinolene, menthatriene,  $\alpha$ -terpineol, apiole and myristicin as the major constituents. Clark and Menary (1983) identified the following compounds in the oil of a plain leaf variety of *P. crispum*:  $\alpha$ -pinene (22.71 %),  $\beta$ -pinene (17.15 %), myrcene (5.08 %),  $\beta$ -phellandrene (11.57 %), terpinolene (3.03 %), 1,3,8-*p*-menthatriene (21.05 %), *p*-cymene (2.24 %), myristicin (4.86 %) and apiole (7.48 %). Pino *et al.* (1997) reported apiole (14.4 %) and myristicin (63.9 %) as the major constituents from parsley oil that was produced from plants grown in Cuba. The leaf has flavonoids (1.9–6.6 %) with the major component being apiin (apigenin-7-O-(6-O-aposyl) glucoside). Parsley is rich in such minerals as calcium, thiamin, riboflavin, potassium and iron and vitamins such as A, C and niacin (Marczal *et al.*, 1977; *Review of Natural Products*, 1991; Tyler, 1994, 1998; Blumenthal, 1998; Gruenwald, 1998). Parsley leaf (herb) also contains furocoumarins, xanthotoxin, isopimpinellin, flavonoids, proteins (2–22 %), fats (4 %), sugars and others (Leung, 1980). Parsley root contains up to 0.5 % essential oil, with apiole and myristicin (Fig. 24.2) as the major constituents. It also has flavonoids (0.2–1.3 %) and furocoumarins.

Zheng *et al.* (1992a) showed myristicin to be an effective cancer or tumour preventive agent. 1,3,8-*p*-Menthatriene is considered to be one of the major compounds to contribute to the parsley aroma (Kasting *et al.*, 1972). Garner *et al.* (1967) described it as having the odour of parsley leaves. MacLeod *et al.* (1985) believe that  $\beta$ -phellandrene, 1,3,8-*p*-menthatriene, 4-isopropenyl-1-methylbenzene and apiole are characteristic aroma constituents of parsley leaf. Spraul *et al.* (1991) and Nitz *et al.* (1992) reported that the main odour characteristic compounds of parsley root oil were sedanenolide and 3-butyl-5,6-dihydro-4H-isobenzofuran-1-one (an isomeric form of sedanenolide).

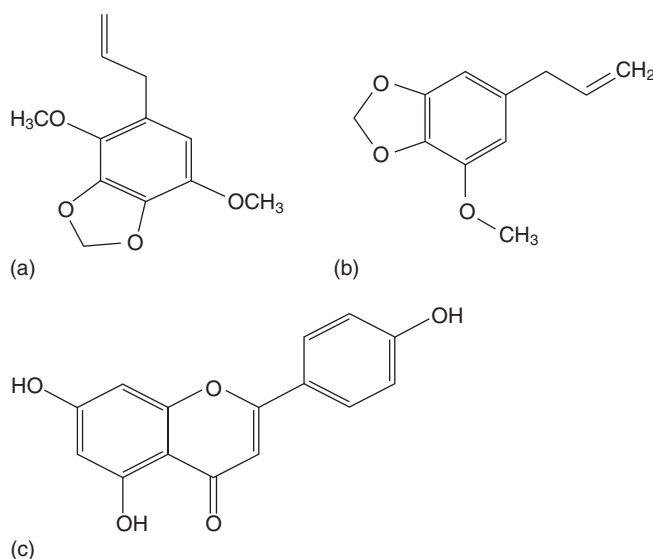


**Fig. 24.2** Chemical structures of the major constituents of parsley: (a) apiole, (b) myristicin, (c) apigenin.

**Table 24.1** Variation in constituents based on origin

| Compounds                     | Iran      | Iraq | Syria     | Turkey    | USA  | Yugoslavia |
|-------------------------------|-----------|------|-----------|-----------|------|------------|
| Myrcene                       | 2.9–13.6  | 15.7 | 2.0–12.2  | 0.9–16.4  | 3.3  | 2.8–13.1   |
| $\beta$ -phellandrene         | 9.7–33.5  | 8.2  | 12.8–14.7 | 5.8–29.8  | 18.3 | 3.6–15.9   |
| Terpinolene                   | 2.7–5.4   | 4.3  | 3.8–6.6   | 2.5–10.7  | 3.5  | 1.9–5.8    |
| + 1-methyl-4-isopropylbenzene |           |      |           |           |      |            |
| 1,3,8- <i>p</i> -menthatriene | 36.1–68.0 | 51.7 | 50.8–57.1 | 29.3–67.4 | 47.5 | 20.1–68.8  |
| Myristicin                    | 0.2–19.4  | 1.2  | 3.1–11.0  | 0.1–36.8  | 19.5 | 0.2–60.5   |
| Apiole                        | 0.1–0.3   | 0    | 4–5.6     | 0–22.1    | 0.1  | 0.1–1.4    |
| MW 268 isomers                | 2.3–10.9  | 7.8  | 3.5–4.7   | 1.7–10.5  | 2.9  | 1.7–7.6    |

Source: Simon and Quinn (1988).

Simon and Quinn (1988) studied the genetic variability of the major essential oil constituents of parsley leaf using the US Department of Agriculture (USDA) parsley collection. They found myristicin as a major compound (20 %) and this was in disagreement with other findings (Lawrence, 1981, 1982) that a high myristicin content (17 %) indicated a partial seed origin. The changes in the oil composition based on parsley origin are presented in Table 24.1. MacLeod *et al.* (1985) also showed a high myristicin content in parsley leaf oil. Unique to the Apiaceae is 1,3,8-*p*-menthatriene (MacLeod *et al.*, 1985), and this suggests a strong phylogenetic relationship among the parsley lines (Simon and Quinn, 1988). The author analysed samples of commercial oils of parsley leaf, herb and seed and the results are presented in Table 24.2. The major constituents were  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -phellandrene, *p*-mentha-1,3,8-triene and myristicin.

**Table 24.2** Composition of commercial sample of parsley essential oil

| Compounds                             | Parsley leaf oil<br>(% of oil) | Parsley herb oil<br>(% of oil) | Parsley seed oil<br>(% of oil) |
|---------------------------------------|--------------------------------|--------------------------------|--------------------------------|
| $\alpha$ -Pinene                      | 26.42                          | 27.41                          | 15.73                          |
| Sabinene                              | 1.10                           | 1.01                           | 0.64                           |
| $\beta$ -Pinene                       | 18.04                          | 17.83                          | 10.01                          |
| Myrcene                               | 4.24                           | 6.51                           | 0.22                           |
| $\alpha$ -Phellandrene                | 0.51                           | 0.66                           | 0.12                           |
| $\beta$ -Phellandrene                 | 6.48                           | 7.12                           | 2.14                           |
| Terpinolene                           | 2.52                           | 2.65                           | 0.01                           |
| <i>p</i> -Mentha-1,3,8-triene         | 16.41                          | 15.96                          | 0.12                           |
| Myristicin                            | 11.92                          | 9.78                           | 39.65                          |
| Elemicin                              | 2.71                           | 1.45                           | 4.84                           |
| 2,3,4,5-Tetramethoxy-<br>allylbenzene | 0.72                           | 2.62                           | 7.82                           |
| Apiole                                | 0.27                           | 0.11                           | 18.32                          |

**Table 24.3** Physiochemical properties of parsley

|                                                                      | Optical rotation | Refractive index | Specific gravity |
|----------------------------------------------------------------------|------------------|------------------|------------------|
| <b>Foods Chemical Codex (2000)</b>                                   |                  |                  |                  |
| Parsley herb oil                                                     | +1 to -9         | 1.503 to 1.530   | 0.908 to 0.940   |
| Parsley seed oil                                                     | -4 to -10        | 1.513 to 1.522   | 1.040 to 1.080   |
| <b>According to Guenther (1976), Vol IV, 656-63</b>                  |                  |                  |                  |
| French parsley herb oil                                              | +6 to -6.10      | 1.5029 to 1.526  | 0.9023 to 1.0157 |
| French parsley seed oil                                              | -4 to -10        | 1.512 to 1.528   | 1.043 to 1.110   |
| American parsley herb oil                                            | -2.13 to -7.40   | 1.5080 to 1.5179 | 0.945 to 1.046   |
| Hungarian parsley herb oil                                           | -1.46 to -6.18   | 1.5053 to 1.5250 | 0.948 to 0.987   |
| <b>According to NF T75-230 AFNOR (1996) and ISO 3527 (ISO, 2000)</b> |                  |                  |                  |
| Parsley fruit oil                                                    | -4 to -11        | 1.510 to 1.522   | 1.043 to 1.083   |
| <b>According to Fenaroli (1975), 428</b>                             |                  |                  |                  |
| Parsley herb oil                                                     | +6               | 1.5029           | 0.911            |
| Parsley seed oil                                                     | -4 to -11        | 1.5100 to 1.5290 | 1.043 to 1.083   |
| Parsley leaf oil                                                     | -2.55 to -6.10   | 1.5087 to 1.5159 | 0.948 to 0.967   |

### 24.5.1 Essential oils and their physicochemical properties

Table 24.3 shows the physiochemical properties of parsley.

#### Extraction

Parsley essential oil is obtained by steam distillation of the seeds or the above-ground parts of the plant. Commercially, there exist two types of parsley oil, *viz.*, parsley herb oil and parsley seed oil.

#### Storage

Parsley seed oil and herb oil should be stored in full, preferably glass, tin-lined or other suitably lined containers in a cool place protected from light.



### *Aroma profile*

Parsley herb oil is a pale yellow or greenish yellow, rarely water-white liquid of a peculiar, warm–spicy, heavy–leafy, yet fresh-herb-like odour. It is very similar to the odour of the freshly cut herb (Arctander, 1982). Parsley seed oil is a yellowish to amber-coloured or brownish liquid, more or less viscous. The odour is warm–woody, spicy, somewhat sweet herbaceous (Arctander, 1982).

## **24.6 Main uses of parsley**

### **24.6.1 Uses in food**

Parsley imparts a wonderful savoury and herbaceous flavour, making it a great culinary foundation. The leaves are cultivated extensively for the purpose of sending to markets fresh. It is also available in dried and powdered form to be used as a culinary flavouring, especially in the winter months when the fresh supply is very low (Fig. 24.3). The seeds, roots and even stems are used. The stem can be dried and powdered and used for culinary colouring and dyeing; the roots of the turnip-rooted variety are used as vegetable and flavouring; there is also a market for seeds to supply to nurserymen.

Parsley leaves can be used to season almost all strong, salty cooked dishes. Chopped fresh leaves are used in soups, stuffings, minces, rissoles and also as a garnish over vegetables and salads. In German cooking, it is the most commonly used kitchen herb. In Turkey, parsley omelettes are very popular, and in the near east tabbouleh, a salad made from shredded wheat and parsley. In France, it is an indispensable component of omelette fines herbes and in Italy of ossobuco alla milanese (Milan veal shank). Italians also use parsley in rich tomato sauces and more delicate seafood preparations. It is an essential ingredient of Mexican salsa verde. Creole cuisine uses parsley to add a herbal lift to otherwise heavy recipes. Used together with celery, bell pepper and onion in fairly large amounts, parsley



**Fig. 24.3** Dried parsley.

comprises the vegetal base to gumbos, rices and etouffees all over Louisiana. Parsley root is eaten on its own as a vegetable cut into slices (especially in the UK) or cooked together with other vegetables.

#### 24.6.2 Uses in traditional medicine

In India, parsley is still used in traditional Ayurvedic medicine for stomach complaints, as a diuretic and as an expectorant. In Europe, parsley has been used to treat asthma, coughs, eye complaints, jaundice, gout, oedema, bladder infections, menstrual problems and plague. In folk traditions, parsley is said to increase female libido, also helping in promoting menstruation and easing the difficulties of childbirth (*Review of Natural Products*, 1991; Tyler, 1994). Parsley juice can be used in treating hives and other allergy symptoms; it also inhibits the secretion of histamine. Parsley has also been used as a liver tonic and to help in the breaking up of kidney stones. The German Commission E has approved parsley as a preventive measure and also for treatment of kidney stones. The parsley root can be used as a laxative and also helps to eliminate bloating. It can reduce weight by reducing excess water gain. The root can be used to relieve flatulence and colic, due to its carminative action. Parsley can be used as a tasty breath freshener owing to its high chlorophyll content. It also speeds the healing of bruises and soothes tired and lustre-lacking eyes. The juice soaked in a pad can relieve earache and toothache. Parsley can be used as a face wash to lighten freckles. Parsley juice relieves itch and stings from insect bites; it works amazingly well as a mosquito repellent. Lactating women have used the leaves of parsley as poultice to relieve breast tenderness. The powdered seeds of parsley are a folk remedy for hair growth and scalp stimulation if massaged into the scalp for three days. It also has strong antioxidant properties (Pizzorno and Murray, 1985).

Parsley has other uses: the essential oil is used in perfumes for men. Head lice can be eradicated if parsley is used as a hair rinse. In modern herbal medicine, parsley is more popular in Europe than in the USA. Preparations from leaves, roots and seeds are used for indigestion, gallstones, kidney stones, urinary infections, jaundice, coughs and asthma. The seeds are prescribed for treatment of gout, rheumatism and arthritis. In the USA, tea made with parsley is recommended for bladder problems in young women.

### 24.7 Functional properties and toxicity

Parsley is reported to possess diuretic, carminative, emmenagogue, antipyretic, anti-cancer and antioxidant properties. Apiole (Fig. 24.2a) was isolated by various chromatographic techniques and identified as the acaricidal constituent of parsley (Song *et al.*, 2011). Myristicin (Fig. 24.2b) from parsley leaf oil was shown to be an active inducer of detoxifying enzyme glutathione S. transferase (GST), and an effective inhibitor of B(a)P-induced tumourigenesis in mice (Zheng *et al.*, 1992a, b). Apiaceous vegetables (including parsley) may inhibit the enzyme cytochrome P-450 1A2, which is understood to activate several procarcinogens, including aflatoxin B1 (Peterson *et al.*, 2006). Apigenin (Fig. 24.2c), a naturally-occurring plant flavone

found in parsley, has become recognized as a beneficial and health-promoting agent because of its low intrinsic toxicity and because of its striking effects on normal versus cancer cells, compared with other structurally-related flavonoids (Gupta *et al.*, 2001). A number of the biological effects of apigenin in numerous mammalian systems *in vitro* and *in vivo* are related to its antioxidant effects and its role in scavenging free radicals.

Further to its antimutagenic and antioxidant properties, it has been shown to exhibit anti-inflammatory, antiviral and purgative effects (Yang *et al.*, 2001; Patel *et al.*, 2007; Shukla and Gupta, 2010). Apigenin specifically from parsley has been found to modulate the effects of deregulated cell cycle checkpoints, thus contributing to the prevention of cancer (Meeran and Katiyar, 2008). Apigenin was shown to inhibit proteasome activity and induce apoptosis in human breast cancer MDA-MB-231 cells (Chen *et al.*, 2005, 2007a). Treatment of nude mice bearing human breast cancer MDA-MB-231 xenografts, with 25 or 50 mg/kg of apigenin for 29 days, showed 22 % and 43 % tumour growth inhibition, respectively, associated with inhibitory proteasome activity and induction of apoptosis (Chen *et al.*, 2007b). Pre-treatment with apigenin (20 and 50 µg/mouse/d) for 2 weeks followed by human prostate cancer 22Rv1 cells implantation in nude mice, tumour volumes were reduced by 39 % and 53 %, respectively. In another PC-3 tumour model, treatment with apigenin resulted in 32 % and 51 % inhibition in tumour growth (Shukla and Gupta, 2006). The proposed mechanism of antitumour activity by apigenin was upregulation of WAF1/p21, KIP1/p27 and INK4a/p16, and down-modulation of the protein expression of cyclins D1, D2, E and cyclin-dependent kinases (CDK) (Shukla and Gupta, 2006). Apigenin was also found to induce G2/M phase arrest in two p53-mutant cancer cell lines, HT-29 and MG63, and simultaneously enhance the levels of Cip1/p21, a CDK inhibitory protein (Takagaki *et al.*, 2005). Treatment of PC-3 and LNCaP cells with apigenin caused a marked reduction in the levels of cyclin D1 protein and decreases in CDK2, CDK4 and CDK6, which leads to G0/G1 phase arrest of the cell cycle and induction of apoptosis (Shukla and Gupta, 2007). Apigenin induced G2/M phase cell cycle arrest and reduced the levels of cyclin A, cyclin B, phosphorylated forms of CDC2 and CDC25 in pancreatic cancer cell lines (Ujiki *et al.*, 2006). It was shown that the apoptosis induced by apigenin in Hep G2 cells was possibly mediated through the p53-dependent pathway and the induction of Cip1/p21 expression, which was probably associated with the cell cycle arrest in G2/M phase (Chiang *et al.*, 2006).

Aliphatic C17-polyacetylenes of the falcarinol type, which occur in common food plants of the Apiaceae family such as carrot, celeriac, parsnip and parsley, have demonstrated interesting bioactivities including antibacterial, antimycobacterial and antifungal activity as well as anti-inflammatory, antiplatelet-aggregatory, neurotogenic and serotonergic effects. In addition, the cytotoxicity of falcarinol-type polyacetylenes towards human cancer cells, bioavailability and their potential anticancer effect *in vivo* indicates that these compounds may contribute to the health effects of certain vegetables and hence could be important nutraceuticals (Christensen, 2011).

Parsley and its constituents have strong antioxidant properties (Pizzorno and Murray, 1985; Fejes *et al.*, 1998; Hempel *et al.*, 1999; Nielsen *et al.*, 1999; Fejes *et al.*, 2000; Calucci *et al.*, 2003; Campanella *et al.*, 2003; Gómez-Coronado *et al.*, 2004;

Meyer *et al.*, 2006; Wei and Shibamoto, 2007a; Chohan *et al.*, 2008; Yildiz *et al.*, 2008; Vora *et al.*, 2009). The flavonoid-rich samples of parsley had good correlation between the chemical property and the antioxidant effect. The essential oils also played a significant role in the scavenging effect (Fejes *et al.*, 1998). The activities of erythrocyte glutathione reductase and superoxide dismutase activities increased during intervention with parsley as compared with the levels on the basic diet (Nielsen *et al.*, 1999). Tocopherol content was found to be 3.45 mg/100 g of parsley fresh leaves (Gómez-Coronado *et al.*, 2004). Parsley seed essential oil inhibited hexanal oxidation by over 95 % after 40 days at a level of 500 ug/ml in the aldehyde/carboxylic acid assay. It also had the greatest inhibitory activity towards malonaldehyde formation from squalene upon UV-irradiation. Myristicin in parsley seed oil showed high antioxidant activity (Wei and Shibamoto, 2007b). Simmering, soup-making and stewing significantly increased the antioxidant capacity of parsley extracts, while grilling and stir frying decreased it (Chohan *et al.*, 2008). Several extracts of parsley were evaluated for their scavenging activity and found to be good scavengers of DPPH and OH radicals and reduced the intensity of lipid peroxidation. The *in vivo* effects were evaluated on some antioxidant systems (activities of LPx, GSH-Px, Px, CAT and XOD, and GSH content) in mice liver and blood after treatment with parsley extracts, or in combination with CCl<sub>4</sub>. The examined extracts exhibited a protective effect (Popovic *et al.*, 2007).

Asparagus-P, a traditional herbal medicinal product (combination of equal parts of dried and pulverized asparagus roots and parsley leaves), has been shown to have anti-inflammatory efficacy and the potential to inactivate reactive oxygen radicals (Dartsch, 2008a). Parsley leaves and hence asparagus-P had a marked dose-dependent antioxidant effect, whereas asparagus roots had only a minor efficacy (Dartsch, 2008b). Jimenez-Alvarez *et al.* (2008a,b) reported that the antioxidant activity of parsley in food systems was related to their total phenolic content and radical scavenging capacity but not to their ability to chelate iron *in vitro*. Ethanolic extract of parsley was postulated to show a protective effect against mitochondrial oxidative damage in the mouse brain. They found a significant decrease in superoxide dismutase and glutathione activity and an increase in catalase activity in D-galactose-stressed mice. However, treatment of the D-galactose-stressed mice with an ethanolic extract of parsley showed protection against the induced oxidative stress in brain regions. The concentration of thiobarbituric acid-reactive product was greatly elevated in D-galactose stress-induced mice, but was significantly reduced in the brain regions of the mice on treatment with parsley (Vora *et al.*, 2009).

#### 24.7.1 Adverse effects

As a widely eaten food, parsley is generally regarded as safe. Though no interactions have been reported between parsley and standard allopathic medications, it may cause allergy in sensitive persons. No health hazards or side-effects are known in conjunction with the proper administration of designated therapeutic dosages. Parsley contains furocoumarins – compounds that may cause photosensitivity in fair-skinned persons exposed to sunlight after coming into skin contact with the freshly harvested herb. An overdose of parsley's essential oil can lead to poisoning because of the toxicity in high doses. Persons with kidney diseases should not take

parsley internally without consulting a physician because parsley is said to irritate the epithelial tissues of the kidney, hence enhancing the flow of blood and filtration rate. Pregnant or lactating women should not use parsley, as the oil rich-seeds contain a chemical which is said to have abortifacient properties (Stransky and Tsankov, 1980; *Review of Natural Products*, 1991; Tyler, 1994; Lagey *et al.*, 1995; Teuscher, 2006).

### 24.7.2 Quality issues and adulteration

The quality of parsley depends on its freshness, green colour, freedom from defects or seed stems and freedom from decay (USDA, 2007). There are generally no problems with adulteration of the fresh or dried crop. Its essential oils are also generally not adulterated.

## 24.8 References

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## Rosemary

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**Abstract:** Rosemary is an aromatic herb that has been known from ancient times as a memory herb. This chapter briefly describes the myths and folklore that are associated with this plant, and goes on to give details of the agricultural techniques used in its production, including biotechnology and developments in post-harvest processing and analytical techniques that have improved the extraction of rosemary oil and oleoresin. The culinary and medical uses of the herb are also outlined in this chapter, as is the use of rosemary as a herbal pesticide. The toxicology of rosemary and its oils is discussed at the end of the chapter, which concludes with suggestions for key topics that require ongoing research.

**Key words:** biomedical uses, chemical profile, oil extraction, pesticide, post-harvest technology, rosemary.

### 25.1 Introduction

Rosemary, the memory herb of the mint family, has been used by man since ancient times. Records of the use of rosemary appear in cuneiform on Sumerian stone tablets of the fifth millennium BC. The Chinese and Greeks used rosemary as a health conditioner; the Greeks, who wore rosemary wreaths in their hair, also believed that rosemary strengthened the brain and enhanced memory. In Egypt, the herb was buried with the pharaohs. Hungary water, an infusion of rosemary in spirits of wine, is thought to have been first prepared for Queen Isabella of Hungary in 1235 to renovate her paralysed limbs. The word rosemary is derived from the Latin word '*rosmarinus*', meaning 'sea dew'. It was also called '*antos*' by the ancient Greeks, meaning the flower of excellence or '*libanotis*' for its smell of incense (Giugnolinini, 1985).

There are many myths and much folklore associated with rosemary. It is believed that placing rosemary sprigs under the pillow would ward off evil spirits and nightmares from the sleeper and that the aroma of rosemary would keep old age at bay (Rose, 1974). During the Middle Ages it was believed that burning rosemary leaves and twigs would scare away evil spirits and disinfect the surroundings; later, it was burned by judges to protect against illness brought in by prisoners. It was also known as a symbol of fidelity and in Europe wedding parties burned rosemary as incense. Rosemary has also been used as a symbol of love and loyalty. As such, in the Middle

Ages, rosemary was associated with wedding ceremonies; either the bride would wear a rosemary head piece, the groom and guests would wear a sprig of rosemary or the couple would plant a rosemary bush on their wedding day. In Hungary, ornaments made of rosemary were once used as a symbol of love, intimacy and fidelity of a couple.

Some of these myths and beliefs had an underlying scientific logic behind them, as present-day studies reveal. Now it is clear that the essential oil and tannins present in rosemary produce an aromatic smoke with cleansing and purifying properties.

### 25.1.1 Origin and distribution

Rosemary (*Rosmarinus officinalis* L.) syn. Compass plant, Family Lamiaceae, is native to the Mediterranean – from Spain to the Balkans and into North Africa (Giugnolinini, 1985). At present, it is widely cultivated in Spain, Morocco, Tunisia, France, Algeria, Portugal and China and, to a limited extent, in India in the Nilgiris and Bangalore. Rosemary chemotypes with distinct morphological characters and oil quality occur in Italy (Mulas *et al.*, 1998).

### 25.1.2 Description

Rosemary is a dense, evergreen, hardy perennial aromatic herb of 60–200 cm in height with small (2–4 cm) pointed, leaves (Fig. 25.1). The upper surface of the leaf is dark green or blue, while the underside of the leaf is white; the leaves are resinous. Branches are rigid with fissured bark and stems brown, square and woody. Flowers are whitish, blue or purple in cymose inflorescence. The leaves, flowering tops and twigs yield an essential oil and oleoresin valued in recipes, traditional medicine, modern medicine and aromatherapy as well as in the perfume and flavour industries. Although it is generally *R. officinalis* that is used for oil extraction, in Morocco *R. eriocalyx* is also used for extracting essential oil (Elamrani *et al.*, 2000).



**Fig. 25.1** Rosemary.

**Table 25.1** Composition of dried rosemary leaves

|               |                    |
|---------------|--------------------|
| Moisture      | 5.7 %              |
| Protein       | 4.5 %              |
| Fat           | 17.7 %             |
| Crude fibre   | 19.0 %             |
| Carbohydrates | 47.4 %             |
| Ash           | 6.0 %              |
| Calcium       | 1.5 %              |
| Phosphorous   | 0.7 %              |
| Iron          | 0.03 %             |
| Sodium        | 0.004 %            |
| Potassium     | 1.0 %              |
| Vitamin A     | 175 IU/100 g       |
| Vitamin B     | 0.51 mg/100 g      |
| Vitamin C     | 61.3 mg/100 g      |
| Energy        | 440 calories/100 g |

Source: Farooqi *et al.*, (2005).

The annual global oil production is 200–300 metric tonnes. Many different varieties and cultivars are grown, each varying in flower colour (blue/pink/white), plant habit (erect/spreading), leaf colour (olive/blue/green), size of leaves, etc.

### 25.1.3 Chemical composition

The composition of dried rosemary leaves is shown in Table 25.1. The main components of rosemary oil are 1,8-cineol (15–20 %), camphor (15–25 %), borneol (16–20 %), bornyl acetate (up to 7 %),  $\alpha$ -pinene (25 %); in addition, the oil contains minor amounts of  $\beta$ -pinene, linalool, camphene, subinene, myrcene,  $\alpha$ -phellandrene,  $\alpha$ -terpinene, limonene, *p*-cymene, terpinolene, thujene, copalene, terpinen-4-ol,  $\alpha$ -terpineol, caryophyllene, methyl chavicol and thymol. The initial distillation fraction contains mostly  $\alpha$ -thujene,  $\alpha$ -pinene, camphene,  $\beta$ -pinene and 1,8-cineol, while camphor and bornyl acetate constitute the bulk of the later distillation (Prakasa Rao *et al.*, 1999). Rosemary oil exhibits variation in composition, both profile and percentage of each component, depending on growing location and/or other factors such as source population, fertilizers and phenology (Boyle *et al.*, 1991; Arnold *et al.*, 1997; Boutekedjiret *et al.*, 1999; Ouahada, 2000; Porte *et al.*, 2000; Wolski *et al.*, 2000; Guazzi *et al.*, 2001). Pintore *et al.*, (2002) reported a total of 58 compounds from rosemary oil from Sardinia and Corsica (Italy) based on gas chromatography retention index (GC–RI), GC mass spectroscopy (GC–MS) and carbon nuclear magnetic resonance (C-NMR) studies. A study of Moroccan rosemary oil not only demonstrated the existence of three different rosemary chemotypes but also identified a total of 91 compounds, based on GC and GC–MS studies (Elamrani *et al.*, 2000).

## 25.2 Production and cultivation of rosemary

Rosemary can be grown either as a field crop or as an indoor plant. The plant thrives well in well-drained soils of pH 6.5–7.0 and with warm, sunny weather conditions

(Doulgas, 1971). It will also grow in a semi-arid tropical climate. The plant is, however, susceptible to severe cold and frost, although frost-resistant varieties are now available (Domokos *et al.*, 1997). Male sterility is known to occur in natural populations of rosemary. Mitochondrial genome polymorphism linked to male sterility has been reported from Spain (Hidalgo-Fernandez *et al.*, 1999). This male sterility may be responsible for spontaneous population evolution and the occurrence of chemotypes in rosemary. Rosemary is a perennial herb propagated either by cuttings or seeds. Cuttings of 10–15 cm length from selected mother plants are ideal for vegetative propagation of rosemary. Treatment of cuttings with growth hormones such as indole butyric acid (IBA), indoleacetic acid (IAA) or saponin are reported to enhance rooting of cuttings (Shah *et al.*, 1996; Silva and Pedras, 1999). The end of winter has been found to be the most successful season for rooting cuttings (Silva and Pedras, 1999). Cuttings with the lower leaves removed are first planted in raised sand beds in a protected nursery. Regular watering is needed to achieve good sprouting. It then takes about 45–50 days for the cuttings to be transplanted to the main field.

Rosemary seeds are very small and black in colour. In India, the seed nursery is usually in operation from September to November. Seed rate is about 0.2–2.5 g seed  $1 \text{ m}^2$  area (Farooqi and Sreeramulu, 2001). Raised seed beds with adequate shade, sufficient watering, good drainage and weeding ensure healthy seedlings. Seedlings are transplantable at 8–10 weeks; the usual spacing adopted for the rosemary plants is  $45 \times 45 \text{ cm}$  as a monocrop.

Soil properties are known to influence the yield and composition of the rosemary oil harvested. Moretti *et al.* (1998a) reported that granite silt soils result in a better herb yield and oil quality when compared with calcareous soils. A fertilizer dose per hectare of 40 kg  $\text{P}_2\text{O}_5$ , 40 kg  $\text{K}_2\text{O}$  and 20 kg N with 20 metric tonnes farmyard manure is recommended for rosemary production in India (Farooqi and Sreeramulu, 2001). Furthermore, N level can be raised to  $300 \text{ kg ha}^{-1}$  in different splits to maximize oil yield. Close spacing coupled with increased nitrogen dose result in higher herbage and oil yield (Prakasa Rao *et al.*, 1999). Studies conducted in Italy (Sardinia) on fertilizer dose and weed management revealed that applying  $80 \text{ kg N} + 60 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  coupled with hand weeding of the major weeds such as *Genista corsica* and *Cytisus* spp. increased herbage yield and oil (Milia *et al.*, 1996). Biofertilizers, such as *Azospirillum*, *Azotobacter* and vesicular arbuscular mycorrhiza (VAM), applied in combination with inorganic fertilizers, have also been reported to have a beneficial effect on the herb and oil yield of rosemary (Anuradha *et al.*, 2002).

Weed control in rosemary is achieved through occasional hand weeding and intercultivation. Other husbandry techniques that have been studied include foliar spraying of iron, use of growth regulators, irrigation and pruning. The application of iron as a foliar spray in irrigated rosemary increased the verbenone concentration in the oil (Moretti *et al.*, 1998b). Studies on the effect of growth regulators (brassinosteroids and uniconazole) on growth, yield and chemical composition of rosemary in Egypt revealed the beneficial effect of growth hormones (Tarraf and Ibrahim, 1999). Irrigation is reported to be beneficial for herbage yield of rosemary. To establish the crop well in the field, two irrigations per week are recommended in India. Subsequently, irrigation once a week will be sufficient (Farooqi



and Sreeramulu, 2001). Drip fertigation with water-soluble fertilizers (80 % WSF) coupled with micronutrients is reported to increase growth, yield and quality traits of rosemary (Vasundhara *et al.*, 2002). Field experiments on water requirements of rosemary in Egypt revealed that irrigation once every 14 days resulted in high herbage and oil yield (Kandeel, 2001). A study on the effect of soil moisture regime, irrigation water (IW): cumulative pan evaporation (CPE) ratios and nitrogen level on herbage and oil yield of rosemary on alfisols in Bangalore, India, indicated that a soil moisture regime maintained at 0.50 IW:CPE ratio with 150 kg N ha<sup>-1</sup> significantly increased herbage and oil yield of rosemary (Singh and Ramesh, 2000). The correct soil moisture/nutrient regime is significant as unstressed rosemary plants are reported to yield higher levels of essential oil and phenolic compounds than those subjected to water/nutrient stress (Solinas *et al.*, 1996). Pruning of rosemary is advisable, though not essential, after 2 or 3 years to enhance shoot and leaf production (Farooqi and Sreeramulu, 2001). As rosemary is a perennial herb, aged plantations (10–12 years old) need to be rejuvenated by cutting back the plant to a height of 4–5 cm above ground coupled with fertigation for better herbage yield.

### 25.2.1 Diseases and pests

The important diseases affecting rosemary are: collar and root rot (*Phytophthora incognita*, *P. drechsleri*), blight (*Rhizoctonia* spp., *Phytophthora rosmarini* and *Orthotyphlus ribesi*), foliar necrosis/leaf spot (*Alternaria alternata*), aerial blight (*Ralstonia solani* AG-4), hook disease (*Botrytis cinerea*) and powdery mildew (*Oidium* spp.) (Perello and Dal Bello, 1995; Minuto and Garibaldi, 1996; Cacciola *et al.*, 1997; Conway *et al.*, 1997; Villevieille *et al.*, 1999). *Alternaria* leaf spot will be severe in humid and less well-ventilated areas, whereas powdery mildew can be severe in shaded conditions. Aerial blight is a major disease in greenhouse-grown rosemary. Various crop husbandry measures can reduce the severity of the impact of these diseases: (i) harvesting before blooming will help to restrict the crop loss due to hook disease; (ii) improved drainage can reduce the risk of root rot; (iii) spraying and drenching with the fungicide Maneb (1 %) are effective against root rot and blight; (iv) sulphur dusting is recommended against powdery mildew. Biological control agents have also been investigated for use on rosemary. Biocontrol of powdery mildew with a commercial formulation of *Ampetomyces quisqualis* gave partial control (Minuto and Garibaldi, 1996). An isolate of the biocontrol agent, *Laetisaria arvalis*, if incorporated in the pots, followed by foliar spray of fungicides at a low dose, was found to check aerial blight of rosemary better than separate applications of fungicide or biocontrol agent alone (Conway *et al.*, 1997). Biocontrol agents such as *Trichoderma* spp., and fluorescent pseudomonads are worth trying, as there is a premium price for organically produced herbs. These biocontrol agents can be components in an integrated disease management approach for rosemary. However, in the case of aerial blight of rosemary no synergistic effect of soil amendment with *Trichoderma harzianum* and foliar spray with iprodione (fungicide) was observed (Conway *et al.*, 1997). Mites are a serious insect pest in rosemary grown in India (Farooqi *et al.*, 2005). Spraying of Ethion (0.05 %) is recommended to control the infestation.



### 25.2.2 Biotechnology

*In vitro* studies of rosemary are in progress in several laboratories worldwide. Published research has focused on several topics: the callus induction potential of various explants from rosemary; the essential oil profile of *in vitro* cultures under salt stress; the *in vitro* production of the pigment shisonin; and the *in vitro* production of carnosic acid in callus cultures and regenerated shoots (Zhu-Ru *et al.*, 1996; Tawfik, 1997; Yang-Rong Hui *et al.*, 1997; Hashimoto *et al.*, 1997; Caruso *et al.*, 2000). However, despite the ongoing research, large-scale downstream processing of value-added products of rosemary using these *in vitro* techniques, or scale-up of the process for commercial exploitation, is still in its infancy.

## 25.3 Post-harvest technology and further processing

Rosemary leaves, flowering tops, flowers and twigs are of economic importance. The first harvesting is carried out about eight months after planting, with the onset of flowering or just before flowering. In the first year, two crops can be taken, whereas in the subsequent years two to four harvests are possible at an interval of 100–120 days. Generally, harvesting of the plants can be carried out until 50 % flowering. At above 90 % flowering, harvesting is not desirable (Farooqi and Sreeramulu, 2001). Tender, non-hardy shoots are also harvested for distillation upon attaining full size. Usually the harvested leaves, flowering tops and shoots are used for downstream processing without drying. However, the leaves and twigs can also be used after drying for oil extraction. Drying studies in normal conditions at 50°C and in dehydrated air (30°C) revealed that in terms of percentage values of the characteristic volatile oil compounds, the dehydrated air dried product is on par with raw leaves (di Cesare *et al.*, 2001). However, Ibanez *et al.*, (1999) reported that, of the different drying methods, the traditional method (drying in a ventilated room) was the best in terms of the yield and quality of the antioxidant principles of rosemary. The genotype of the plant, the age of the leaf and growing conditions are known to affect the oil quality, especially the quantities of the antioxidant principle, carnosic acid, in the oil (Hidalgo *et al.*, 1998). Boutekedjiret *et al.*, (1999) reported a phenology-dependent variation in yield and quality. For best oil yield, the flowering stage of rosemary is preferable, although the oil quality from plants at this growth stage is slightly inferior. To achieve high yields of good-quality oil, the grower has to select an appropriate growth stage when harvesting.

### 25.3.1 Extraction of rosemary oil

Rosemary oil is colourless to pale yellow and its properties are shown in Table 25.2. Rosemary oil is usually recovered by steam or water distillation, although supercritical fluid extraction (SFE) using CO<sub>2</sub> as a solvent is also now being used (Coelho *et al.*, 1997; Bicchi *et al.*, 2000). Detailed studies aimed at standardizing the various parameters for supercritical CO<sub>2</sub> extraction of the oil – such as pressure, temperature, particle size, CO<sub>2</sub> rate, etc. – are in progress (Carvalho *et al.*, 2005; Bensebia *et al.*, 2009; Ivanovic *et al.*, 2009; Zermane *et al.*, 2010). A new extraction process, the Fast Control Pressure Drop method, has also been reported (Rezzoug *et al.*, 2005).

**Table 25.2** Physical properties of rosemary oil

|                             |               |
|-----------------------------|---------------|
| Specific gravity (25°/25 C) | 0.894–0.912   |
| Optical rotation (20°C)     | –5° to +10C   |
| Refractive index (20°C)     | 1.464–1.476   |
| Esters as bornyl acetate    | Minimum 1–5 % |
| Total alcohol as borneol    | Minimum 8 %   |
| Solubility (90 % ethanol)   | 1 vol. (EOA)  |

This process involves exposing the rosemary leaves for a brief period of time to steam pressure varying from 0.5–3 bar followed by an instantaneous decompression to vacuum (about 15 mbar). Most of the oil (90 %) comes out within the first 60 minutes of the conventional distillation, although distillation can be continued for 120 minutes for full recovery of rosemary oil under field distillation conditions (Prakasa Rao *et al.*, 1999).

It has been found that the oil obtained from leaves and flowering tops is of better quality than the oil obtained from whole-plant distillation. It has also been reported that, in terms of the yield and quality profile of the rosemary oil, steam distillation is preferable to water distillation (Boutekedjiret *et al.*, 1997). Blanching (microwave blanching for 1 min) is observed to have a positive effect on retention of the antioxidant principles, green colour and texture of rosemary; however, blanching leads to total loss of volatile oils (Singh *et al.*, 1996).

The oils and extracts obtained from rosemary by steam distillation, water distillation and controlled instantaneous decompression were analysed by GC and GC–MS in order to determine their chemical composition (Boutekedjiret *et al.*, 2004). It was observed that steam distillation seemed to be the most appropriate process for oil isolation of rosemary since it gave the highest yield, and an oil composition similar to values quoted in the literature and in commercial standards. The composition and antioxidant properties, as well as antimicrobial activities, of oil extracted using supercritical CO<sub>2</sub> have also been confirmed (Genena *et al.*, 2008).

The oil content of fresh rosemary leaves is 1 % and in shade-dried leaves it increases to 3 % (Farooqi and Sreeramulu, 2001). In theory, the yield from 1 ha is approximately 10–12 tonnes of herbs per year, yielding 25–100 kg oil (Farooqi and Sreeramulu, 2001). However, in field distillation conditions, the oil yield varies from 0.5–0.9 % (Prakasa Rao *et al.*, 1999), so annual yields may be lower.

### 25.3.2 Extraction of active compounds

For the extraction of the active compounds of rosemary, conventional solvent extraction techniques using solvents such as hexane, benzene, ethylene, chloroform, dioxane and methanol (Chang *et al.*, 1977) besides distillation and SFE are routinely employed. SFE using CO<sub>2</sub> has been reported to be superior to liquid solvent sonication for the maximum recovery of carnosic acid in its pure form (Tena *et al.*, 1997). Recent research on improving the yield and quality of rosemary extract with new techniques has shown encouraging results. Superheated water under pressure between 125 and 175°C has been shown not only to extract high-quality oxygenated fragrance and flavour compounds from rosemary rapidly but also to produce higher

yields than steam distillation (Basile *et al.*, 1998). Ibanez *et al.*, (1999) proposed a two-step SFE and fractionation of essential oil-rich oleoresin and antioxidant compounds by varying the pressure and temperature requirements. Enzyme-assisted ensiling (ENLAC) prior to polyphenol extraction is reported to double the yield of polyphenol from rosemary (Weinberg *et al.*, 1999).

Rosemary oleoresin, also known as rosemary oil extract, is a green to brownish green semi-solid to pasty extract which is fluid when warmed to 50°C. Different grades of rosemary oleoresin are available, with the grade being dependent on the volatile oil concentration, in particular the carnosic acid concentration (Heath, 1981). It is the carnosic acid that is believed to be responsible for the potent antioxidant properties of rosemary oleoresin. These properties will be discussed in a later section of the chapter.

## 25.4 Main uses of rosemary

### 25.4.1 Culinary uses and food processing

Rosemary leaves and flowering tops have many culinary uses: lamb roast, mutton preparations, marinades, bouquet garni, baked fish, rice, soups, salads, occasionally with egg preparations, dumplings, apples, summer wine cups and fruit cordials, in vinegar and oil (Bonar, 1994). The fresh and dried leaves of rosemary are used frequently in traditional Mediterranean cuisine as they have a bitter astringent taste and are aromatic. Dried and powdered leaves are added to cooked meat, fish, poultry soup, stews, sauces, dressings, preserves and jams. Mixed with sage, the leaves are also used in pork preparations. When burned the leaves give off a distinct mustard smell which is favoured in barbecuing.

Rosemary is a most effective herb with a wide range of uses in food processing. In Europe and the USA, rosemary is commercially available for use as an antioxidant, though not technically listed as natural preservative or antioxidant, especially in Europe (Yanishlieva-Maslarova and Heinonen, 2001). Rosemary has potential applications in the suppression of warmed over flavour (WOF) (Valenzuela and Nieto, 1996). The main antioxidant principles in rosemary are carnosic acid, 12-methoxy carnosic acid and carnosol, as well as the antioxidative diterpenes such as epirosmarinol, isorosmanol, rosmaridiphenol, rosmariquinone and rosmarinic acid (Richheimer *et al.*, 1996). The antioxidant properties of rosemary are attributed to its ability to scavenge superoxide radicals, lipid antioxidation, metal chelating, etc. Extracts and essential oil of rosemary can be used to stabilize fats, oils and fat-containing foods, e.g. butter, against oxidation and rancidity (Zegarska *et al.*, 1996; Pokorny *et al.*, 1998) and also to stabilize fermented meat products (Korimova *et al.*, 1998). A useful review by Yanishlieva-Maslarova and Heinonen (2001) discusses the literature on rosemary and sage antioxidant principles, covering their extraction, properties and applications, and the chemistry involved. Deodorized liquid, commercial antioxidant formulations of rosemary, either as mono-herbal or as poly-herbal (rosemary, thyme, sage, oreganum) formulations, i.e. 'Herbor 025', 'Spice Cocktail', etc., are available (Aruoma *et al.*, 1996).

Rosemary oil also has applications in meat preservation. The addition of rosemary oleoresin to ground chicken had an overall positive effect on raw meat

appearance during storage and on the flavour of the cooked meat (Koekamnerd *et al.*, 2008). The antioxidative properties of rosemary oleoresin and its inhibition of off-flavours in pre-cooked roast beef slices have also been reported (Murphy *et al.*, 1999).

#### 25.4.2 Aromatherapy and cosmetics

Rosemary oil is used in aromatherapy to stimulate the central nervous system and blood circulation, to relieve muscular pain. Add a few drops of rosemary essential oil to any massage oil, such as sweet almond oil, blend together and massage gently into the area affected by pain.

It also has beneficial effects in promoting hair growth. Massaging a cocktail of essential oils of rosemary, thyme, cedar wood and lavender in carrier oils of jojoba and grape seed over a period of 7 months into the scalp of patients with *Alopecia areata* was found to be a safe and effective treatment (Hay *et al.*, 1998). Rosemary oil stimulates hair follicles and circulation in the scalp and thus may also be helpful in premature baldness ([http://www.holistic-online.com/Herbal-Med/\\_Herbs/h228.htm](http://www.holistic-online.com/Herbal-Med/_Herbs/h228.htm)). Rosemary is used in curing acne (Baumann, 2007).

In addition to its use in aromatherapy, rosemary also has several cosmetic applications. When used in a shampoo, rosemary oil can help to control dandruff and greasy hair; it is also known to impart a black colour to hair. Rosemary cleansing cream can be used for oily skin (Bonar, 1994). Rosemary oil is a component in soaps, room fresheners, deodorants, perfumes, skin lotions, etc., either in formulations with other herb oils or singly. The traditional Hungary water is a perfume based on rosemary oils. The flower, calyx and leaves of rosemary are used in potpourri and herb pillows (Bonar, 1994).

#### 25.4.3 Traditional medicinal uses

Hungary water prepared from rosemary is considered to be a revitalizing agent and the herb has an old reputation for improving memory. The presence of rosemary in one's body is believed to enhance clarity of mind and memory, akin to the belief surrounding sweet flag (*Acorus calamus*) in India. In traditional medicine, herbalists recommend rosemary oil for a wide range of disorders including pulmonary diseases. Rosemary oil has been described as having the following properties: stomachic, wound healing (poultice), choleric, antidiabetic, diuretic, antidepressant and antispasmodic (Oury, 1984; Giugnolinini, 1985; Erenmemisoglu *et al.*, 1997). The whole plant, in the form of decoction, infusion, extract in ethanol (for external application) and essential oil, is administered against digestive disorders, vaginitis, leucorrhoea, respiratory diseases, varicose vein, heart pain, inflammation and dizziness by the native people of Mexico and Central America (Santos García-Alvarado *et al.*, 2001). In Russia and the Central Asian countries of the former Soviet Union, leaves of rosemary preparation (gallenical and powder made into cigarettes) are used to treat asthma (Mamedov and Craker, 2001). The abortifacient (anti-implantation) effect of rosemary extract is also known (Lemonica *et al.*, 1996). A distilled water obtained from rosemary flower is used as an eye lotion (Farooqi *et al.*, 2005).

#### 25.4.4 Modern research into medicinal properties

Rosemary is today credited with having many varied medicinal properties. Modern analysis techniques have enabled the individual components of the oil to be identified and their effects investigated. The medicinal properties and the chemical constituents/part of the plant that are now thought to be involved in these effects are listed below:

- carminative properties – flavanoids;
- antidepressant, antispasmodic properties – volatile oils;
- rubefacient properties – phenolics;
- antimicrobial properties – diterpenes;
- emmenagogue properties – oleanolic acid;
- anti-inflammatory properties – carnosol;
- carcinogen blocking and liver detoxifying properties – carnosol and whole-plant extract;
- antirheumatic properties – ointment of rosemary oil;
- abortifacient properties (aqueous extract).

##### *HIV treatment*

Liquid deodorized extract of rosemary ('Herbor 025') and oily extract of mixture of herbs such as rosemary, thyme, sage and oreganum ('Spice Cocktail') inhibited human immunodeficiency virus (HIV) infection at very low concentrations though they were cytotoxic. Carnosol and carnosic acid were found to be the main active constituents of the extracts (Aruoma *et al.*, 1996). Purified carnosol @ 8  $\mu$ M exhibited anti-HIV activity besides being cytotoxic.

##### *Cardiovascular effects*

The cardiovascular effects of rosemary extract on the isolated intact rabbit heart demonstrated significant positive inotropic effect and coronary vasodilatation (Khatib *et al.*, 1998). Administering an infusion of dried rosemary leaves resulted in decrease of blood glucose levels in normoglycaemic and diabetic mice and had no toxic effects (Erenmemisoglu *et al.*, 1997).

##### *Cancer prevention*

Rosemary is now gaining importance in cancer treatment. Rosemary has been shown to have potential as a source of anticancer molecules and as a treatment to enhance the bioavailability of cancer drugs (Plouzek *et al.*, 1999; Huang *et al.*, 2009; Rasha and Abdella, 2010; Yesil-Celiktas *et al.*, 2010). The major anticancer compounds identified from rosemary are: carnosol, carnosic acid, ursolic acid, bifulinic acid, rosmaridiphenol and rosemanol. Carnosol is found to reduce cellular nitric oxide in mice, a free radical that can damage DNA (Chan *et al.*, 1995). It is also reported, in rats, that administering a whole-plant extract of rosemary is more effective in preventing the carcinogen 7,12-dimethyl benz(a)anthracene (DMBA) from binding to breast cell DNA than administering carnosol or ursolic acid alone (Singletary *et al.*, 1996).

Extract of rosemary is found to increase the intracellular accumulation of the common chemotherapy drugs such as dexamethasone (DOX) and vinblastine (VIN) in

drug-resistant MCF-7 human breast cancer cells leading to increased availability of the drugs for bioactivity (Plouzek *et al.*, 1999). Adding rosemary extract to cooked beef reduces the formation of heterocyclic amines, a carcinogen (Puangsombat and Smith, 2010).

#### *Diuretic and other effects*

Haloui *et al.*, (2000) reported a diuretic effect of aqueous extract of rosemary (8 % concentration) on Wister rats. Carnosic acid in rosemary is reported to shield the brain from free radicals, lowering the risk of strokes and neurodegenerative diseases like Alzheimer's and Lou Gehrig's (Lipton, 2007). Hyperglycaemic and insulin release inhibitory effects of the herb have also been reported (Al-Hader *et al.*, 1994). Rosemary extract also prevents binding of aflatoxins to human liver cells and benzo(a)pyrene to bronchial tissue (Offord *et al.*, 1995).

#### *Administration*

Rosemary can be administered in the form of infusion, decoction, ethanol extract (external use), tinctures, rosemary wine, drug-containing volatile oil, powdered drug, liquid extract, dry extract and rosemary oil (mainly for external use). The infusion is prepared by adding 2 g of herb to 1 l of boiling water (Santos García-Alvarado *et al.*, 2001).

### **25.4.5 Herbal pesticide**

In the agricultural industry, rosemary is known to possess insect-repellent and antimicrobial properties which are beneficial in growing and storage of crops. Comparative laboratory studies have been carried out where different herbal powders, including rosemary powder, were dusted on stored grains of wheat and French bean; analysis of the results of these treatments on the pest species *Sitophilus granarius* and *Acanthoscelides obtectus* revealed that grain wheat can be very effectively protected against *S. granarius* by dusting the grain with rosemary powder (Kalinovic *et al.*, 1997). Aphids are also repelled by the odour of rosemary and the mint *Mentha pulegium*. Based on GC-MS analysis, a few components of rosemary oil – i.e. 1,8-cineole, D-1-camphor,  $\alpha$ -1-camphor and  $\alpha$ -pinene – have been found to be the major principles repelling the aphids (Hori and Komatsu, 1997). The antirepellent property of rosemary oil to *Myzus persicae* is attributed to 13 compounds; the most important being linalool, D-1-camphor and  $\alpha$ -terpineol (Hori, 1998).

Rosemary oil (1 %) is found to reduce the fecundity rate of the predacious mites *Amplyseius zaheri* and *A. barkeri* (Momen and Amer, 1999). The repellent and oviposition-detering activities of rosemary oil on the spider mites *Tetranychus usticae* and *Eutetranychus orientalis* have also been reported (Amer *et al.*, 2001). Essential oil of rosemary in vapour form is shown to reduce fecundity, decrease egg hatchability, increase neonatal larval mortality and adversely affect offspring emergence of *Acanthoscelides obtectus* (Papachristos and Stomopoulos, 2002).

Essential oil of rosemary has also been reported to have antimicrobial activity against an array of bacterial and fungal species including *Listeria monocytogenes* and *Aspergillus niger* (Baratta *et al.*, 1998; Faliero *et al.*, 1999). Gram-negative bacteria such as *Staphylococcus aureus* and *S. epidermidis* have been found to be more



susceptible to rosemary oil than are other gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* (Pintore *et al.*, 2002). Rosemary leaves themselves are a source of antibacterial molecules; in laboratory studies, an active compound effective against the plant pathogen *Streptomyces scabies* has been isolated from the leaves of rosemary (Takenaka *et al.*, 1997).

## 25.5 Toxicology and quality control

Rosemary is generally considered safe and devoid of toxic side-effects if taken in recommended doses. However, certain guidelines for its use should be followed. (i) rosemary oil if internally consumed or ingested in excess can trigger convulsions; (ii) pregnant and lactating women are advised not to use rosemary, as are people with epilepsy; (iii) rosemary oil should be used with caution by persons suffering from hypertension, blood pressure or insomnia; (iv) rosemary leaves in excess quantity can cause coma, spasm, vomiting and, in some cases, pulmonary oedema.

There have been occasional reports of allergic reactions such as skin irritation when rosemary has been applied externally. In addition, rosemary essential oil may have epileptogenic properties as a handful of case reports over the past century have linked its use with seizures in otherwise healthy children (Burkhard *et al.* 1999). Rosemary may also interfere with iron absorption; hence it should be avoided by persons with iron deficiency anaemia (Samman *et al.*, 2001).

### 25.5.1 Adulteration

Rosemary oil can be adulterated with white camphor oil, fractions of eucalyptus oil, Spanish sage oil, oil of turpentine and petroleum. The quality of rosemary oil can be ascertained based on its solubility in alcohol and by using an iodine test. Rosemary oil does not detonate with iodine but just dissolves with heating (Diete and Brannt, 1892).

## 25.6 Conclusion

Rosemary is emerging as an important herb, being a potential source of anticancer molecules, a functional food, a botanical nutraceutical and a functional pesticide. Despite the multifaceted importance of the herb, it has yet to receive adequate research attention. In many places, it is grown either as a minor herb on marginal lands or still grows wild. Varietal improvement, quality profiling including adulteration detection, toxicology and organic cultivation practices are some areas that require immediate attention, in addition to post-harvest technology.

## 25.7 References

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## Saffron

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**Abstract:** Saffron, with its intriguing history, is the world's most valuable spice and has a great reputation. Saffron spice consists of dried *Crocus sativus* L stigmas, used either whole, cut or ground. It is important to note that this plant cannot survive without human handling, which is why it does not grow in the wild. This chapter presents the origins, history, classification, biology and morphology of saffron. Its chemical composition and dehydration procedures are described, together with its production and distribution, functional properties in relation to food, textiles, medicine (both traditional and modern) and others. Quality issues and applicable standards and specifications are described.

**Key words:** saffron spice, *Crocus sativus* L., colour, aroma, flavor, pharmacological effects.

### 26.1 Introduction

Saffron is considered the most valuable, renowned and intriguing spice. Much of this intrigue derives from several fabulous myths and legends regarding its origin, which are frequently disputed between the current production areas. Saffron is the only known plant that uses its own stigmas for spreading. Unable to reproduce naturally, this mutant plant with its tiny stigma (2.5 cm) is dependent on human handling and care for its survival. Hundreds of animal and plant species, with complete reproductive systems, have disappeared from our planet, yet this tiny, sterile plant has been maintained for centuries, without the least evolution, thanks to an uninterrupted chain of gardeners, farmers, travellers and merchants who used it for its colouring and medicinal properties, as well as for its flavour.

Another explanation for the intrigue inspired by saffron is that, despite the plant's inability to reproduce sexually, it has retained its beautiful colour, heady perfume and abundant pollen production, all of which are evolutionary tools to ensure its perpetuation by pollinating bees. The saffron plant blooms in mid-autumn when other plants are preparing for the winter. It can flourish when no other plant does in the most hostile edaphic and weather conditions, but only for a very short period of time.

Saffron is valued not only for its price, but also for its attributes; it is the only spice capable of giving food colour, flavour and aroma. The most expensive spice in



the world, the high price of saffron is due to its production costs. The methods of plant cultivation and its subsequent transformation have not changed for the last four centuries and a great deal of labour is required to collect the stigmas at almost ground level. However, while it is true that its price per weight unit is the highest of the spices, the high concentration of active ingredients in saffron makes its use as a spice inexpensive, as only very small amounts need to be used to spice foods. Saffron is also valuable because it is used as a textile pigment and body cosmetic as well as for relieving the symptoms of several human diseases.

Beginning with the Sumerians, saffron has played an important role in religious practices and rituals in various cultures throughout history. Often adopted as a symbol of light, wisdom and spirituality, monarchs, as well as political and religious leaders (including the Dalai Lama), wore, and still wear, saffron yellow tunics (Suvatbandhu, 1968). Saffron's reputation has lasted to this day, when many of its health claims have been proven by scientists, and it is used as a spice in cuisines all over the world.

### 26.1.1 Origin

There are several proposals concerning the origins of saffron. It may have originated in Egypt since it is represented in hieroglyphs, which experts have dated as being from approximately 4000 bc (Pérez-Bueno, 1995). Saffron is also cited by Salomon in the 'Canticle of Canticles' (1100–1000 bc) under the name of 'karkom', a word of Indian derivation, leading some authors to locate its origin in the region of Kashmir, where its cultivation has traditionally been most significant (Toussaint-Samat, 1991). Other authors propose Greece as the birthplace of saffron due to the large quantity of species from the *Crocus* genus that grow in this area (Ordoudi and Tsimidou, 2004) and also because of the important Minoic archaeological remains, which are in an excellent state of preservation. Wall paintings in the palace of Minos (Knossos, Crete) date back to 1700–1600 bc and those at Akrotiri, on the island of Thera (Santorini, Cyclades), are dated at 1500 bc and depict saffron fields and young women harvesting the flower.

It is likely that, due to the importance of saffron in the various cultures established in the Mesopotamia region, that this is the true origin of the of this plant and that Greece was the physical bridge for it to enter Europe. Some experts do not doubt that saffron was known to the Sumerian civilization (sixth millennium bc). Saffron is found on a list of foods in the abundant Sumerian documentation transcribed on clay tablets from the period of Ur (3000–2000 bc). Later (approximately 2400 bc) references appear for its use as a clothes dye for tunics (Pérez-Bueno, 1995). It was also important in the Acadia culture (2350–2150 bc), whose first king of note, Sargon, was born in 'Azupiranu'. The name of this population nucleus on the banks of the Euphrates River could mean 'Saffron city' (Polunin and Smythies, 1981), which might be the etymological origin of the term saffron. After the end of the Sumerian and Acadian civilizations, saffron continued to be popular in Mesopotamia during the development of Babylonian culture. Several texts speak of its use as a condiment during the reign of Hammurabi (1800–1700 bc). It was also used by dyers and painters (Pérez-Bueno, 1995).



### 26.1.2 Botanical classification and geographical distribution

Saffron (*Crocus sativus* Linnaeus) belongs to the class Liliatae (monocotyledons), sub-class Liliidae, order Liliales, Iridaceae family and genus *Crocus*. The *Crocus* genus comprises about 85 species distributed throughout Mediterranean Europe and Western Asia. The plants of this genus are well adapted to areas with cold winters and warm, dry summers. Saffron is considered an undemanding plant due to its enormous capacity for adaptation (Rubio, 1997). It tolerates an enormous variety of ecological edaphic, as well as climatic, conditions. As a cultivated plant, saffron can be found in altitudes ranging from sea level to almost 2000 m, although it is more acclimatized to hillsides, plateaux and mountain valleys in altitudes between 600 and 1200 m, supposedly its original conditions. This plant can be cultivated in arid or semi-arid areas where the water deficit is extreme in summer. As far as temperature is concerned, it can withstand rigorous environmental temperatures of  $-15^{\circ}\text{C}$  or  $-20^{\circ}\text{C}$  in winter and  $35\text{--}45^{\circ}\text{C}$  in summer, which does not mean it is insensitive to them (Pérez-Bueno, 1989).

### 26.1.3 Reproductive biology

Saffron (*C. sativus* L.) is a triploid species, with a  $3n = 24$  number of chromosomes. This causes irregular meiosis in the formation of gametes giving rise to a high percentage of infertile pollen and ovules (Chichiricco and Grilli, 1984; Chichiricco, 1987, 1989; Grilli and Chichiricco, 1991). Besides the absence of pollen, germination in the stigma renders the species sterile (Grilli, 1999). Contradictory opinions exist about the genetic origins of this saffron and it is not known for certain how it came into being. When nuclear DNA from *C. sativus* L. is compared to other diploid species from the same family, the most probable predecessor seems to be *C. cartwrightianus* Herbert, although *C. thomasii* Ten. and *C. hadriaticus* Herbert are also possible ancestors (Grilli, 2004). However, some authors are now beginning to think that saffron is an allopolyploid, whose genetic matter would have come from two species and not from only one (Jacobsen and Øgaard, 2004).

Saffron corms continue multiplying by vegetative reproduction until they have no more space, at which stage flower production is progressively reduced until it disappears entirely. Humans maintain plant reproduction by removing corms from the ground and separating them so that the stigmas can be extracted and used as a spice. Saffron blooms in autumn when other plants are preparing to protect themselves against the rigours of winter and, again in contrast to other plants, its activity slows down in spring. Some authors have defined five or six stages (Chrungoo and Farooq, 1985; Jirage *et al.*, 1994; Álvarez, 2003) to describe the biological cycle of saffron which lasts two years from the time that the buds form through the plant, reaching its full development a year later and finally to its disappearance to give rise to one or more offspring.

### 26.1.4 Plant morphology

Saffron (Fig. 26.1) is a perennial, herbaceous, 10–25 cm high plant that develops from corms. From a botanical point of view, a corm is a short, thick shoot, similar to an onion, except that it is a solid structure, which does not consist of numerous

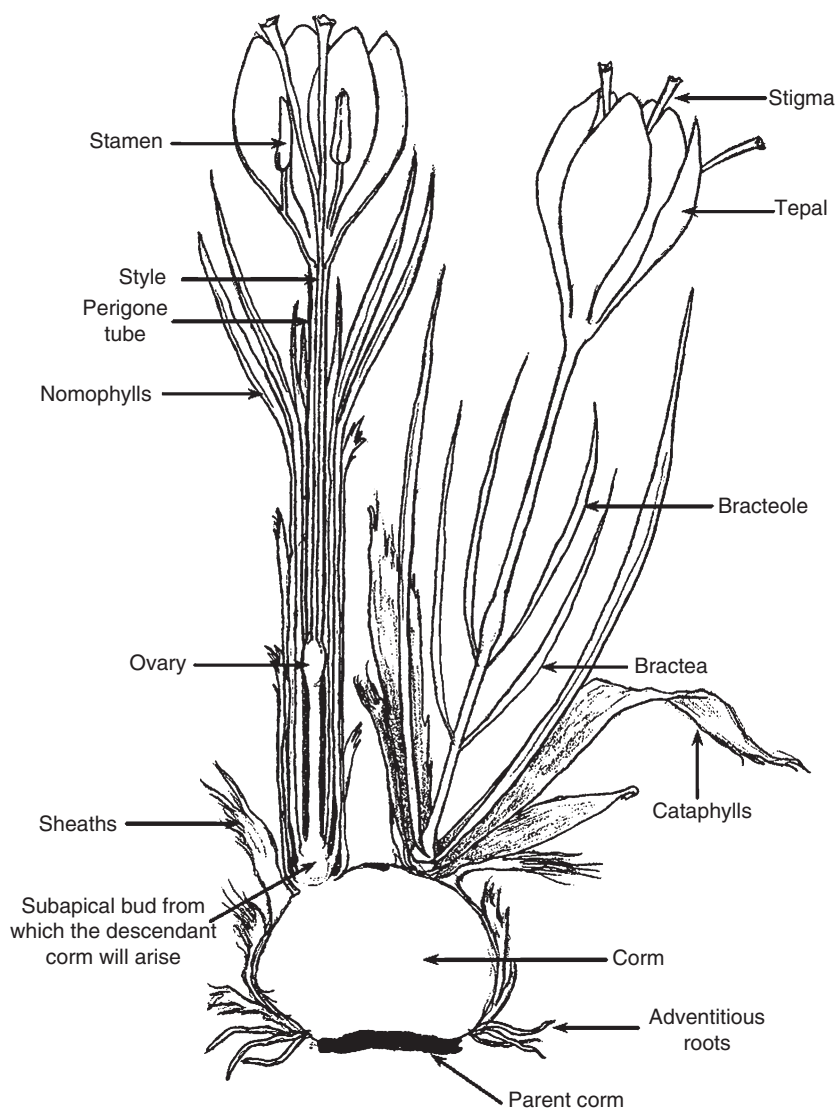


**Fig. 26.1** Diagram of the organography of *Crocus sativus* L.

concentric layers. It is a subsoil organ that measures 5–7 cm in diameter and is protected by fibrous reticulated leaf tunics coming from the degeneration of various foliar structures that constituted the sprouts from which it was formed (Oromí, 1992). As previously mentioned, saffron multiplies by vegetative reproduction.

When the corm is activated and buds begin to protrude, three to five structures of foliar origin, called cataphylls, form a tube of a whitish colour and membranous consistency (from 5–20 cm) which surrounds and protects the other foliar and floral structures that grow longitudinally and simultaneously in their interior (Botella *et al.*, 2002). Between five and 11 green leaves or nomophylls are found per sprout and are usually called bristles, horsehair or esparto grass (Luceño, 1999). They are very narrow, between 1.5 and 2.5 mm wide, dark green in colour, linear, with a white

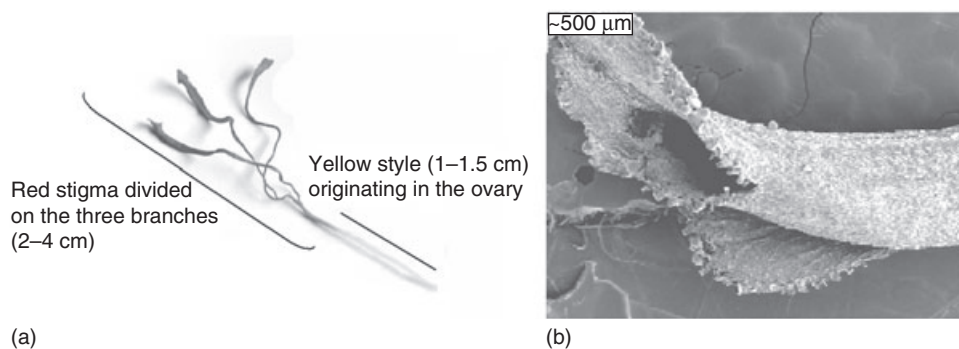


**Fig. 26.2** (a) *Crocus sativus* L. stigma and style after drying. (b). Photograph from an electron microscopy scan of the distal part of a *Crocus sativus* L. stigma filament, where grains of pollen are observed.

band on their inner side and a vein on the outer side. They can measure about 50 cm although excessive irrigation and certain types of management can make them reach 1 m in length (Moya, 1998). Flowers, known as the saffron rose, sprout from the bractea axils. They consist of six tepals, three inner and three outer ones, united in a long tube that starts at the top of the ovary. Tepals are violet-coloured with darker veins. Flowers are erect and regular in form and, depending on water availability, the flowers can appear before or simultaneously to green leaves. There are one to three flowers per stem and two or three stems per plant. Three yellow stamens emerge from the upper part of the afore-mentioned tube.

The long, yellowish-white, filiform style starts at the subsoil ovary apex, crossing through the perianth tube and ending in a sole stigma of three bright red branches or filaments (Fig. 26.2a), the part of the plant for which man cultivates it. The filaments, 25–32 mm each, are tubular (Fig. 26.2b) in appearance and somewhat rolled up. They become progressively thicker from their base, to end in wide papils in the form of a scalloped trumpet, where grains of pollen are usually found. Their length is disproportionate in comparison to the other floral organs, so they rest on the tepals or hang outside the flower.

## 26.2 Chemical composition

The stigmas are the reason why men cultivated the saffron plant, but until the stigmas are dehydrated, losing 80 % of their weight, they do not turn from a plant into a spice. Fresh stigmas do not have the qualities for which the saffron spice is prized. Raw stigmas have neither flavour nor aroma and their colouring strength is very poor. There is some evidence that high-temperature procedures would promote the production of compounds responsible for flavour and aroma (Loskutov *et al.*, 2000; Pardo *et al.*, 2002; Carmona *et al.*, 2006b), supporting the idea that during the dehydration stage, important physical and chemical changes take place. For this reason, the traditional methodologies in different countries employing low temperatures are being adapted to the methods employed in Italy, Greece or Spain, focusing

**Table 26.1** Crocetin ester identification by several authors

| Crocetin esters structure    |                            |              | Crocetin esters identified in the bibliography |                           |    |                                 |    |                              |    |                                   |    |                                    |    |                            |    |                               |    |
|------------------------------|----------------------------|--------------|------------------------------------------------|---------------------------|----|---------------------------------|----|------------------------------|----|-----------------------------------|----|------------------------------------|----|----------------------------|----|-------------------------------|----|
| n° glucose moieties (R + R') | Moiety distribution (R/R') | Isomer       | Named as                                       | Ichi <i>et al.</i> , 1995 |    | Tarantilis <i>et al.</i> , 1995 |    | Pfister <i>et al.</i> , 1996 |    | Straubinguer <i>et al.</i> , 1997 |    | Van Calsteren <i>et al.</i> , 1997 |    | Knapp <i>et al.</i> , 2002 |    | Carmona <i>et al.</i> , 2006c |    |
|                              |                            |              |                                                | Gj                        | Cs | Cs                              | Gj | Cs                           | Gj | Cs                                | Gj | Cs                                 | Cs | Gj                         | Cs | Cs                            | Gj |
| 5                            | trig/gen                   | <i>trans</i> | <i>trans</i> -5-tG                             |                           |    |                                 |    |                              |    |                                   |    |                                    |    |                            |    | X                             | X  |
|                              |                            | <i>cis</i>   | <i>cis</i> -5-tG                               |                           |    |                                 |    |                              |    |                                   |    |                                    |    |                            |    | X                             | X  |
|                              | nea/gen                    | <i>trans</i> | <i>trans</i> -5-nG                             |                           |    | X                               |    | X                            |    |                                   |    |                                    |    |                            |    | X                             | X  |
|                              |                            | <i>cis</i>   | <i>cis</i> -5-nG                               |                           |    | X                               |    |                              |    |                                   |    |                                    |    |                            |    | X                             | X  |
|                              | nea/glu                    | <i>trans</i> | <i>trans</i> -4-ng                             |                           |    |                                 |    |                              |    |                                   |    |                                    |    |                            |    | X                             | X  |
| 4                            |                            | <i>cis</i>   | <i>cis</i> -4-ng                               |                           |    |                                 |    |                              |    |                                   |    |                                    |    |                            |    | X                             | X  |
|                              | gen/gen                    | <i>trans</i> | <i>trans</i> -4-GG                             |                           |    | X                               |    | X                            |    | X                                 |    | X                                  |    |                            |    | X                             | X  |
|                              |                            | <i>cis</i>   | <i>cis</i> -4-GG                               |                           |    | X                               |    | X                            |    |                                   |    | X                                  |    |                            |    | X                             | X  |
|                              | gen/glu                    | <i>trans</i> | <i>trans</i> -3-Gg                             | X                         |    | X                               |    |                              |    | X                                 |    | X                                  |    |                            |    | X                             | X  |
| 3                            |                            | <i>cis</i>   | <i>cis</i> -3-Gg                               |                           |    | X                               |    |                              |    |                                   |    | X                                  |    |                            |    | X                             | X  |
|                              | gen/-                      | <i>trans</i> | <i>trans</i> -2-G                              | X                         |    | X                               |    | X                            |    | X                                 |    | X                                  |    |                            |    | X                             | X  |
|                              |                            | <i>cis</i>   | <i>cis</i> -2-G                                | X                         |    |                                 |    |                              |    |                                   |    |                                    |    | X                          |    | X                             | X  |
| 2                            | glu/glu                    | <i>trans</i> | <i>trans</i> -2-gg                             | X                         |    | X                               |    |                              |    | X                                 |    |                                    |    |                            |    | X                             | X  |
|                              |                            | <i>cis</i>   | <i>cis</i> -2-gg                               |                           |    | X                               |    |                              |    | X                                 |    |                                    | X  |                            |    | X                             | X  |
|                              | glu                        | <i>trans</i> | <i>trans</i> -1-g                              | X                         |    | X                               |    |                              |    | X                                 |    |                                    |    |                            |    | X                             | X  |
|                              |                            | <i>cis</i>   | <i>cis</i> -1-g                                | X                         |    |                                 |    |                              |    |                                   |    |                                    |    |                            |    | X                             | X  |
|                              |                            |              |                                                |                           |    |                                 |    |                              |    |                                   |    |                                    |    |                            |    |                               |    |

Gj = *Gardenia jasminoides* Ellis; Cs = *Crocus sativus* L.

especially on the last one, since Mancha saffron has a long reputation of being the most aromatic and flavourful variety.

### 26.2.1 Dehydration procedure

In Italy, the dehydration process is carried out by placing fresh stigmas in a sieve situated some 20 cm above oak embers. Halfway through the process, which lasts between 15 and 20 minutes, they are turned over to assure homogeneous drying. The process is considered complete when stigmas contain between 5 and 20 % moisture, the moment when they possess some elasticity when squeezed between one's fingers (Tammaro, 1999). In Greece, the mixture of fresh stigmas and part of the stamens is spread out in a thin layer of 4–5 mm on 40 × 50 cm trays with the bottom covered by silk cloth. These trays, 50 cm apart, are placed on vertical supports in rooms prepared for dehydration with a room temperature kept between 35 and 45°C and a relative humidity never surpassing 50 % (Ordoudi and Tsimidou, 2004). The drying process is complete when moisture is reduced to 10–11 %, usually after 12 hours (Goliaris, 1999). In Spain, stigmas are placed on a sieve with a silk bottom that is placed over the heating source, which can be a gas cooker, live vine-shoot charcoal or, to a lesser extent, an electric coil (Carmona *et al.*, 2005). The process is finished when the sample has lost between 85 and 95 % of its moisture, after being dried at between 50 and 80°C for 30 or 60 min (Pardo *et al.*, 2002; Del Campo *et al.*, 2010). In other countries such as England and France where saffron was cultivated in the past and where nowadays its cultivation is anecdotic, high temperatures were also used during dehydration (Pellowe, 1989; Aucante, 2000).

The countries that are adapting their traditional methods are mainly India, Iran and Morocco, where drying takes place at room temperature. In India, stigmas dry in the sun for 3–5 days until humidity content is reduced to 8–10 % (Nauriyal *et al.*, 1997; Sampathu *et al.*, 1984). Around Kashmir, the usual procedure is to dry the whole flower in the sun and later to separate the three filaments from the stigma (Hassnain, 1998). In the case of Iran or Morocco, stigmas are placed in a cloth on the ground in thin layers and dried for 2 hours in the sun or for 7–10 days in the shade (Ait-Oubahou and El-Otmani, 1999).

### 26.2.2 Compounds responsible for colour, taste and aroma

The dehydration process releases the characteristic compounds of saffron or possibly even generates them. The most relevant compounds can be classified into three groups, those ones responsible for colour, taste and aroma. Unusual water-soluble carotenoids derived from crocetin ( $C_{20}H_{24}O_4$ , 8,8'-diapo- $\Psi,\Psi'$ -carotenedioic acid), where glucose, gentiobiose, neapolitanose or triglucose are the sugar moieties and where *trans*- or *cis*-configuration is found, are the substances responsible for the intense colour that saffron gives in aqueous solutions. This family of compounds (Table 26.1) represents between 0.5 and 32.4 % on a dry basis.

Picrocrocin (4-( $\beta$ -D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) is considered to be the foremost contributor to saffron's bitter taste, although other compounds such as picrocrocin-related ones, kaempferols and amino acids could contribute to this taste too (Carmona *et al.*, 2007a). The

picrocrocin content found in saffron spice on a dry basis has been reported as ranging from 0.8 to 26.6 % (Iborra *et al.*, 1992; Alonso *et al.*, 2001a; Sánchez *et al.*, 2008). The taste detection threshold of picrocrocin has been set at 10 mg L<sup>-1</sup> (Sánchez *et al.*, 2009). The flavonoid fraction of saffron is made up of glycosides of kaempferol (Winterhalter and Straubinguer, 2000), being five new compounds (kaempferol-3-O-sophoroside-7-O-glucoside, the kaempferol-7-O-sophoroside, the kaempferol-3-O-sophoroside, the kaempferol-3,7,4'-triglucoside, and a kaempferol tetrahexoside) recently identified by Carmona *et al.* (2007a). A recent study of free amino acids revealed that alanine, proline and aspartic acid are the major amino acids in saffron spice (Del Campo *et al.*, 2009). Some picrocrocins, picrocrocin related derivatives and flavonoids found in saffron are illustrated in Fig. 26.3.

The compounds that make up the saffron aroma and its glycosidic precursors have received increased attention in recent years (Winterhalter and Straubinguer, 2000, Carmona *et al.*, 2007b). More than 160 volatile compounds have been reported in the saffron spice, although some authors asserted that many of them could be generated as artefacts when exhaustive isolation procedures are employed (Carmona *et al.*, 2007b). Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde; compound 1 – Fig. 26.4) is the major compound in the volatile fraction of saffron (Semiond *et al.*, 1996; Alonso *et al.* 1998a), representing around 70 % and between 1.07 and 5.670/000 of dry matter weight (Alonso *et al.*, 2001b). Other main compounds in terms of aroma chemistry are 3,5,5-trimethyl-2-cyclohexene-1-one (isophorone; compound 2 – Fig. 26.4), 2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone; compound 5 – Fig. 26.4), 3,5,5-trimethyl-3-cyclohexen-1-one (an isomer of isophorone; compound 18 – Fig. 26.4), 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (an isomer of safranal; compound 43 – Fig. 26.4), 2,2,6-trimethyl-1,4-cyclohexanedione (compound 4 – Fig. 26.4), HTCC and 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one (compound 19 – Fig. 26.4).

There is some evidence that the three types of compounds – crocetin esters, picrocrocin (and its related compounds) and volatiles – could be inter-related. In addition to the scheme presented in Fig. 26.5 in which crocetin esters are converted into picrocrocin (and its related compounds), which afterwards will be transformed into the volatile compounds (preferably safranal), it has been demonstrated that crocetin esters can convert directly to volatile compounds without passing through the picrocrocin stage (Carmona *et al.*, 2006c, 2007b).

In addition to most characteristic compounds the general composition of saffron spice is shown in Table 26.2.

### 26.2.3 Changes during storage

Maggi *et al.* (2010) studied how saffron's volatile compounds and its sensory characteristics change during storage. They analysed 73 samples with different storage times (<1 year, 3–4 and 8–9 years) in order to determine aromatic descriptors obtained by gas chromatography–olfactometry (GC–MS/FID–O). Saffron stored for less than 1 year contained a higher proportion of saffron, flower and spicy descriptors, while the oldest stored saffron (3–4 and 8–9 years of storage) contained volatiles with vegetal, caramel and citrus aromas. The aromas that differed most due to saffron storage were spicy, freshly cut grass and vegetable (Carmona *et al.*, 2007b, Maggi *et al.*, 2010).

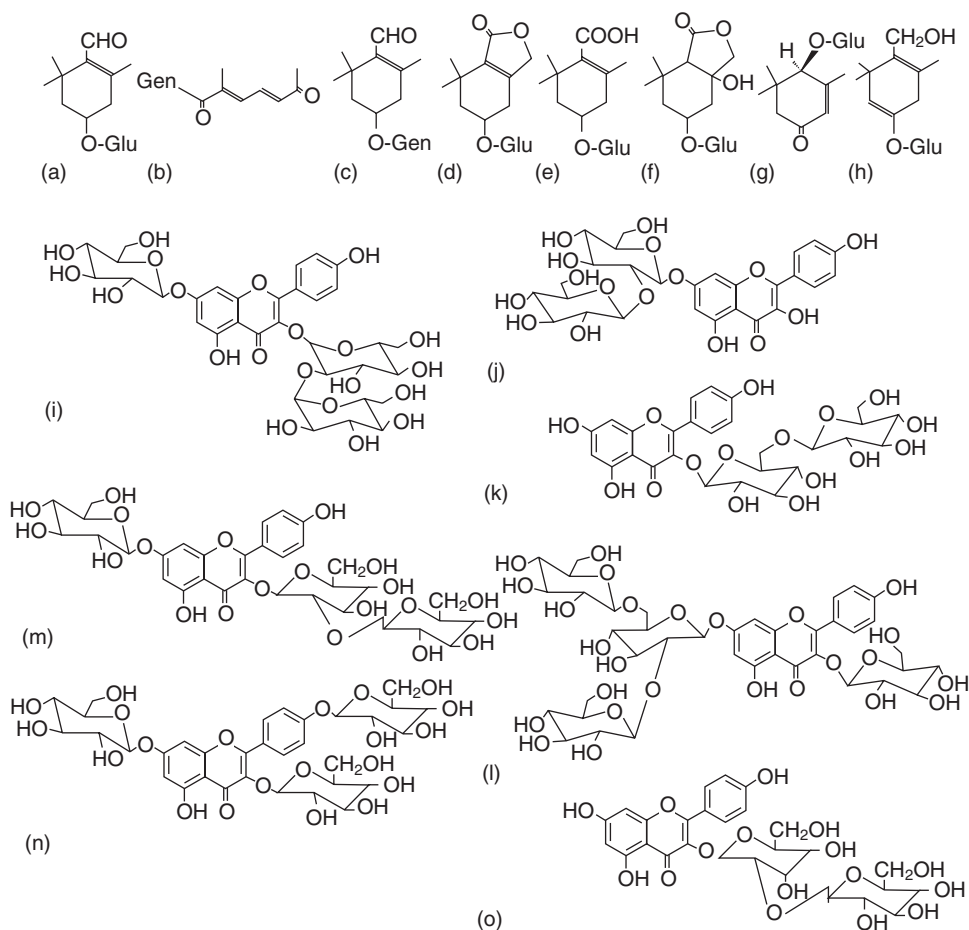


**Fig. 26.3** Structure of picrocrocin, picrocrocin-related derivatives and flavonoids found in saffron by various authors. (a) Picrocrocin (b) 2-methyl-6-oxo-2,4 heptenate of O- $\beta$ -D-gentibioside; (c) 4-hydroxy-2,6,6-trimethyl-1-cyclohexen carbaldehyde 4-O- $\beta$ -D-gentibioside; (d) 5-hydroxy-7,7-dimethyl-4,5,6,7-tetrahydro-3H-isobenzofuranone 5-O- $\beta$ -D-glucopyranoside; (e) 4-hydroxy-2,6,6-trimethyl-1-cyclohexene carboxylic 4-O- $\beta$ -D-glucopyranoside acid; (f) 3',5'-dihydroxy-7,7-dimethyl perhydro-isobenzofuranone 5-O- $\beta$ -D-glucopyranoside; (g) 4-hydroxy-3,5,5-trimethyl-2-cyclohexenone 4-O- $\beta$ -D-glucopyranoside; (h) 4-hydroxymethyl-3,5,5-trimethyl-2-cyclohexenone 4-O- $\beta$ -D-gentibioside; (i) kaempferol-7-O-glucopyranosyl-3-O-sophoroside; (j) kaempferol-7-O-sophoroside (k) kaempferol-3-O-gentibioside; (l) kaempferol 3-glucoside 7-neoplitanoside; (m) kaempferol-3-sophoroside-7-glucoside; (n) kaempferol-3,7,4'-triglucoside; (o) kaempferol-3-galactoside-glucoside.

## 26.3 Production and distribution

### 26.3.1 Producer countries

The high adaptability of the saffron plant is manifested when comparing crop conditions in the different saffron-producing countries. In Castile-La Mancha, it grows at an altitude of about 600 m with a pluviosity of 300–400 mm and a continental climate of very hot summers and very cold winters with regular frosts. In Italy, it is cultivated in Aquila, Cerdeña and Emilia-Romagna (Negbi, 1999) and recently the crop was reintroduced in San Gimignano (La Toscana) (Galigani and Garbati, 1999).



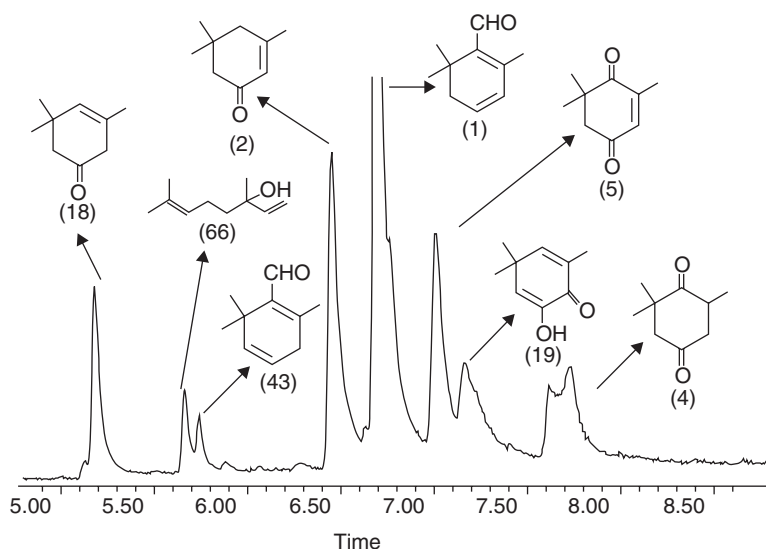


**Fig. 26.4** Structure of main volatiles found in saffron.

Note: (1) Safranal; (2) 3,5,5-trimethyl-2-cyclohexene-1-one (isophorone); (4) 2,2,6-trimethyl-1,4-cyclohexanedione; (5) 2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone); (18) 3,5,5-trimethyl-3-cyclohexen-1-one (an isomer of isophorone); (19) 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one; (43) 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (an isomer of safranal); (66) linalool.

In the region of Aquila, it grows in sub-mountainous areas between 650 and 1100 m, which involves lower temperatures and an annual precipitation of 700 mm (Tammara, 1999). In Azerbaijan, it is cultivated on the peninsula of Apsheron near Baku in a region with a reduced pluviometry of 223 mm (Azizbekova and Milyaeva, 1999). In Morocco, it is cultivated in several areas although around Taliouine is the preferred one. Here, saffron grows at an altitude between 1200 and 1400 m, near the Atlas Mountains with relatively cold winters and extremely low precipitation of between 100 and 200 mm, making watering the crop obligatory (Chitt *et al.*, 1985; Wallach, 1989; Ait-Oubahou and El-Otmani, 1999). In contrast, in Greece, saffron grows in the area of Kozani where rain is frequent and winter temperatures rarely go below 0°C (Ordoudi and Tsimidou, 2004). In India, it is principally produced in Kashmir and Jammu, at an altitude of 1600 m, but also in other areas such as Uttar Pradesh and Himachal Pradesh (Dhar and Mir, 1997). In Iran, it is grown in the region of Khorasan and, to a lesser extent, in others such as Fars, Kerman and Yazd.

In recent years, the annual world production of saffron has been about 200 tons and the main producer is Iran (which accounts for approximately 90 % of total production), followed by India (5 %), Greece (2 %), Spain (1 %) and Morocco (less than 1 %) (Carmona *et al.*, 2006c). Concern has been a growing about guaranteeing and defending the quality of saffron historically produced in certain regions (Alonso *et al.*, 2007) and many Protected Designations of Origin (PDO) and quality labels have been created in Europe: *Azafrán de La Mancha*, *Artesanía Alimentaria de Aragón*, *Munder Saffran*, *Zafferano di Sardegna*, *Zafferano purissimo de San Gimignano*, *Zafferano delle Colline Fiorentine*, *Zafferano dell'Aquila* and *Krokos Kozanis*.

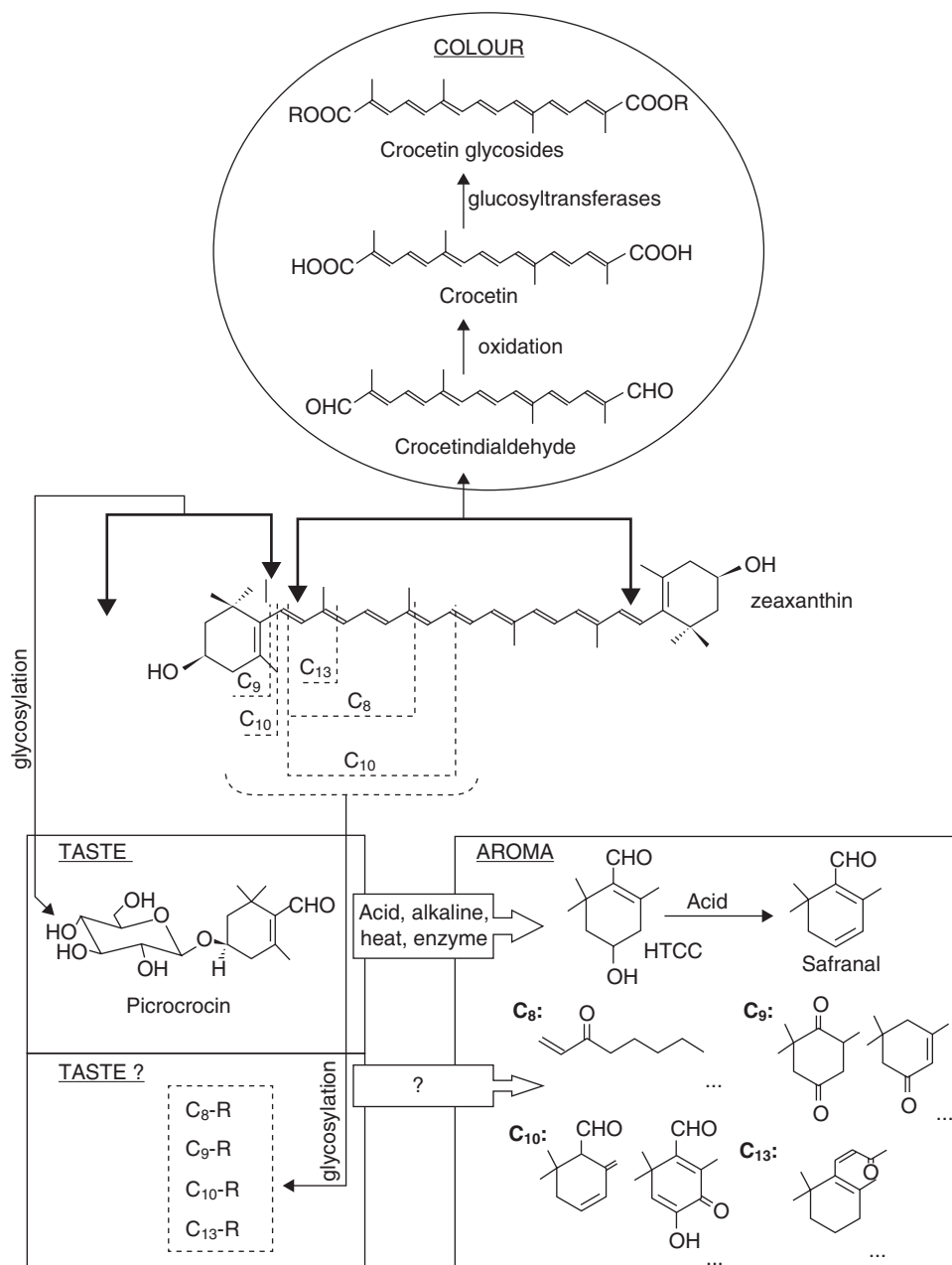


**Fig. 26.5** Generation mechanism for picrocrocin, related compounds and volatile compounds.

**Table 26.2** Approximate composition of saffron spice

| Component                 | %                    |
|---------------------------|----------------------|
| Moisture                  | 6–12                 |
| Mineral matter            | 4–8.5                |
| Raw fibre                 | 4–5                  |
| Proteins                  | 10–14                |
| Total sugars <sup>1</sup> | 26–27                |
| Gentibiosides             | 2–3                  |
| Glucose                   | 7–8                  |
| Fructose                  | 1–2                  |
| Xylose and ramnose        | traces               |
| Pentosanes                | 6–7                  |
| Starch                    | 6–7                  |
| Pectins                   | 6                    |
| Gums and dextrans         | 9–10.5               |
| Essential oil             | 0.3                  |
| Fats <sup>2</sup>         | 5–8                  |
| Vitamins <sup>3</sup>     | 0.3–138 <sup>4</sup> |

Notes:

<sup>1</sup> Including those from pigments after hydrolysis.

<sup>2</sup> Stigmasterol, campesterol, b-sitosterol among others.

<sup>3</sup> Riboflavin and thiamine.

<sup>4</sup> in µg/g.

Sources: Data elaborated from Lewis *et al.* (1981); Sampathu *et al.* (1984); Rios *et al.* (1996); Basker (1999).

### 26.3.2 Traditional production

Edaphic characteristics vary significantly between different production areas, but it is of little concern since the saffron plant grows well in soils of very diverse composition. The crop is maintained for 4 years in Spain, 3–5 years in Azerbaijan (Azizbekova and Milyaeva, 1999), 7 years in Greece (Goliaris, 1999), 5–12 years in Morocco (Ait-Oubahou and El-Otmani, 1999), 6–15 years in Iran (Ordoudi and Tsimidou, 2004), and for as many as 20 years in India. In Italy, however, the crop is lifted every year to prevent problems with fungal pests due to high pluviometry (Tammamo, 1999).

In general, saffron is planted by hand between June and September. Yield is improved when planting is earlier (Sadeghi *et al.*, 2003). Planting close to the surface at 10 cm produces a noticeable increase in flowering or in the total capacity to take root in comparison with identical conditions at 20 cm below the surface (Muñoz, 2000). In Castile-La Mancha, planting is done in furrows, maintaining a separation of approximately 35 cm in between. Two rows of corms are planted in each furrow, separated 8–10 cm apart and arranged either parallel or alternately, which is characteristically known as ‘tresbolillo’. Corms in each line are separated less than 5 cm apart (Alonso *et al.*, 1998b) with from 30–60 corms/m<sup>2</sup> (Pérez-Bueno, 1995; Rubio, 1997). In Morocco, a handful of two or three corms are planted in the furrow at a depth of 15 cm, separated by 10 or 15 cm, which involves using some 3 tm/h (Ait-Oubahou and El-Otmani, 1999). In Greece, corms are planted with a separation of between 11 and 13 cm and at a depth of 15–17 cm in furrows 20–25 cm apart, which means a planting density of 230–250 000 corms/ha (Goliaris, 1999). In Italy, corms

are planted at a depth of 8–10 cm either without leaving a separation between corms or having at most a 2 or 3 cm separation which is the greatest planting density, between 60 and 70 corms/m<sup>2</sup> requiring 13 to 15 tm/ha (Tammaro, 1999).

Flowering occurs in Castile-La-Mancha between mid October and mid November, but can be earlier or later depending on rain and temperatures during the preceding months (Garrido *et al.*, 1985). The time of flowering varies between 15 and 25 days, of which between 2 and 6 constitute the so-called 'cover period' when flowering is at a maximum. The extension of this phenomenon and its distribution over time depends on the corm size (Gómez *et al.*, 1988). Flowers emerge throughout the night and are found in the form of buds at dawn. Sunlight makes them open throughout the day (Rueda, 2003). In cases where the flower is closed, it is held between one's fingers and lightly pushed towards the ground so the perianth tube breaks far below the aerial part that is beginning to appear. In other countries where the opened flower is picked, the thumbnail is used to cut the flower.

Maximum foliar development is attained in February. In April, once frosts are over, the leaves, which will have begun to dry up, were traditionally cut for use as forage for livestock. However, the majority of farmers have given up this practice and now leave them to dry in the field (Pérez-Bueno, 1995). Once the plant enters the dormant stage, the land is ploughed again to eliminate weeds, or plantation is lifted to clean and collect the corms previously planted for re-planting in a different field.

Fungi attacks are the biggest problem for saffron crops due to the variety of strains. The most dangerous of all is *Rhizoctonia violacea*, also known as 'violet root rot,' since it is very difficult to eradicate once it appears. It is not widely present in Castile-La Mancha due to extreme temperatures and dry climate, but in other latitudes saffron cultivation would be impossible without the use of fungicides. Other relevant losses are caused by field mice (*Pitymis duodecimcostatus*), which devour and destroy corms, or nematodes (*Ditylenchus dipsaci*) (Ait-Oubahou and El-Otmani, 1999). The symptoms caused in leaves are similar to those caused by a frost; growth is slowed and the plant is too small in size (Moore *et al.*, 1989).

### 26.3.3 Modern alternatives to traditional production

To improve upon the traditional methods of saffron production in the field, several alternative options have been considered, such as the mechanization of field crop, forced flowering under controlled conditions and *in vitro* cultivation.

#### *Mechanization of field crop*

There are two tasks in which mechanization has made important progress: the planting of the corms and the collection of the flower. Technology applied to other bulbous plants can also be applied to saffron and commercial planting machinery for crocus, freesia and gladiolus can be used for corms of saffron without requiring any adaptation. Adapted garlic, onion or potato planters are currently used at least in Italy, Greece and Spain (Galigani and Garbati, 1999; Goliaris, 1999).

There are several more or less sophisticated machines which are used to collect flowers in the field. Using a cutting bar, the flowers are cut and transported by a conveyor lift up in containers or boxes arranged for that purpose (Alonso *et al.*, 2007). This method increases the yield of cut flowers and reduces production costs.

There are, however, some drawbacks. The level of impurities increases and the stigmas of open flowers sometimes stain the ground. These machines also cut the leaves that have sprouted in the flower, which could further affect the development and future growth of corms (Tammaro, 1999).

#### *Forced flowering under controlled conditions*

This approach requires large quantities of corms that are usually produced in a mechanized field. Corms for the flowering process under controlled conditions are bound to have flourished at least twice in the field. These corms should be stored in trays in ventilated chambers at 0 °C for the buds to stop or at 30 °C and between 70 and 80 % relative humidity, and for no more than 150 days, otherwise the corms could begin to germinate and, therefore, produce a limited number of flowers. CO levels should not exceed 2500 ppm. Another parameter to be controlled is the ethylene level which can break corm dormancy, stimulate flowering and cause physiological disorders (Molina *et al.*, 2004).

Floral initiation occurs in corms of more than 20 mm in diameter, at temperatures between 23 and 27 °C and with irrigation. Corms placed in the trays are filled with an inert substrate such as vermiculite or arlita, allowing levelling, helping to retain moisture and serving as an anchor for shoots and roots. The density of planting corms in trays is directly linked to their size, but a guideline average is 472 corms/m<sup>-2</sup> (Molina *et al.*, 2004). It is advisable to maintain a temperature of about 17 or 18 °C before flowering occurs. Under these conditions, a 45–60 day waiting period is required for flowering. The corms should be illuminated only during the flowering period to avoid etiolation, the disproportionate growth of leaves and floral tube, which depletes stocks and prevents the corms from producing flowers. The flowering period can last about 100 days, while the average duration of flowering for each batch of corms is 13 days (Molina *et al.*, 2005). The corms are kept in the trays until the end of flowering, before being transplanted back to the field for multiplication.

#### *In vitro cultivation*

Numerous attempts using diverse starting materials – corm tissue, apical and axillary buds, leaves, nodes and several floral organs – have been carried out in this field looking for two main goals: multiplication of the corms and obtaining the stigma (Homes *et al.*, 1987; Himeno *et al.*, 1988; Isa and Ogasawara, 1988; Milyeva *et al.*, 1988, 1995; Fakhari and Evans, 1990; Plessner *et al.*, 1990; Sarma *et al.*, 1990, 1991; Visvanath *et al.*, 1990; George *et al.*, 1992; Dhar and Sapru, 1993; Agüero and Tizio, 1994; Ahuja *et al.*, 1994; Piqueras *et al.*, 1999; Ebrahimzadeh *et al.*, 2000).

The approach of *in vitro* corm multiplication resulted in a lower rate of saffron plant reproduction compared with field multiplication and the high economic cost is simply not viable. This application could, however, prove useful for supplying the farmer with a quality, pathogen-free seed, with a consistent behaviour over time.

In relation to *in vitro* stigma production, abnormal tubular structures similar to stigmas were obtained from various flower and ovary parts, containing crocetin esters, but in smaller proportions (Sano and Himeno, 1987; Sarma *et al.*, 1990, 1991). It did not, however, prove possible to obtain intact saffron stigmas, which could be commercialized through the usual distribution channel. This would limit its application to obtaining metabolites of interest in saffron. Techniques must therefore be

greatly improved, and methods of continuous cultivation systems in the liquid phase that do not need constant renovation of flower explants need to be developed. However, even in the event that conditions were optimized for crocin production, for example by using cultivation systems in two stages (Chen *et al.*, 2003; Zeng *et al.*, 2003), industrialization would still be difficult.

#### 26.3.4 Worldwide international market

According to the United Nations Comtrade Database in 2005, Iran followed by Spain were the principal exporters of saffron in the world market and Spain, Italy then the USA were the principal importers. Despite the low local production, Spain is at the forefront of world trade with approximately one quarter of the total exports. Spanish traders have always worked with the highest quality product on the market and therefore have more experience than their competitors, and can boast the world's leading market in saffron, although production has fallen considerably in recent years. In many countries, the official statistics when looking for 'saffron' include false samples, such as, for example the 'false crocus' or petals of *Cartamus tinctorius*. Saffron stamen and flower styles of *C. sativus* are also included under saffron spice. It is for this reason that within the statistical data web page 'United Nations Comtrade Database' saffron appears with very different prices on the market ranging from a few \$/kg to more than 4000 \$/kg during 2009. The highest priced importer country for several decades has been Spain, with values of 155 M\$ in the years 2007–9, followed by Italy with 84 M\$, the USA with 50 M\$, France with 44 M\$ and Sweden with 29 M\$. For exporters, Iran is the number one with values of 292 M\$, followed by Spain with 180 M\$, France 14 M\$, Portugal 13 M\$ and Sweden 11 M\$. Spanish traders are very active as can be deduced from Table 26.3 which shows the number of countries alongside the amount of saffron spice exported during the years 1997–2009.

#### 26.3.5 Current presentations on the market

Primary packaging is made of different materials in various forms: cellulose, plastics (polyethylene, polypropylene, polystyrene, PET, PVC, cellophane), glass, aluminium, etc. These materials do not always guarantee the best preservation, but are necessary due to market conditions and practices. The best materials for preservation are the

**Table 26.3** Number of countries to which each country has exported over the last decade

| Year | Spain | Iran | Greece | India | Italy | Germany | France |
|------|-------|------|--------|-------|-------|---------|--------|
| 1997 | 60    | 36   | 13     | 14    | 16    | 22      | 21     |
| 1999 | 55    | 36   | 13     | 21    | 21    | 26      | 24     |
| 2001 | 61    | 39   | 15     | 14    | 20    | 17      | 19     |
| 2003 | 68    | 44   | 16     | 12    | 25    | 22      | 20     |
| 2005 | 69    | 45   | 22     | 16    | 31    | 23      | 19     |
| 2007 | 65    | 36   | 14     | 15    | 37    | 23      | 26     |
| 2008 | 70    | 37   | 17     | 20    | 37    | 26      | 26     |
| 2009 | 67    | 45   | 21     | 25    | 31    | 29      | 27     |

ones which protect saffron from light and moisture. It is customary to package saffron in specific small formats (less than 5 g). When it is sold as threads, packaging is completely manual, from the filling of the containers to the labelling. It is extremely difficult to automate this process without risking the rupture of the fragile filament. Packaging of ground saffron is automated instead.

There is a market for bulk sales, for firms that produce ready meals, working in the catering industry or packaging under their own brand. In this case, containers commonly consist of polyethylene bags which are then placed in metal or cardboard containers. Companies which provide for this type of customer are introducing advanced packaging under controlled atmosphere, to extend the life of the saffron and thus slow down the oxidation processes that degrades the chemical and organoleptic properties of saffron.

## 26.4 Functional properties and uses of saffron

### 26.4.1 Historical uses

The most ancient cultures on record which used saffron were established in Mesopotamia, and used it principally as a condiment, in religious rites and celebrations (Pérez-Bueno, 1995). The Phoenicians then used it to dye clothes. The Romans used it in the composition of numerous perfumes and ointments (Giaccio, 2004; Carmona *et al.*, 2006c), and as a ludic element in a multitude of celebrations. In voluptuous orgies highly concentrated infusions were served, which the Empire's sybarites drank with delight, persuaded of its virtues against drunkenness and obesity and its aphrodisiac properties (Capel and Girbes, 1988; Boisvert and Aucante, 1993; Pérez-Bueno, 1995). Saffron was also used by Cleopatra (69–30 BC), and during her reign it was a cosmetic, phitotherapy and a nail, hair and lip dye. The Egyptians and Hebrews used it to carry out ablutions in temples and sacred places (Capel and Girbes, 1988). In India, it was used in religious ceremonies and as a sign of a good omen applied on the forehead (Madan *et al.*, 1965; Alarcón and Sánchez, 1968).

Specialists in ancient dyes state that saffron was known as 'the great dye' that did not need a mordant. It was lasting and permanent through fermentation, but when boiled it did not hold up after the first wash (Cannon and Cannon, 2003). Through the former it was used to dye wool, hide and leather (Boisvert and Acuante, 1993).

### 26.4.2 Uses in food

Undoubtedly the most important current use of saffron is in food. This spice forms part of some of the best known traditional dishes from several Mediterranean countries: *Risotto alla Milanese* in Italy, *Bouillabaisse* in France and *Paella Valenciana* in Spain. It is also an indispensable ingredient in various pastries and breads, many of them commemorative of a lay or religious festival, such as the German *Gugelhupf*, Saint Lucia buns in Sweden, buns and breads in Cornwall (England), Christmas bread in Estonia, sweets on the Greek islands, rice pudding in Iranian Shiite celebrations or the Jewish Sabbath *Challah* bread (Pellowe, 1989; Pérez-Bueno, 1995; Winterhalter and Straubinger, 2000). In industry, besides these



traditional sweets, it is used to produce several liqueurs and vermouths, sauces and milk products (Attri *et al.*, 1993; Sen and Rajorhia, 1994).

Usually the spice is either not used in the kitchen or not used properly. Contrary to what is believed due to the great amount of volatiles found in the spice, very few are transmitted to food. The best option when using this spice in food is to reheat it, in order to transform the aroma precursors into volatile compounds. Filaments should then be ground to improve extraction followed by several hours of maceration to obtain complete extraction of the colouring and flavouring substances, which is highly recommended. If this process of maceration is not possible due to lack of time, the saffron should be added at least 30 minutes before the end of cooking. Some authors suggest otherwise, claiming that saffron should be added just before serving, because the aromas evaporate during the cooking process, but it has been shown that without a longer extraction the potential of saffron is largely untapped and the amount of aromas that evaporate are more than offset by those generated and extracted from the extra cooking time (Carmona *et al.*, 2006c).

#### **26.4.3 Uses in the textiles industry**

Interest in saffron as a dye, which had practically disappeared with the development of synthetic colouring, seems to have returned in areas where natural rather than synthetic ingredients are being sought. It is therefore being used to dye high-quality textiles manufactured with silk, cotton or wool (Takaoka *et al.*, 1992; Tsatsaroni and Eleftheriadis, 1994; Liakopoulou-Kyriakides *et al.*, 1998; Tsatsaroni *et al.*, 1998). It is also used to dye rugs, hats and traditional clothes in Sardinia. As a dye, it is also utilized in combination with hematoxylin, erythrosine and others, to achieve human and animal histological staining (Desmettre *et al.*, 2001; Rostoker *et al.*, 2001; Alyahya *et al.*, 2002; Edston *et al.*, 2002).

#### **26.4.4 Uses in traditional medicine**

The therapeutic uses of saffron stretch far back in time. Saffron is cited in the most important Egyptian medical papyrus known, the Ebers Papyrus (1500 bc), as a remedy for kidney problems. It was also much used by the Greeks. Dioscorides considered it a sexual stimulant, anti-inflammatory and as an impediment to drunkenness which is also the reason why it was used profusely by the Romans, although during this era its pharmacological effects lost importance in favour of its magical-medicinal properties as an amulet (Font, 1973). It recovered its reputation in the highly-developed Arabian medicine. The great Avensoar (980–1037), the most accredited physician of the Arabian world, recommended the consumption of saffron as a potent remedy against tuberculosis (Pérez, 1995).

In general, saffron, its extracts and tinctures have been used in traditional medicine as an antispasmodic, eupeptic, sedative, carminative, diaphoretic, expectorant, stomachic, stimulant, aphrodisiac, emenagogue and abortive (Basker and Negbi, 1983; Ríos *et al.*, 1996). Its use reached the greatest peak in the treatment of specifically feminine illnesses. Nowadays, it is known that its therapeutic effect in these cases is due to its congestive action on the uterus. This is why it was prescribed for genital illnesses and for the regulation and relief of menstruation (Font, 1993). Its

abortive action is well known. Midwives in the Middle Ages applied a dose of saffron to women in labour for its sedative and anti-spasmodic action, to ease expulsion and calm the pain (Martindale, 1943; Morales, 1945). However, it has also been used for the treatment of ocular and cutaneous conditions; for wounds, fractures and joint pain; to prevent the plague and other epidemics; to cure anaemia, migraines and insomnia, for throat maladies, tuberculosis, jaundice, ulcers, sores and as a cardiotonic (Fernández and Escribano, 2001). It was also used as a kind of panacea as Pliny the Elder claims in his work *Naturae Historiarum XXXVII libri* (Alonso *et al.*, 1988). Between the seventeenth and nineteenth centuries, the use of saffron for its medicinal characteristics met with progressive decline, until it practically stopped being used.

#### 26.4.5 Scientific bases for saffron healthy properties

The properties of saffron mean that it could be applied as a possible therapeutic agent for various diseases as demonstrated by several biomedical studies (Licón *et al.*, 2010). The main biological activity of saffron is based on its great antioxidant ability; in fact, the antioxidant properties of saffron are well known and have been widely studied since this property is responsible for many of its biomedical attributes. Great efforts have been made to find natural products that possess anti-inflammatory, anticarcinogenic and wound healing properties, among others (Assimopoulou *et al.*, 2005), and many of these studies have looked at the potential properties in saffron. Saffron extracts exhibited a remarkable intracellular antioxidant activity. Moreover, the antioxidant efficiency observed in ethanol saffron extracts was equivalent to 116 mg  $\alpha$ -tocopherol/g (Chen *et al.*, 2008). It can therefore, be assumed that this property is responsible for preventing many diseases in which the mechanisms involve oxidation, such as neurodegenerative injury (Urrutia *et al.*, 2007) and cardiovascular diseases (described below) and injuries in kidney or brain tissues caused by ischemia-reperfusion (I/R) (Hosseinzadeh *et al.*, 2007). In addition, treating thermally induced burn wounds with saffron extract cream (20 %) results in a significantly increased re-epithelialization that could be explained by the antioxidant effects of this spice (Khorasani *et al.*, 2008).

The potential chemopreventive and antitumoural properties of saffron and several other spices against cancer have been extensively studied during the last decade, proposing different hypotheses for the mode of action of its constituents. The cytotoxic effect of saffron extract (200–2000  $\mu$ g/ml) was evaluated by Tavakkol-Afshari *et al.* (2008) in HepG2 and HeLa malignant cell lines, resulting in a decrease in the viability of malignant cells in a concentrated and time-dependent manner, which was confirmed by Feizzadeh *et al.* (2008). Saffron doses inducing 50 % cell growth inhibition (IC<sub>50</sub>) values against HeLa and HepG2 were determined as 800 and 950  $\mu$ g/ml after 48 hours, respectively. It was concluded that saffron can cause cell death in which apoptosis or programmed cell death plays an important role (Tavakkol-Afshari *et al.*, 2008). The cytotoxic and antitumour properties of saffron petals have also been studied with results showing the IC<sub>50</sub> values of stigma and petal extract against a tumour at 5.3 and 10.8 mg/ml (Hosseinzadeh *et al.*, 2005), respectively. On the other hand, the genotoxic potential of antitumour drugs limits their efficacy in the treatment of cancers, so a study was designed to ascertain the

chemoprotective potential of saffron against the genotoxicity of cisplatin, cyclophosphamide and mitomycin, three well known antitumour drugs. Saffron doses of 20, 40 and 80 mg/kg b.w. significantly inhibited the cellular DNA damage induced by the antitumour drugs, suggesting that saffron could be used as an adjuvant in chemotherapeutic applications (Premkumar *et al.*, 2006).

Another important property which could lead to saffron being seen as beneficial spice for health is its antimicrobial activity. Many spices such as garlic and basil are already well-known antibacterial agents (Dog, 2006). This property has been studied using various parts of the saffron plant; ethyl acetate extracts of stigma, stamen and leaves were tested on *Staphylococcus aureus*, *S. epidermis*, *Escherichia coli*, *Micrococcus luteus*, *Candida albicans*, *Cladosporium* sp. and *Aspergillus niger*. The antifungal activity of the stigma was higher than that of the stamen; in contrast, the antibacterial activity of the stamen was higher than the rest of the parts studied (Vahidi *et al.*, 2002).

The anti-*Helicobacter pylori* activity of saffron extracts, safranal and crocin was investigated using aqueous and methanol extracts and four antibiotics as control. All isolates were susceptible to methanol and aqueous saffron extracts, the minimum inhibitory concentrations of methanol saffron extract, crocin and safranal being 677, 26.5 and 16.6 µg/ml, respectively (Nakhai *et al.*, 2008). In another series of studies, the anti-ulcer properties of saffron were determined and results suggested that saffron inhibits gastric acid secretion and stimulates mucus secretion creating a barrier to prevent damage (Al-Mofleh *et al.*, 2006). In this work, saffron extracts at 250 mg/kg b.w. produced a significant decrease in the volume of gastric secretion and ulcer index in the animals tested. In addition, Kianbakht and Mozaffari (2009) studied the effects of pre-treated rats with saffron extract (25, 100 and 250 mg/kg b.w.), crocin (2.5, 5 and 10 mg/kg b.w.) and safranal (0.25, 2 and 5 ml/kg b.w.), and found that these extracts prevent gastric lesions, increase lipid peroxidation and decrease glutathione levels induced by indomethacin, effects that are comparable to omeprazole, an inhibitor protons pump, which is used as an anti-ulcerogenic agent.

#### 26.4.6 Other current uses

Assimopoulou *et al.* (2005) suggests that saffron could be used in functional foods, drinks with antioxidant activity and in pharmaceutical and cosmetic preparations, and as a food supplement with antioxidant properties. Despite these potential applications, saffron's current use is almost limited to alternative medicine. It is used as a tonic and as a promoter of defences in Ayurvedic medicine, for some disorders of the central nervous system in Chinese medicine and for homeopathic preparations (Ghosal *et al.*, 1989; Dhar and Mir, 1997; Abe and Saito, 2000; Soeda *et al.*, 2001; Ordoudi and Tsimidou, 2004). Saffron is also used in some pharmaceutical preparations to treat cases of early ejaculation, to relieve premenstrual syndrome symptoms or as an analgesic and sedative for tooth and gum pain (Ríos *et al.*, 1996). Care should be taken with the posology of saffron in any of its forms, since it can cause serious symptoms at high doses: terrible headaches, acceleration of cardiac rhythm, dizziness and hallucinations that lead to death by internal haemorrhage according to some authors and by paralysis of the central nervous system according to others (Ávila, 1999; Fernández and Escribano, 2001). Unanimity does not exist on the dose

that causes problems, but the following values could be taken as a reference. The maximum single dose of saffron would be 1.5 g, the abortive dose, 10 g daily and the lethal dose, around 20 g a day (Ávila, 1999).

## 26.5 Quality issues

The authenticity of saffron is an extremely important matter for the industry and for consumers in view of security and protection, quality assurance, active properties and, last but not least, economic impact. Despite the fluctuation in prices in international markets, saffron was and still remains the most expensive spice. Consequently, a wide range of adulterants and substitutes has appeared throughout its history (Table 26.4).

Laws to protect the purity of saffron have been imposed throughout history to pursue fraudulent conduct. In the fifteenth century, an armed police force was created in Italy called 'Uficio dello Zaferano'. Established in Venice, its mission was to ensure that the spice would not be adulterated. In France, Henry II (1519–59) established confiscation, burning of the merchandise and corporal punishment for

**Table 26.4** The most frequent adulterations saffron has undergone throughout history

| Way to adulterate                       | Adulteration consisting of:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Without adding extraneous substances    | <ul style="list-style-type: none"> <li>• Mixture with extract or old saffron</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Adding other parts of the saffron plant | <ul style="list-style-type: none"> <li>• Addition of stamens or styles cut in strips and dyed</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Adding substances to increase weight    | <ul style="list-style-type: none"> <li>• Increase in moisture</li> <li>• Impregnation with syrup, honey, glycerine</li> <li>• Addition to the above-mentioned syrup, barium sulphate, sodium sulphate, calcium sulphate, calcium carbonate, potassium hydroxide, potassium nitrate, potassium sodium bitartrate, sodium borate, lactose, starch or glucose</li> </ul>                                                                                                                                                                                                                                                                                                                     |
| Adding parts from other plants          | <ul style="list-style-type: none"> <li>• Flowers from <i>Carthamus tinctorius</i></li> <li>• Flowers from <i>Calendula officinalis</i></li> <li>• Stigmas from other <i>Crocus</i> species, generally shorter and without dyeing properties (<i>Crocus vernus</i>, <i>Crocus speciosus</i>, etc.)</li> <li>• Flowers cut in strips from <i>Papaver rhoeas</i> L., <i>Punica granatum</i>, <i>Arnica montana</i> and <i>Scolimus hispanicus</i></li> <li>• Stamens from some carnation species</li> <li>• Ground red pepper</li> <li>• Herbaceous plants cut in pieces and coloured with an azoic colourants</li> <li>• Root hairs from <i>Allium porrum</i></li> <li>• Curcuma</li> </ul> |
| Adding animal substances                | <ul style="list-style-type: none"> <li>• Dried, salted meat fibres</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Adding artificial products              | <ul style="list-style-type: none"> <li>• Coloured gelatine fibres</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Adding organic colourants               | <ul style="list-style-type: none"> <li>• Martins yellow, tropeolin, fuchsine, picric acid, tartrazine, erythrosine, scarlet or ponceau 4R, azorubin, orange-yellow etc.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

the adulterators (Pérez-Bueno, 1995). Henry VIII (1509–47), fanatically devoted to its aroma, forbade the use of saffron as an article for dyeing clothing and hair and any act of falsification at that time received the death penalty. In the Middle Ages, there were also saffron inspectors in Nuremberg, Germany, with authority to burn or bury alive those who adulterated saffron (Laux *et al.*, 1996).

### 26.5.1 Evolution of the ISO 3632 standard

Saffron quality in international trade has been mainly determined by specifications recommended by the ISO 3632 standard. It was first published in 1975 and has undergone four revisions: in 1980, 1993, 2003 and 2011. After all these revisions, ISO/TS 3632 is very complete and, although it is not compulsory, at present this is the internationally accepted reference specification for saffron quality control and is used in most transactions. The strict requirements of the standard have resulted in the disappearance of very low quality saffron and have made it much easier to detect adulteration. According to ISO/TS 3632-1 (2003), saffron is considered to be pure when it complies with the requirements in Table 26.5 and when no matter has been added to the natural product. At the international level, ISO/TS 3632 classify saffron into three categories with regard to the specified physical and chemical parameters, although it does not establish the content of crocetin esters, picrocrocin and/or safranal. Among these categories, category I corresponds to the highest quality saffron (see Table 26.5).

Also, at a national level, pure saffron is graded according to its characteristics giving rise to different commercial categories. In Spain, examples of this classification (from the highest to the lowest category) are the categories ‘Selecto or superior’, ‘Río’, ‘Sierra’, ‘Common or Standard’, ‘Coupe’ and ‘Ground’ (NCCEA, 1999); in India: ‘Lacha’ and ‘Mongra’; or in Iran: ‘Sargol’ and ‘Pushali’. Table 26.5 also shows the specifications that all saffron under the Azafrán de La Mancha PDO (EC, 2001) should meet. Saffron from this PDO is presented to the consumer in filaments and never powdered in order to make its authentication easier. Furthermore, there are two new Spanish normatives: UNE 34802 (AENOR, 2011a) determines the picrocrocin content and UNE 34803 (AENOR, 2011b) the safranal content of saffron for the purpose of ensuring quality standards.

### 26.5.2 Authenticity indicators

The best authenticity biomarker in saffron is picrocrocin. So far, saffron is the only plant in which it has been found and this is why it is an excellent marker of saffron authenticity and purity even against the most sophisticated adulteration with *Gardenia jasminoides*. This plant contains members from the same crocetin ester family, but in different proportions. It is a fraudulent practice to increase the colour of saffron. Recently, a rapid method for picrocrocin routine control has been proposed using UV–Vis technique (Sánchez *et al.*, 2009) which could be very useful in the future for saffron authentication. If the picrocrocin content is below the usual levels (Del campo *et al.*, 2010), the sample can be suspected of tampering and should be analysed in detail with specific antifraud methodologies.

In addition to picrocrocin, several major and minor constituents can be used as biomarkers for origin differentiation. The crocetin esters profile is determined using

**Table 26.5** Requirements of saffron according to ISO/TS 3632-1 (2003) and characteristics of saffron produced under the *Azafrán de La Mancha* PDO (EC, 2001)

| Characteristics                                                          | ISO/TS 3632-1 (2003)<br>Specifications |                |                 | 'Azafrán de<br>La Mancha'<br>Specifications |                     |
|--------------------------------------------------------------------------|----------------------------------------|----------------|-----------------|---------------------------------------------|---------------------|
|                                                                          | Category<br>I                          | Category<br>II | Category<br>III | Bulk<br>saffron                             | Packaged<br>saffron |
| Floral waste, max. %                                                     | 0.5                                    | 3              | 5               | 0.5                                         | 0.5                 |
| Foreign matter, max. %                                                   | 0.1                                    | 0.5            | 1.0             | 0.1                                         | 0.1                 |
| Water and volatile matter, max. %                                        |                                        |                |                 |                                             |                     |
| Saffron in filaments                                                     | 12                                     | 12             | 12              | 7–9                                         | 11                  |
| Powdered saffron                                                         | 10                                     | 10             | 10              |                                             |                     |
| Total ash, as dry matter, max. %                                         | 8                                      | 8              | 8               | –                                           | 8                   |
| Acid-insoluble ash, %, as dry matter,<br>max.                            | 1.0                                    | 1.0            | 1.5             | –                                           | 1                   |
| Soluble extract in cold water, as dry<br>matter, max. %                  | 65                                     | 65             | 65              | –                                           | 65                  |
| $E_{1\text{ cm}}^{1\%}$ 257 nm on dry basis, min.                        | 70                                     | 55             | 40              | 70                                          | 70                  |
| $E_{1\text{ cm}}^{1\%}$ 330 nm                                           |                                        |                |                 |                                             |                     |
| Min.                                                                     | 20                                     | 20             | 20              | 20                                          | 20                  |
| Max.                                                                     | 50                                     | 50             | 50              |                                             |                     |
| Colouring strength, $E_{1\text{ cm}}^{1\%}$ 440 nm on<br>dry basis, min. | 190                                    | 150            | 100             | 200                                         | 200                 |
| Artificial water-soluble acid<br>colourants                              | Absent                                 | Absent         | Absent          | Absent                                      | Absent              |
| Safranal content (% of total volatile<br>components), min.               |                                        |                |                 | 65                                          | 65                  |
| Ethereal extract, % (w/w)                                                |                                        |                |                 | –                                           | 3.5–14.5            |

several techniques: Fourier transform-near infrared (FT-NIR) spectroscopy, Fourier transform-mid infrared (FT-MIR) spectroscopy, liquid chromatography or  $^1\text{H}$  nuclear magnetic resonance (NMR) (Zalacain *et al.* 2005a,b; Yilmaz *et al.*, 2010), have all proven useful for origin discrimination purposes among Iranian, Greek and Spanish saffron disputes. Gas chromatography (GC) offers the possibility of establishing the fingerprint of saffron volatiles which can permit geographical differentiation. Several authors have established it for different countries, such as Iran (Jalali-Heravi *et al.*, 2010), Greece (Kanakis *et al.*, 2004) and Spain (Alonso *et al.*, 2007; Maggi *et al.*, 2009). The contents of three compounds, 3, 5, 5-trimethyl-2-cyclohexenone, 2, 6, 6-trimethylcyclohexane-1, 4-dione and acetic acid, are capable of differentiating saffron according to its country of origin. Iranian and Moroccan samples show high contents of acetic acid while low or undetectable content is observed in Greek and Spanish samples. These differences are attributed to the different post-harvest processes that saffron undergoes depending on its origin



(Carmona *et al.*, 2006a). GC can be also applied to train an electronic nose based on metal oxides to perform the differentiation (Carmona *et al.*, 2006a).

There are also significant differences in the content of minor constituents such as the kaempferol-3-O-sophoroside in saffron from Spain, Greece, Iran and Morocco (Carmona *et al.*, 2007a), as well as free amino acids and ammonium ion content (Del Campo *et al.*, 2009). Finally, a recent study (Maggi *et al.*, 2011) has proved that analysis of the stable isotopes of the bio-elements H, C and N of saffron from the production areas of Western Macedonia in Greece, Khorasan in Iran, Sardinia in Italy and Castilla-La Mancha in Spain, combined with multivariate analysis, is a reliable tool in determining the geographical origin of saffron.

### 26.5.3 Fraud detection

It was not until the 2003 revision of ISO 3632 that the UV-vis fingerprint of saffron aqueous extracts was included. Several works have demonstrated its ability to detect certain adulterations with colourants to increase its colouring strength (Zalacain *et al.*, 2005a). Thin-layer chromatography (TLC) is also proposed in ISO/TS 3632 (2003) for the identification of the main components after soaking the saffron in methanol. It reveals the pigments existing in saffron, picrocrocin,  $\beta$ -hydroxycyclocitral and safranal, and consequently it can be used as an indicator of the authenticity of the product. In this Standard, another test using TLC for the detection and identification of artificial water-soluble acidic colourants after their isolation and elution on a polyamide microcolumn is also proposed.

High-pressure liquid chromatography (HPLC) coupled to diode array detection (DAD) or mass spectroscopy (MS) detection is the standard technique for the analysis of saffron components. ISO/TS 3632-2 test methods include HPLC–DAD for the identification of water-soluble acidic colourants. Before analysing with HPLC, colourants are extracted, isolated and eluted using chromatography on a polyamide microcolumn. The HPLC technique has also been proposed for use against other types of fraud (no water-soluble compounds) usually employed to improve the saffron characteristics, but without increasing colouring strength. This has been approved by the Spanish AENOR committee (PNE 34804, 2011) and will be submitted to ISO for their approval in the near future.

HPLC can be used to fight against fraud with gardenia, the most refined form of adulteration since this plant contains the same kind of crocetin esters. This adulteration can be detected using the profile of crocetin esters together with the presence of compounds from gardenia that are not found in saffron, geniposide compound, and the absence of or low content of picrocrocin (Carmona *et al.*, 2006b).

Recent advances together with the knowledge about this spice generated over centuries make it possible to guarantee that consumers are actually getting what they are paying for. Nevertheless, adulterers are always one step ahead, and authorities and analysts must be alert to ensure the quality and safety of saffron.

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## Thyme

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**Abstract:** People have used thyme (*Thymus vulgaris* L. – Labiatae) for many centuries as a flavouring agent, culinary herb and herbal medicine. It is indigenous in the Mediterranean region, especially on the Iberian Peninsula and in Northwest Africa and is grown commercially in a number of countries for the production of the dried leaves, thyme oil, thyme extracts, and oleoresins. This chapter presents the history, botany and morphology of thyme, together with its chemical structure, production and harvesting. The main uses of thyme in food processing and its functional properties and toxicity are described along with its pharmacopoeial status and applicable quality specifications.

**Key words:** Thyme, *Thymus vulgaris*, thyme oil, thyme herb, botany, chemical structure, production, health issues, quality issues.

### 27.1 Introduction

The common English word ‘thyme’ covers both the genus and the species most widely used, *Thymus vulgaris* L. (common thyme, garden thyme). From the aromatic and medicinal points of view, *T. vulgaris* is indeed the most important species and is widely used as a flavouring agent, a culinary herb and a herbal medicine. Therefore *T. vulgaris* is the central species in this chapter and ‘thyme’ here refers to *T. vulgaris* unless another botanical name is mentioned. However, other *Thymus* species shall be included here because they are used for similar purposes or as a substitute for *T. vulgaris*, especially *T. zygis* L. (Spanish thyme), *T. serpyllum* L. (wild thyme, mother-of-thyme) and *T. pulegioides* L. (large thyme or larger wild thyme). The commercial products that are obtained from these four species include essential oils, oleoresins, fresh and dried herbs and landscape plants.

#### 27.1.1 History and etymology

People have used thyme for many centuries for its flavouring and medicinal properties. The first recorded evidence can be found in Dioscorides’ work (first century AD) about medicinal plants and poisons, which mentions ‘Thymo’, ‘Serpil’ and ‘Zygis’ and in Pliny’s *Natural History* (first century AD). While in the Mediterranean region thyme has always been widely used as a spice, it was only in the early Middle

Ages that Benedictine monks brought it over the Alps to Central Europe and England where it began its glorious career. From this time on, it was found in all herb books, those by Pear Matthioli (1505–77) and Leonhart Fuchs (1501–66) being the most famous. The most favoured interpretation of the etymology of the name is that it comes from the Greek word ‘thymos’ which means ‘courage, strength’.

### 27.1.2 Systematic botany and distribution

The genus *Thymus* belongs to the Labiate family (Lamiaceae), sub-family Nepetoideae, tribe Mentheae. The distribution of the genus can be described as Eurasian with the Mediterranean region, especially the Iberian Peninsula and North-west Africa, being its centre. Today, about 250 taxa (214 species and 36 sub-species) are accepted, subdivided into eight sections (Jalas, 1971; Morales, 2002). *T. vulgaris* L. and *T. zygis* L. belong to the Western Mediterranean section *Thymus* and *T. serpyllum* L. and *T. pulegioides* L. to the section *Serpyllum* which is the most extensive section considering the numbers of species and the areas of distribution.

*T. vulgaris* is native to Southern Europe, from Spain to Italy. It is commonly cultivated there as well as in most mild-temperate and subtropical climates, which include Southern and central Europe. *T. zygis* is indigenous to the Iberian peninsula (Portugal and Spain) and the Balearic Islands; it is little known elsewhere and is cultivated in Spain. *T. serpyllum* and *T. pulegioides* are the dominant *Thymus* species in Northern and middle Europe; in the east they reach Siberia. It is difficult to differentiate these two species; the plant material on the market comes from wild collections in the Balkans and the Ukraine.

### 27.1.3 Morphological description

Common thyme (*T. vulgaris* L.) is a perennial, evergreen subshrub, 10–30 cm in height. The small grey-green leaves are opposite, oblong-lanceolate to linear, 5–10 mm long and 0.8–2.5 mm wide and gland-dotted. Their margins are recurved. The flowers are light violet, two-lipped, 5 mm long with a hairy glandular calyx, borne with leaf-like bracts in loose whorls in axillary clusters on the branchlets or in terminal oval or rounded heads. The Spanish thyme (*T. zygis* L.) is smaller with narrower leaves, which are clustered at the nodes. The flowers are whitish and in clusters spaced at intervals in an elongated inflorescence. Wild thyme (*T. serpyllum* L.) and large thyme (*T. pulegioides* L.) differ considerably in appearance, being more herbaceous, woody only at the base, partly procumbent and with leaves that are flat, linear to elliptical, sub-sessile and ciliate at the base. The inflorescence is usually capitate. The corolla is purple, the calyx campanulate with upper teeth as long as wide, usually ciliate. Their distinct phenotypic variety makes botanical classification difficult, and some authors handle *T. serpyllum* L. as a collective species with the addition ‘s.l.’ (*sensu latiore*), and include therein *T. pulegioides*.

## 27.2 Chemical composition of thyme

The chemical character of thyme is represented by two main classes of secondary products, the volatile essential oil (Stahl-Biskup, 2002; Lawrence, 2004; Figuereido

*et al.*, 2010, and references therein) and the non-volatile (poly)phenols (Vila, 2002 and references therein). Since we are dealing with a natural product, the yield of essential oils and of the polyphenols, as well as the proportions of individual constituents, vary. This is caused by intrinsic (seasonal and ontogenetic variations) and extrinsic (soil, climate, light) factors. The data presented here are a result of an evaluation of numerous publications with respect to what is important to know when thyme is treated as a herb and as a spice for commercial purposes.

### 27.2.1 Essential oil

The essential oil is responsible for the typical spicy aroma of thyme. As in all representatives of the Lamiaceae, it is stored in glandular peltate trichomes situated on both sides of the leaves. They show a very typical anatomy with a gland head of 8–16 secretory cells sitting on one basal stalk cell. The secretory cells secrete the oil into the sub-cuticular space. If the cuticle is ruptured, e.g. by rubbing or grinding, the volatile oil spreads into the air producing the typical spicy aroma of thyme. On hot days, traces of the volatiles penetrate the cuticle and form an aromatic cloud around the plants as can be perceived in the fields of thyme in Mediterranean regions.

Dried plant material of thyme contains 1–2.5 % of an essential oil. Its composition, including the chemical structures of the components, is given in Fig. 27.1. Most of the volatiles detected in thyme oil belong to the monoterpene group with thymol, a phenolic monoterpene, as the main representative (30–55 %). It causes the typically strong and spicy smell which is associated with thyme. Thymol is always

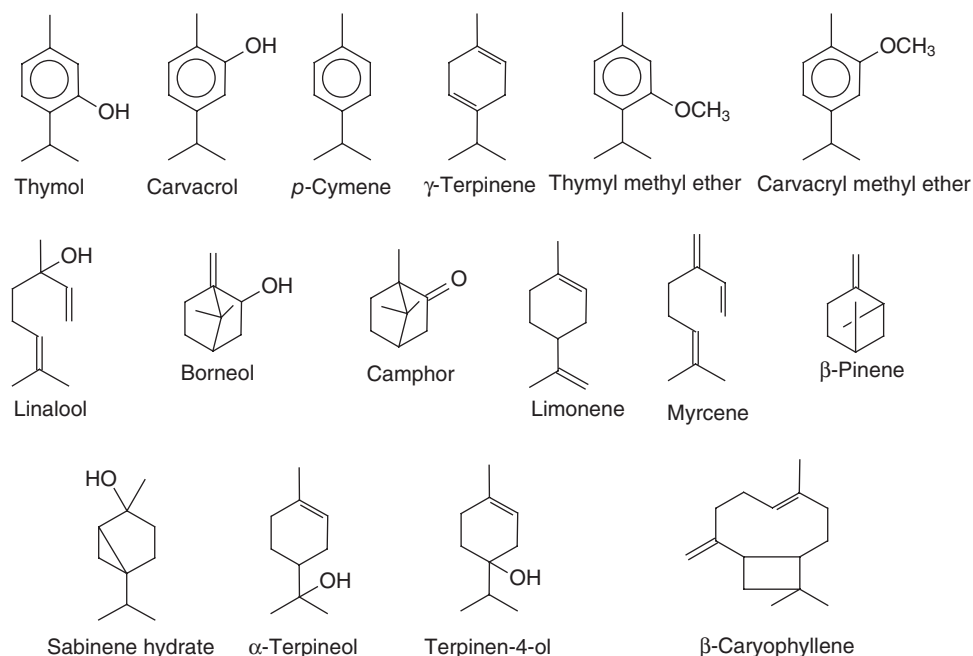


**Fig. 27.1** Terpenes in the essential oil of thyme.

accompanied by some monoterpenes which are closely connected by biogenetical processes, namely carvacrol (1–5 %), an isomer of thymol, as well as *p*-cymene (15–20 %) and  $\gamma$ -terpinene (5–10 %). The latter two are precursors in the biogenetic pathway of thymol (and carvacrol). Often the methyl ethers of thymol and carvacrol are present. Further monoterpenes are linalool (1–5 %) and, in smaller percentages (0.5–1.5 %), borneol, camphor, limonene, myrcene,  $\beta$ -pinene, *trans*-sabinene hydrate,  $\alpha$ -terpineol and terpinen-4-ol. Sesquiterpenes are not very important in thyme oils. Only  $\beta$ -caryophyllene (1–3 %) is worth mentioning.

The composition of thyme oil given so far is that of commercially used thyme. However, it is important to mention that *T. vulgaris*, the main source of commercial thyme, is a chemically polymorphous species. In the South of France today, seven different genetically-based chemotypes of *T. vulgaris* are known. They are named according to their dominant monoterpene in the essential oil: a thymol type, a carvacrol type, a linalool type, a geraniol type, an  $\alpha$ -terpineol type, a *trans*-thuyanol (= *trans*-sabinene hydrate) type and a cineole type (Keefover-Ring *et al.*, 2009). In Spain (Catalonia), only two chemotypes are present, a cineole type and a linalool type (Torrás *et al.*, 2007). Only the thymol chemotype is of commercial interest.

The chemical composition of the essential oil from *T. zygis*, the most important source of thyme oil in Spain, is quite similar to that of *T. vulgaris* with a remarkably high content of thymol. No practicable criterion tells the difference between both oils. Also *T. zygis* is chemically polymorphous showing five different chemotypes on the Iberian Peninsula (Sáez, 1995).

The dried herb of wild thyme, *T. serpyllum*, yields 0.2–0.6 % essential oil. Again, we are dealing with a chemically polymorphous species. For commercial use, plant material with a high phenolic content in the oil is required, mainly represented by carvacrol (20–40 %) and thymol (1–5 %). Further monoterpenes are *p*-cymene (5–15 %),  $\gamma$ -terpinene (5–15 %), borneol, bornyl acetate, 1,8-cineol, citral, geraniol, linalool and others. Also, *T. pulegioides* is chemically polymorphous. Again, only the phenolic chemotypes (thymol and/or carvacrol) are of commercial interest.

### 27.2.2 Polyphenols

Aside from the essential oil, the tannins, mainly represented by rosmarinic acid (Fig. 27.2), contribute to the commercial use of the herb (Kivilompolo and Hyötyläinen, 2007). The contents of rosmarinic acid reported in the literature vary between 0.15 and 4 %. Also the 3'-*O*-(8''-*Z*-caffeoyl)-rosmarinic acid has been isolated from the leaves (Dapkevicius *et al.*, 2002). Free phenolic acids are mainly represented by caffeic acid, gentisic acid, *p*-cumaric acid, syringic acid, ferulic acid and *p*-hydroxybenzoic acid (Proestos *et al.*, 2005; Kivilompolo and Hyötyläinen, 2007).

In common thyme (*Thymus vulgaris* L.), about 25 different flavonoids could be detected and are listed below (Wang *et al.*, 1998; Vila, 2002 and references therein):

|                  |                                                                                                                                                                                                      |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| flavones:        | apigenin, luteolin, 6-hydroxyluteolin                                                                                                                                                                |
| methyl flavones: | cirsilineol, 8-methoxycirsilineol, cirsimaritin, 5-desmethylnobiletin, 5-desmethylinensetin, gardenin B, genkwanin, 7-methoxyluteolin, salvigenin, sideritoflavone, thymonin, thymusin, xanthomicrol |

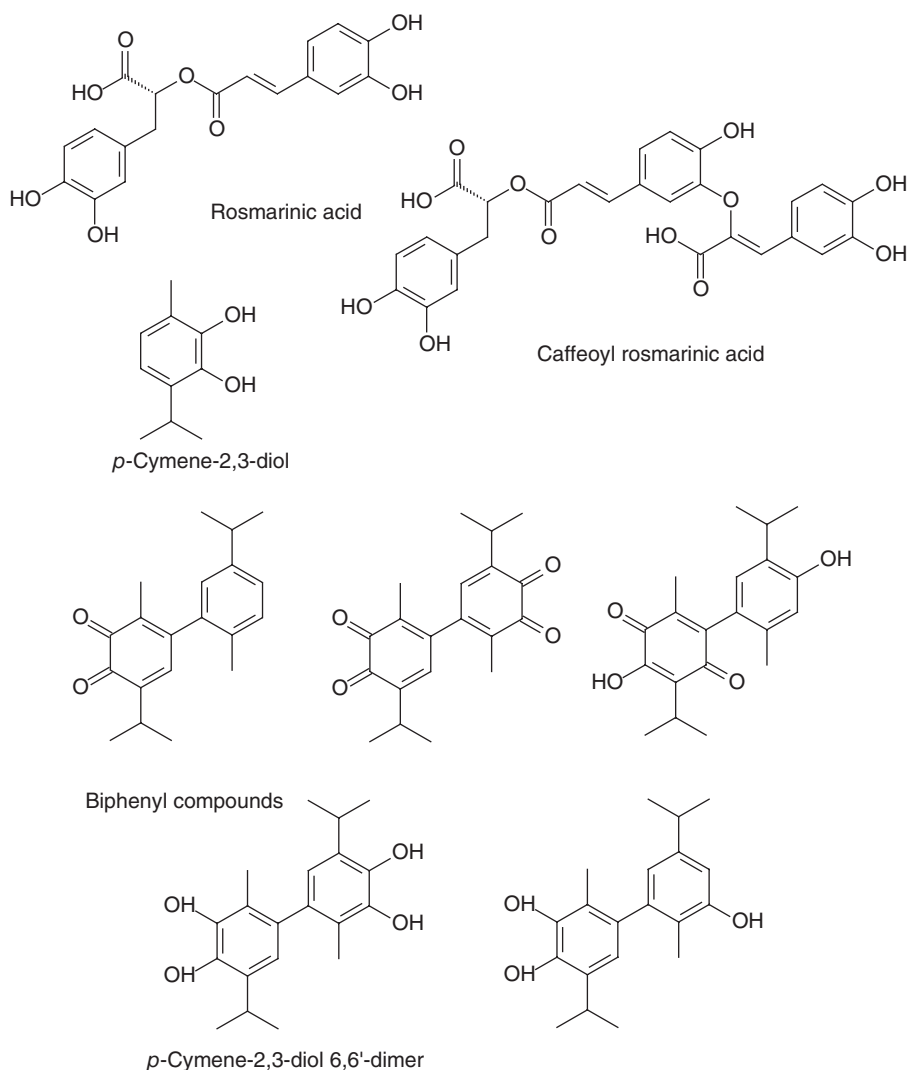


**Fig. 27.2** Polyphenols and biphenyl compounds in thyme (exclusive flavonoids).

|                     |                                                                                                                                                                                                                                                                                                           |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| flavanonols:        | taxifolin, 2,3-dihydrokaempferol                                                                                                                                                                                                                                                                          |
| flavanones:         | eriodictyol, naringenin                                                                                                                                                                                                                                                                                   |
| methyl flavans:     | 2,3-dihydroxanthomicrol, sakuranetin                                                                                                                                                                                                                                                                      |
| flavonols:          | kaempferol, quercetin                                                                                                                                                                                                                                                                                     |
| flavone glycosides: | apigenin-7-O- $\beta$ -D-glucoside, apigenin-7-O- $\beta$ -D-rutinoside, apigenin-6,8-di-C- $\beta$ -glucoside, apigenin-7-O- $\beta$ -glucuronide, eriodictyol 7-O- $\beta$ -D-rutinoside, hesperidin, luteolin-7-O- $\beta$ -D-glucoside, luteolin-7-O- $\beta$ -D-diglucoside, vicenin-2 (C-glucoside) |

These are present mostly in the form of their aglycones. The flavones apigenin and luteolin are the most important flavonoids present in both forms, as aglycones and as O-glycosides. They are accompanied by a great variety of methylated flavones

whereas flavonols and flavanones are of inferior importance. Vicenin-2, the 6,8-di-C-glucoside of apigenin, turned out to be a chemosystematic marker of the genus *Thymus*, occurring only in certain taxonomic groups, e.g. in the sections *Pseudothymbra* and *Thymus*.

### 27.2.3 Biphenyl compounds

Biphenyl compounds from thyme have attracted attention because of their antioxidative activity and deodorant effects (Miura *et al.*, 1989; Nakatani *et al.*, 1989). Five different biphenyl compounds have been isolated from an acetone extract of the leaves (Fig. 27.2). The biogenetic connection with the terpene phenols is obvious as well as that of *p*-cymene-2,3-diol, which is present in thyme in concentrations of 0.8 % (Schwarz *et al.*, 1996).

### 27.2.4 Other compounds

The 15 monoterpene glycosides in the aerial parts of common thyme show the biosynthetic relation to thymol, carvacrol,  $\alpha$ -terpineol and borneol (Kitajima *et al.*, 2004a; Takeuchi *et al.*, 2004). Moreover, a hydroxyjasnone glucoside was found (Kitajima *et al.*, 2004b). Thyme contains triterpenes in the form of ursolic acid (0.94 %) and oleanolic acid (0.37 %) (Jaeger *et al.*, 2009) as well as 7.5 % polysaccharides (labile in acids) and 1 % soluble carbohydrates (stable in alkalines).

## 27.3 Production of thyme

Various growing, harvesting, post-harvest handling and processing aspects have to be properly controlled in order to obtain high yields of herb suitable for good-quality ingredients in food and other applications. The information in this section is focused mainly on *T. vulgaris*, which is the only *Thymus* species cultivated and processed commercially for use in food processing in reasonable amounts.

### 27.3.1 Main producing areas

Thyme is grown commercially in a number of countries for the production of essential oil, extracts and oleoresins, dried leaves and other applications. Thyme-producing countries are Spain, Portugal, France, Germany, Italy and other continental European states as well as North Africa, Canada and the USA. Spain, Jamaica and Morocco are the main suppliers of dried leaf to the US market, while Spain and France supply the oil market.

There is much confusion concerning the amounts and species of *Thymus* in trade. In Spain, which is the leading producer with most production from the wild, this is increased by the fact that local names change from one region to another. Little distinction is made in Turkey between a number of species of *Origanum* and *Thymus*, and also *Thymbra spicata*. Fifteen species of Lamiaceae are traded in Turkey under the name 'kekik' which is one of the main medicinal and

aromatic plants exported from Turkey, the annual quantity being between 3 and 4 million kg.

In Spain, almost all thyme comes from wild plants, mainly growing in the South-east. France, Hungary and Poland are other countries which still harvest large amounts of wild *Thymus*, although large-scale cultivation programmes are in progress. Cultivation provides greater control over quality and supply; however, its feasibility depends on a species ability to thrive as a monocrop, while its economic viability depends on the volumes required and market prices.

Since 1990, improvements have been made for thyme cultivation in France (Verlet, 1992), Finland (Galambosi *et al.*, 2002), Switzerland and Germany (Rometsch, 1993; Carlen *et al.*, 2009), enabling the crop to compete with wild thyme.

### 27.3.2 Propagation

Thyme can be grown from seed. It is also easy to root from cuttings taken from non-woody, fast-growing shoots. Another method is to separate out sections of rooted stems and re-plant. Direct sowing of very small thyme seeds in fields is problematic, and therefore the majority of thyme plots are established by seedlings prepared in a greenhouse or by selected clones struck as cuttings in individual cells. The germination rate of thyme is comparatively low (72 %), therefore, vegetative propagation is preferable to seeding (Nicola *et al.*, 2004).

To plant a large area of thyme, it would be more economical to buy prepared seedlings from an established nursery than to produce seedlings in an under-equipped home nursery. It should be possible to generate the desired 160 000–240 000 seedlings per ha from 50–80 g of seed (Fraser and Whish, 1997). Culinary varieties of thyme have to be replaced or propagated every 2 or 3 years as they become woody and straggly, and produce few tender leaves. Due to an increasing demand for organic farming, the cutting techniques on *T. vulgaris* with season-long testing of natural rooting hormones for organic farming and synthetic auxins with different application procedures to increase rooting were studied in Italy. It was established that products registered for organic farming gave similar or superior results as compared to products of synthetic origin (Nicola *et al.*, 2004).

The recommendations of the spacing for thyme plants are different. Planting space was shown to have a significant effect on various plant parameters except oil content. The maximum yield of dry and fresh herbage, yield and content of oil and thymol yield were obtained in 15 cm space and beginning of blooming stage. Maximum thymol content was observed in the beginning of blooming and 45 cm space, whereas 15 cm spacing and harvesting in the beginning of blooming was the best treatment in respect of yield of dry matter, oil and thymol per unit area (Badi *et al.*, 2004). The effects of 15, 30 and 45 cm intra-row spacing and four harvest times (vegetative, beginning of blooming, full blooming and fruit set) on plant growth and herbage biomass of thyme were studied in Jordan, and it was found that during the first growing year the effects of spacing were not significant. However, spacing had significant effects on plant height, canopy diameter, herbage fresh and dry weight and number of leaves on long shoots in a second year harvested thyme (Al-Ramamneh, 2009).



### 27.3.3 Cultivation

Thyme prefers a light, dry calcareous soil; it succeeds in poor soils and tolerates drought once it is established. Additionally agricultural lime to the soil is recommended before sowing if the pH is less than 5.5. Successful growing of most thyme species is possible in any climate having a mean annual temperature from 7–20 °C. Thyme thrives in full sun, but also tolerates partial shade. The accumulation of essential oil in thyme directly or indirectly depends on light.

Thyme should be treated as a leafy vegetable when considering water requirements. The effects of watering levels expressed in evapotranspiration equivalents were studied for two *Thymus* species. It was suggested that in case of *T. hyemalis* winter harvesting could be used for the extraction of oil, with a low level of water supply, whereas spring harvesting could be employed for collection of leaves as a food condiment (Jordan *et al.*, 2003). The same species were grown in two different cultivation areas of South-east Spain in order to determine how edaphoclimatic conditions affect the essential oil yield and chemical composition; no statistically significant differences were found for the total essential oil (Martinez *et al.*, 2005). Watering levels had an effect on *T. zygis* ssp. *gracilis* polyphenolic extract and essential oil (Jordan *et al.*, 2009). It was also shown that thyme herbage biomass and oil production may be improved by coordinating irrigation frequency and planting density (Khazaie *et al.*, 2008).

The optimal amount of fertilizers and the schedule of their application should be adjusted to every particular growing site. In some cases, the effect of fertilizers on the yield of essential oil and its composition were not remarkable (Shalaby and Razin, 1992; Baranauskiene *et al.*, 2003). However, organic fertilizers increased *T. vulgaris* crop and essential oil yields in Egypt sandy soil (Ateia *et al.*, 2009) and shifted the major thyme components thymol, carvacrol, and  $\gamma$ -terpinene to the high margins specified by the European Pharmacopoeia (Edris *et al.*, 2009). It was also demonstrated that young thyme plants exhibit increases in photosynthesis and biomass production at elevated CO<sub>2</sub> concentration (Tisserat *et al.*, 2002).

Weed control in thyme crops, as with all herbs, is difficult. The best method to reduce weeds is to grow a dense stand of pasture prior to planting the crop, then follow up by fallowing the land prior to planting. The use of a chemical fallow and smothering pasture crops would help to reduce the weed seed reserves prior to planting. Mulching is a useful weed control method; however, as thyme plants can produce a dense cover, the crop will outrival many weeds. Unfortunately, the spreading nature of thyme is impeded by the use of inorganic mulch, and therefore if mulch is to be applied, a long lasting organic form would be more suitable (Fraser and Whish, 1997). It was demonstrated that mulching favoured plant growth and plant diameter (Fontana *et al.*, 2006). In northern areas, plants are mulched to protect them from winter injury. However, it was reported that fresh thyme yield was reduced after mulching, which encouraged the development of a fungal disease of the soil. Cultivation of thyme is reported to be associated with fungal infections, leaf diseases, root rot and spider mites.

### 27.3.4 Harvesting and post-harvest handling

In general, thyme is most aromatic during the period of blooming or at the beginning of full bloom. However, the period of vegetation and blooming can be different

in various geographical zones depending on their climatic conditions. In Spain, the harvest takes place during the blooming period from February to August, depending on the species. In France, thyme can be harvested twice a year, once in May and then again in September. In the central regions of Russia, thyme is harvested during the second year of plant vegetation, usually the first cutting in June during flowering, the second one in September–October.

Most wild growing plants are collected by hand. The main objective of thyme harvesting is to collect the most valuable anatomical parts, the leaves and flowering parts. Woody stems, which are of minor value, must be avoided as far as possible. The plant's low-growing habit makes mechanical harvest difficult.

The most important quality characteristics of thyme, i.e. the yield of essential oil and its chemical composition, are highly dependent on harvesting time, and this has been clearly established in several studies performed with different *Thymus* species (Venskutonis, 2002a; Badi *et al.*, 2004). For instance, the highest essential oil content from *T. vulgaris* from Iran was obtained in the early flowering stage, and the lowest at seed ripening stage (Omidbaigi *et al.*, 2008). Consequently it is possible to optimize the yield and the quality of the essential oils of thyme by harvesting the plant material at the right development stage (Christensen and Grevsen, 2006).

All the post-harvest principles that apply to leafy green tissues apply to the handling of fresh herbs. Temperature is the most important factor in maintaining quality after harvest. The optimum post-harvest temperature for fresh thyme is 0°C (a shelf-life of 3–4 weeks). With a temperature of 5°C, a minimum shelf-life of 2–3 weeks can be expected (Cantwell and Reid, 1986). Therefore, after harvesting, the appropriate cooling is needed to prolong shelf-life of fresh thyme.

Some novel processes to prolong shelf-life of fresh herbs and spices and retain their flavour and appearance for considerably longer time have been developed and tested on various culinary herbs and spices. These processes and their possible applicability to thyme have been reviewed elsewhere (Venskutonis, 2002b).

Natural drying is the simplest way to prepare thyme for storage and further processing. Natural drying of the whole *T. vulgaris* herb is particularly problematic because the shrub consists of comparatively fast-drying leaves and slowly-drying rather hard stems. A more sophisticated method is that of solar drying. It maintains the rich green colour making the product look attractive.

Traditional hot air drying should be tailored to minimize flavour loss and to perform the process at reasonable time and energy costs. It is well-established that higher drying temperatures need shorter drying processes. However, they cause bigger losses of volatiles. Raghavan *et al.* (1995) compared cross-flow and through-flow drying methods on Indian thyme at 40, 50 and 60°C and found that through-flow drying at 40°C gave the best results.

Freeze drying is based on evaporation of water directly from ice under a high vacuum. The products obtained by this method are usually of a better appearance (colour) and aroma quality. The high cost is the main disadvantages of freeze drying, which limits the wider use in a commercial scale.

### 27.3.5 Packaging and storage

The main tasks for packaging are to protect the herb against external conditions and to increase the stability against negative internal changes (enzymatic,

non-enzymatic, chemical reactions, etc.). In general, dried thyme should be stored in cool, dry conditions away from light. Ideally, it should be in air-tight packaging to reduce oxidation. Storage below  $-18^{\circ}\text{C}$  is a guarantee for unlimited storage time; for instance, freezing of thyme in a forced-air freezer and stored at  $-20^{\circ}\text{C}$  for 12 months best maintained the composition of essential oil as compared to air-dried and freeze-dried herb (Usai *et al.*, 2011). Dried herb can be stored at  $5-7^{\circ}\text{C}$  for more than 12 months, whereas at room temperature the stability considerably decreases. Finely milled thyme does tend to lose volatiles more rapidly than medium or coarsely ground material and must be stored in well-closed containers. Storage in multi-layered paper sacs having an impervious lining is also satisfactory.

## 27.4 Main uses in food processing

Thyme herb or processed products can be used in culinary and/or food processing as a separate flavouring or in the composition of compounded seasonings, spice, essential oil, oleoresins or other product blends. The list of thyme applications includes almost all foods: beverages, cheese, fish, meat, salad dressings, sauces, vegetables, egg dishes, game and poultry, soups and honey. Usually, owing to its sensory characteristics, thyme is not suitable for sweet products.

The main uses of thyme in culinary and food processing are defined by the following properties of thyme components: (i) odour and taste, (ii) antioxidant and (iii) antimicrobial activities. Also, fresh green thyme leaves can be used in culinary art as a decorative green herb. It is evident that food flavouring remains as the main thyme application area, while its antimicrobial and antioxidant properties can be considered as the supplementary benefits of thyme products, which have been added to the foods. The possibility to successfully use all three benefits provided by thyme components, namely flavour and prevention of microbial and oxidative spoilage, depends on product requirements, processing parameters and food producer skill.

### 27.4.1 Fresh and dried herb

The use of fresh thyme herb in food is rather limited due to a very short shelf-life (see Section 27.3.4). Although proper temperature and storage conditions can prolong shelf-life of freshly cut thyme up to 4 weeks, green herb is mainly used in catering and home cooking. Some studies have shown that even simple cutting of the plants generates the changes in their aroma composition.

Whole dried thyme herb as such can find numerous culinary applications, however, its direct use in food processing is rather limited. The main concerns are related to the evenness of distribution throughout the food product and to the release of volatile compounds into the product. Therefore, in most cases, industry requires additional treatments in order to meet specified quality parameters. Comminution is a simple and the most widely used treatment before final application of dried thyme. The leaves can be chopped, cut or sliced, broken or rubbed and ground; the spice manufacturers may adopt their own empirical classification.

Grinding ruptures the oil structures containing the volatile oil, and the oil becomes available for reaction (e.g. oxidation) or evaporation. Grinding also generates some

**Table 27.1** Advantages and disadvantages of dried ground thyme

| Advantages                                                   | Disadvantages                                                                   |
|--------------------------------------------------------------|---------------------------------------------------------------------------------|
| Slow flavour release in high-temperature processing          | Variable flavour strength and profile                                           |
| Easy to handle and weigh accurately                          | Unhygienic, often contaminated by filth                                         |
| No labelling declaration problems                            | Easy adulteration with less valuable materials                                  |
| Presence of natural antioxidant and antimicrobial components | Flavour loss and degradation on storage                                         |
|                                                              | Undesirable appearance characteristics in end products                          |
|                                                              | Poor flavour distribution (particularly in thin liquid products such as sauces) |
|                                                              | Unacceptable hay-like aroma                                                     |
|                                                              | Dusty and unpleasant to handle in bulk                                          |

heat, which tends to vaporize the volatile oil, leading to a reduction in flavour strength. Therefore, it is necessary to keep the temperatures during the grinding process as low as possible. Granulation, usually producing optimal products in many respects, was shown to cause a remarkable loss of essential oil (Kowalski and Wawrzykowski, 2009a).

The moisture content of ground thyme is of importance to both stability and flavour value. It must be dry enough to prevent a musty odour and flavour, and yet be moist enough to retain the optimum odour and flavour character. In terms of use for food flavouring, the most common advantages and disadvantages of dried ground thyme are summarized in Table 27.1 (Heath and Reineccius, 1986).

Microbial contamination of thyme can encounter serious problems in some microbiologically sensitive foods. Therefore, sterilization procedures are often applied before final application. Sterilization is performed by chemical (ethylene oxide, methyl bromide, ozone) or physical (irradiation, UV-irradiation, microwaving, high-frequency electric current, high pressure) treatments. It should be considered that treatment with ethylene oxide has been banned in many countries, including the EU.

**27.4.2 Thyme extractives and processed products**

Owing to the above-mentioned disadvantages of dried ground thyme, manufacturers increasingly are recognizing the advantages of seasonings based on herb extractives. The most important extraction products that are obtained from thyme are essential oils, herb oleoresins and extracts. The yield of hydrodistilled oil may be increased by ultrasound-assisted maceration (Kowalski and Wawrzykowski, 2009b), while microwave-assisted hydrodistillation saves energy and reduces isolation time (Golmakani and Rezaei, 2008).

Thyme essential oil and oleoresin possess sweetly aromatic, warmly pungent odour and sharp, rich, warmly phenolic flavour. The advantages and disadvantages of its uses in food processing are common to many other herb essential oils and oleoresins and are summarized in Tables 27.2 and 27.3 (Heath and Reineccius, 1986).

To avoid the disadvantages of thyme essential oils and oleoresins, they can be further processed to obtain such advanced products as solubilized (providing clear

**Table 27.2** Advantages and disadvantages of thyme essential oil

| Advantages                                             | Disadvantages                                                       |
|--------------------------------------------------------|---------------------------------------------------------------------|
| Hygienic, free from all microorganisms                 | Flavour good but incomplete and unbalanced compared to natural herb |
| Flavouring strength within acceptable limits           | Does not contain non-volatile antioxidants                          |
| Flavour quality consistent with source of raw material | Some compounds readily oxidize                                      |
| No colour imparted to the end-product                  | Readily adulterated                                                 |
| Free from enzymes                                      | Very concentrated so difficult to handle and weigh accurately       |
| Stable in storage under good conditions                | Not readily dispersible, particularly in dry products               |

**Table 27.3** Advantages and disadvantages of thyme oleoresins

| Advantages                                    | Disadvantages                                                          |
|-----------------------------------------------|------------------------------------------------------------------------|
| Hygienic, free from all microorganisms        | Flavour quality good but as variable as the raw material               |
| Can be standardized for flavouring strength   | Flavour profile dependent on the solvent used                          |
| Contain natural antioxidants                  | Very concentrated so difficult to handle and weigh accurately          |
| Free from enzymes                             | Sometimes difficult to incorporate into food mixes without 'hot spots' |
| Long shelf-life under good storage conditions |                                                                        |

solution when mixed with water), dispersed, plated or 'dry-soluble', encapsulated, heat-resistant and fat-based products (Table 27.4). For instance, with dispersed products, standardized flavour profile and flavouring strength can be obtained. Usually such products are tailored for a specific food application and contain blends of essential oils and/or oleoresins. Thyme extractives are used as a part of such blends in numerous flavourings and seasonings

## 27.5 Functional properties and toxicity

All over the world, thyme, *T. vulgaris*, is highly regarded. Thyme has developed from a simple traditional herb into a drug that is taken seriously in phytotherapy. Herbal thyme, thyme extracts and thyme oil are used to treat symptoms of bronchitis and whooping cough as well as catarrh in the upper respiratory tract. This development is based on numerous experimental *in vitro* studies revealing well-defined pharmacological activities (Zarzuelo and Crespo, 2002; and references cited therein) of both the essential oil and the plant extracts, the antimicrobial, antioxidative and antispasmodic properties being the most important ones. The non-medicinal use of thyme is no less important because thyme serves as a preservative for foods and is a culinary ingredient widely used as a seasoning in many parts of the world. Furthermore, thyme oil is an ingredient in many cosmetic preparations.

**Table 27.4** Examples of standardized thyme products

| Product                                                             | Producer                                                                     | Characteristics                                                                                                                                                                                                                                                                |
|---------------------------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Standardized oleoresin<br>Thyme FD0718                              | Bush Boake Allen Limited,<br>London, UK                                      | Volatile oil content (% v/w)<br>54–60                                                                                                                                                                                                                                          |
| Standardized oleoresins<br>Thyme HX2089                             | Lionel Hitchen Essential Oil<br>Company Limited, Barton<br>Stacey, Hants, UK | Volatile oil content (% v/w)<br>50<br>dispersion rate kg = 100 kg<br>of spice 1                                                                                                                                                                                                |
| Dispersed spices – salt thyme                                       | Bush Boake Allen Limited,<br>London, UK                                      | Volatile oil content<br>0.3–0.4 % (v/w)                                                                                                                                                                                                                                        |
| Dispersed spices – dextrose<br>thyme                                | Bush Boake Allen Limited,<br>London, UK                                      | Volatile oil content<br>0.3–0.4 % (v/w)                                                                                                                                                                                                                                        |
| Dispersed spices – rusk<br>Thyme FD5781                             | Bush Boake Allen Limited,<br>London, UK                                      | Volatile oil content<br>0.6–0.8 % (v/w)                                                                                                                                                                                                                                        |
| Standardized emulsion<br>oleoresins<br>Thyme HF107                  | Felton Worldwide SARL,<br>Versailles, France                                 | Strength compared to<br>ground spice 4×                                                                                                                                                                                                                                        |
| Standardized emulsion<br>oleoresins<br>Thyme FD6136                 | Bush Boake Allen Limited,<br>London, UK                                      | Strength compared to<br>ground spice 5×                                                                                                                                                                                                                                        |
| Encapsulated standardized<br>oleoresins<br>Thyme FD4040             | Bush Boake Allen,<br>'Saronseal Encapsulated<br>spices'                      | Strength compared to<br>ground spice 10×                                                                                                                                                                                                                                       |
| Thyme Extract                                                       | Acetar Bio-Tech Inc.<br>Xi'an, China                                         | Obtained from comminuted<br><i>Thymus serpyllum</i> L. herb;<br>fine brownish-yellow<br>powder; standard<br>concentrate ratio: 10:1 or<br>20:1; active ingredient:<br>thymol, carvacrol, linalool,<br>p-cymol, etc.                                                            |
| Thyme Extract P070                                                  | MDidea Brand,<br>Ningxia, China                                              | An extract of the flowers<br>and leaves of <i>Thymus<br/>vulgaris</i> ; shelf-life 18–24<br>months; light brown fine<br>powder; 100 % pass 80<br>mesh screen; light aroma<br>smell; characteristic sweet<br>taste; bulk density<br>0.48–0.54 g/ml                              |
| Oleoresin thyme (extracted<br>with supercritical carbon<br>dioxide) | Oleoresins World Actives,<br>South Sherman,<br>Connecticut, USA              | Brown–dark green, liquid at<br>room temperature; odour:<br>fresh spice; volatile oil<br>5.8–6 %; carvacrol content<br>58.7 %; residual solvent<br>less than 25 ppm;<br>shelf-life: 12 month(s);<br>application dosage: 1 kg<br>oleoresin thyme = 30 kg<br>thyme in powder form |

### 27.5.1 Antimicrobial activity of thyme oil

Thyme oil reveals a broadband spectrum of antimicrobial activity in various *in vitro* test systems (agar diffusion test, dilution test, vapour phase test). The results of about 40 screening studies with thyme oil published between 1975 and 2008 are documented in a comprehensive table (Pauli and Schilcher, 2010). It presents the microbial activity of thyme oil on 34 gram-negative bacteria, 36 gram-positive bacteria, 62 fungi and 27 yeasts, including human, animal, plant and micromycetal food poisoning pathogens. The data show that thyme oil is able to effectively inhibit a broad spectrum of microorganisms (for details see references in Pauli and Schilcher, 2010). More recent publications support these findings by documenting the antimicrobial effect of thyme oil against several foodborne pathogens (Gutierrez *et al.*, 2008; Paparella *et al.*, 2008; Barbosa *et al.*, 2009; Nedorostova *et al.*, 2009), mycotoxigenic fungi (Nguefack *et al.*, 2009) and yeasts (das Neves *et al.*, 2009), as well as food poisoning microorganisms (Sokovic *et al.*, 2009), phytopathogenic fungi (Kim *et al.*, 2008; Alzate *et al.*, 2009) and post-harvest fungal infectors (Kumar *et al.*, 2008). Furthermore, the essential oil of thyme showed a wide range of antibacterial activity against microorganisms that had developed resistance to antibiotics (Nelson, 1997), among others against the methicillin-resistant *Staphylococcus* (Nelson, 1997; Chao *et al.*, 2008) and the vancomycin-resistant *Enterococcus faecium* (Nelson, 1997). Inhibition of drug-resistant clinical herpes simplex virus type 1 and 2 could be proven for thyme oil and thyme extracts (Nolkemper *et al.*, 2006; Schnitzler *et al.*, 2007).

When discussing the antimicrobially active constituents of thyme oil, the monoterpenes thymol and carvacrol play an outstanding role (Hoeflerl *et al.*, 2009). They bind to the amine and hydroxylamine groups of the proteins of the bacterial membrane altering their permeability and resulting in the death of the bacteria (Juven *et al.*, 1994). Aside from that, the strong antimicrobial activity of thyme oil is based on additive effects, which might at least enhance the rapidity of the antimicrobial action (Iten *et al.*, 2009). Also, the antifungal activity of thyme oils is attributed to thymol and carvacrol causing degeneration of the fungal hyphae which seems to empty their cytoplasmic content (Zambonelli *et al.*, 1996).

### 27.5.2 Thyme as an antioxidative agent

The antioxidative property of thyme is important in both the medicinal and non-medicinal context. Oxidative stress contributes to the pathogenesis of many human diseases; therefore, the intake of antioxidative agents is important for the prevention of chronic diseases. In the non-medicinal context, the antioxidant character is responsible for a preservative activity, especially in preventing oxidation of lipids in food.

Several recent papers confirm what has been known for a long time, namely the antioxidative ability of thyme oil (Bozin *et al.*, 2006; Stoilova *et al.*, 2008; Wang *et al.*, 2008; Viuda-Martos *et al.*, 2010; Wei and Shibamoto, 2010) and thyme extracts (Kulisic *et al.*, 2006; Sarosi and Bernath, 2006; Chizzola *et al.*, 2008; Szabo *et al.*, 2008; Amarowicz *et al.*, 2009; Lagouri and Nisteropoulou, 2009). Discussing the chemical principle of the antioxidative activity of the oil, the terpene phenols thymol and carvacrol have always been in the focus of interest. Indeed, it could be demonstrated



that both exhibit antioxidative activities (Schwarz *et al.*, 1996; Nakatani, 2000). The antioxidative activity of ethanolic and water extracts of thyme is due to non-volatile phenolic components, the most potent ones being rosmarinic acid and 3'-O-(8''-Z-caffeoyl)-rosmarinic acid (Dapkevicius *et al.*, 2002). Also, the biphenyl compounds are responsible for the antioxidative power of thyme, namely *p*-cymene 2,3-diol (Schwarz *et al.*, 1996) and the *p*-cymene 2,3-diol 6,6'-dimer (Haraguchi *et al.*, 1996; Dapkevicius *et al.*, 2002). Due to their electron-donating properties, the flavonoids of thyme contribute also to its antioxidative activity. In this respect, the aglycones eriodictyol and 7-O-methyl luteolin (Miura and Nakatani, 1989; Haraguchi *et al.*, 1996; Miura *et al.*, 2002) and two flavone glycosides, namely luteolin-O-glucoside and eriodictyol rutinoside (Wang *et al.* 1998), were shown to be the most effective flavonoids.

### 27.5.3 Antispasmodic activity of thyme

Contractions of the isolated guinea pig trachea, induced by various spasmogens, were antagonized by an ethanolic thyme extract (Meister *et al.*, 1999; Boskapady *et al.*, 2006), an effect which is at least partially caused by its activity on  $\beta_2$ -receptors (Wienkötter *et al.*, 2007). The phenols thymol and carvacrol contribute to the anti-spasmodic effect in most models. Thymol, however, is not important for the overall antispasmodic effect, when all spasmic triggers are considered, e.g. thymol alone had no effect on endothelin-induced trachea contraction (Engelbertz *et al.*, 2008). That explains why extracts with very low thymol levels are active too, meaning that other not yet identified components in thyme extract seem to be important (Begrow *et al.*, 2010). Years ago some authors focused on the flavonoids and found that the flavones as well as thyme extracts were effective in test systems with smooth muscles of guinea pig ileum and of rat vas deferens (Van den Broucke and Lemli, 1983).

### 27.5.4 Further effects

Several further effects of thyme and thyme preparation have been reported. An extract of *T. vulgaris* showed antiparasitic properties against *Leishmania mexicana* inhibiting its mitochondrial DNA polymerase with thymol to be mainly responsible of this effect (Schnitzler *et al.*, 1995). Nematicidal effects could be proven for the essential oil (Kong *et al.*, 2007; Zouhar *et al.*, 2009) as well as acaricidal activity studied with the two-spotted spider mite, *Tetranychus urticae* (El-Gengaihi *et al.*, 1996; Lee *et al.*, 1997; Aslan *et al.*, 2004; Eldoksch *et al.*, 2009; El-Zemity *et al.*, 2009), poultry red mite, *Dermanyssus gallinae* (Ghrabi-Gammar *et al.*, 2009; George *et al.*, 2010), *Tyrophagus putrescentiae*, a stored food mite (Jeong *et al.*, 2008), house dust mite, *Dermatophagoides pteronyssinus* (Saad *et al.*, 2006). Insecticidal effects were proven for the essential oils of *T. vulgaris* and *T. serpyllum* by direct toxicity of adult insects and by inhibiting reproduction through ovicidal and larvicidal effects (Regnaultroger and Hamraoui, 1994). Other test objectives were the pulse beetle, *Callosobruchus maculatus* (Erler *et al.*, 2009), *Lycoriella mali* (Choi *et al.*, 2006), the granary weevils, *Sitophilus zeamais* and *Acanthoscelides obtectus* (Bittner *et al.*, 2008), three stored-product insects (Rozman *et al.*, 2007), the flour beetle, *Tribolium confusum* (Sener *et al.*, 2009) and the house fly, *Musca domestica* (Pavela 2007, 2008),

the cabbage aphid, *Brevicoryne brassicae* (Gorur *et al.*, 2008), the Western corn rootworm, *Diabrotica virgifera* (Lee *et al.*, 1997) and *Spodoptera littoralis* (Farag *et al.*, 1994). Also the larvicidal activity against the mosquito larvae, *Culex quinquefasciatus* (Pavela, 2009) and *Anopheles gambiae* (Tchoumboungang *et al.*, 2009) was documented by several authors as well as the mosquitocidal and mosquito repellent activity of thyme oil (Hori, 2003; Park *et al.*, 2005; Choi *et al.*, 2006; Zhu *et al.*, 2006; Knio *et al.*, 2008; Pavela *et al.*, 2009). Thyme was shown to have herbicidal and phytotoxic activity and therefore allelopathic activity (Angelini *et al.*, 2003; Arminante *et al.*, 2006; Uremis *et al.*, 2009; Rolim de Almeida *et al.*, 2010).

### 27.5.5 Toxicity and mutagenicity

Little is reported on toxic effects of thyme on mammals. In an acute toxicity test, a concentrated extract of thyme reduced locomotor activity and caused a slight slowing down of respiration in mice when 0.5–3.0 g extract/kg body weight (= 4.3–26.0 dried plant material) was administered to the mice (Qureshi *et al.*, 1991). Sub-chronic toxicity was observed after administration of a concentrated ethanol extract of plant material to mice over three months causing an increase in liver and testes weight; 30 % of the male animals died (Qureshi *et al.*, 1991). The essential oil of thyme oil showed an acute oral toxicity of LD 50 = 4.7 g/kg rat. This effect is attributed to the terpene phenols thymol and carvacrol (Dilaser, 1979), which also cause skin irritations and irritation of the mucosa, explaining the severe irritation of mouse and rabbit skin when it is exposed to undiluted thyme oil. In a mutagenicity test system with *Salmonella typhimurium*, thyme oil showed neither mutagenic nor DNA-damaging activity (De Martino *et al.*, 2009).

### 27.5.6 Thyme in phytotherapy

Thyme and thyme preparations have achieved a reliable place in phytotherapy for the treatment of catarrhs of the upper respiratory tract and bronchial catarrhs. This is due to the intensive efforts of committees which evaluated the published scientific literature of herbal drugs in order to elaborate scientific standards for herbal medicinal products. The first monographs on thyme with data concerning the therapeutic indications, posology, methods of administration and side-effects were formulated by the German Commission E in 1990 and 1992 (Blumenthal 1998), later by the European Scientific Cooperative on Phytotherapy (ESCOP, 2003) and the World Health Organization (WHO, 1999).

Today the Herbal Medicinal Product Committee (HMPC 2007/2010), located at the EMA (European Medicines Agency, London), is responsible for the European harmonization of herbal medicinal products. The HMPC decides about the status of herbal drugs in Europe on the basis of the monographs of the above-mentioned commissions and of recent scientific knowledge. Concerning thyme, the HMPC published the following decisions: (i) thyme as herbal substance or comminuted herbal substance for tea preparation or as other herbal preparations in liquid or solid dosage forms for oral use is classified as a 'traditional herbal medicinal product used as an expectorant in cough associated with cold'; (ii) the essential oil of thyme also has the status of a traditional herbal medicinal product and is recommended

as ‘an expectorant in cough associated with cold’ (oral use) and ‘for the relief of symptoms in coughs and colds’ (cutaneous use and as bath additive). When applied correctly, the herbal thyme and thyme oil are considered to be safe aside from possible hypersensitive reactions (for details see [www.ema.europa.eu](http://www.ema.europa.eu)).

## 27.6 Quality issues

### 27.6.1 Specifications

The medicinal and non-medicinal uses of thyme and thyme preparations demand high-quality standards. Concerning the non-medical use, the International Standard Organisation (ISO) elaborated specifications for ‘Dried Thyme’ (ISO 6754: 1996) which were accepted also by the ‘Association Française de Normalisation’ with the title ‘Thym séché’ (AFNOR NF ISO 6754). According to this standard ‘Dried Thyme’ is won from *Thymus vulgaris* L. It must fulfil special requirements concerning the odour, and the absence of mould and insects; furthermore, the limits for foreign matters and chemical specifications (water, total ash, ash insoluble in hydrochloric acid, essential oil content) are given.

Internationally accepted specifications also exist for the commercially important thyme oil. Table 27.5 presents the specifications of the ‘Food Chemical Codex’ (FCC). Specifications for ‘Oil of Thyme containing thymol, Spanish type [*Thymus zygis* (Loefl.) L.]’ are given by the ISO (ISO 14715:2010) which were also accepted by the ‘Association Française de Normalisation’ (AFNOR). In comparison with the requirements of the European Pharmacopoeia (Ph. Eur. 7.3 ed. 2011: Thyme oil, Thymol type), the ISO standard is a bit more stringent, stipulating percentage limits for three further oil components ( $\alpha$ -pinene, *trans*- $\beta$ -sabinene and  $\beta$ -caryophyllene) and formulating more restricted limits for myrcene,  $\gamma$ -terpinene and linalool. The lower limit for methyl carvacrol is higher. The percentage limits of thymol and carvacrol and four further components are the same in both the ISO standard and the Ph. Eur. 7.3.

**Table 27.5** Specifications for thyme oil

|                          |                                                                                                                                                                                                                                                                                               |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Appearance               | A colourless, yellow or red liquid with a characteristic, pleasant odour and a pungent, persistent taste. It is the volatile oil obtained by distillation from the flowering plant <i>Thymus vulgaris</i> L., or <i>Thymus zygis</i> L., and its var. <i>gracilis</i> Boiss. (Fam. Labiatae). |
| Phenol content           | Not less than 40 %                                                                                                                                                                                                                                                                            |
| Angular rotation (20 °C) | Between $-3^{\circ}$ and $-0,1^{\circ}$                                                                                                                                                                                                                                                       |
| Refractive index (20 °C) | 1.495–1.505 at 20 °C                                                                                                                                                                                                                                                                          |
| Specific gravity         | 0.915–0.935                                                                                                                                                                                                                                                                                   |
| Solubility               | 1 mL dissolves in 2 mL of 80 % ethanol (v/v)                                                                                                                                                                                                                                                  |
| Water-soluble phenols    | Shake 1 mL of oil with 10 mL of hot water and, after cooling, pass water layer through a moistened filter. Add 1 drop of ferric chloride TS to the filtrate, not even a transient blue or violet colour appears.                                                                              |
| Packaging and storage    | Store in a cool place in full, tight, light-resistant containers                                                                                                                                                                                                                              |

Source: FCC (2010).

### 27.6.2 Thyme in pharmacopoeias

For the medical use of thyme, only the standards of the pharmacopoeias are relevant. In the European Pharmacopoeia (Ph. Eur. 7<sup>th</sup> edition 2010) quality standards for thyme (*Thymi herba*), and wild thyme (*Serpylli herba*) are given. New quality standards for thyme oil, thymol type (*Thymi typo thymolo aetheroleum*) are published in the supplement 7.3 (2011) of the Ph. Eur. 7<sup>th</sup> edition. The German Pharmacopoeia (DAB, 2011) contains a monograph for ‘thyme liquid extract’ (*Thymi extractum fluidum*). Pharmacopoeial summaries for quality assurance can also be found in the WHO monographs (WHO, 1999). The pharmacopoeial monographs begin with a definition of the drug including the plant source and the quantitative requirements concerning the biologically active compounds.

- Thyme – *Thymi herba* (Ph. Eur. 7.0)  
Thyme consists of the whole leaves and flowers separated from the previously dried stems of *Thymus vulgaris* L. or *Thymus zygis* L. or a mixture of both species. It contains not less than 12 ml/kg of essential oil (anhydrous drug). The sum of the contents of thymol and carvacrol (both C<sub>10</sub>H<sub>14</sub>O; M 150.2) in the essential oil is a minimum of 40 % (anhydrous drug).
- Thyme oil, thymol type – *Thymi typo thymolo aetheroleum* (Ph. Eur. 7.3)  
Thyme oil is obtained by steam distillation from the fresh flowering aerial parts of *Thymus vulgaris* L., *T. zygis* L. or a mixture of both species.
- Wild thyme – *Serpylli herba* (Ph. Eur. 7.0)  
It consists of the whole or cut dried, flowering aerial parts of *Thymus serpyllum* L. s.l. Content: minimum 3.0 ml/kg essential oil (dried drug).
- Thyme liquid extract – *Thymi extractum fluidum* (DAB, 2011)  
It is prepared by a suitable method using 1 part powdered thyme and 2 to 3 parts of a mixture of 1 part ammonia 10 %, 20 parts glycerol and 109 parts purified water. It contains a minimum of 0.03 % phenols, calculated as thymol.

In the monographs of the herbal drugs, macroscopic and microscopic descriptions are given which serve as a basis for the identification of the drugs. Component-related identifications of the drugs are performed by thin-layer chromatography (TLC) of a methylene chloride extract of the drugs containing the essential oils, including the terpene phenols, thymol and carvacrol, which serve as reference substances. Descriptions of the TLC-fingerprints are given in a tabular form. Further pharmacopoeial requirements concern the cleanliness which must be verified by special tests (Table 27.6). The content of essential oils is ascertained by means of water distillation with a standardized Clevenger-type apparatus. The volume of the separated essential oil is measured in the graduated tube of the apparatus. For thyme, a determination of the phenol content (thymol plus carvacrol) in the essential oil is postulated (a minimum of together 40 %).

The identity of thyme oil has also to be completed by TLC with thymol, terpinen-4-ol and linalool as references. A second identification test has to be carried out by means of gas-liquid chromatography (GLC) with eight references. The cleanliness tests for thyme oil require that the relative density of the oil must range between 0.915 and 0.935, the refractive index between 1.490 and 1.505. The qualitative assay is called the ‘chromatographic profile’ and is done by GLC with a fused-silica column 60 m long and about 0.3 mm in internal diameter coated with poly(dimethyl)

**Table 27.6** Pharmacopoeial tests for cleanliness (European Pharmacopoeia 7.0, 2010)

|                                    | Thyme                                                                                                                                                                                                                                                 | Wild thyme                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Foreign matter                     | Maximum 10 % of stems and maximum 2 % of other foreign matter. Stems must not be more than 1 mm in diameter and 15 mm in length. Leaves with long trichomes at their base and with weakly pubescent other parts ( <i>T. serpyllum</i> L.) are absent. | Maximum 3 %, determined on 30 g; foreign matter may also consist of acicular to linear–lanceolate leaves with a strong bent margin, the adaxial surface showing covering trichomes shaped as pointed teeth with warty walls, the abaxial surface showing many types of warty covering trichomes: unicellular, straight or slightly curved, bicellular or tricellular, often elbow-shaped, and bicellular or tricellular, more or less straight ( <i>T. vulgaris</i> , <i>T. zygis</i> ). |
| Water                              | Maximum 100 ml/kg determined on 20.0 g of powdered drug                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Loss on drying                     |                                                                                                                                                                                                                                                       | Maximum 10.0 %, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C for 2 h                                                                                                                                                                                                                                                                                                                                                                                        |
| Total ash                          | Maximum 15.0 %                                                                                                                                                                                                                                        | Maximum 10.0 %                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Ash insoluble in hydrochloric acid | Maximum 3.0 %                                                                                                                                                                                                                                         | Maximum 3.0 %                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

**Table 27.7** Percentage content of the components in thyme oil, thymol type as postulated by the European Pharmacopoeia 7.3, 2011 ‘Chromatographic profile’

| Component              | Percentage ratio |
|------------------------|------------------|
| $\alpha$ -Thujene      | 0.2 %–1.5 %      |
| $\beta$ -Myrcene       | 1.0 %–3.0 %      |
| $\alpha$ -Terpinene    | 0.9 %–2.6 %      |
| <i>p</i> -Cymene       | 14.0 %–28.0 %    |
| $\gamma$ -Terpinene    | 4.0 %–12.0 %     |
| Linalool               | 1.5 %–6.5 %      |
| Terpinen-4-ol          | 0.1 %–2.5 %      |
| Carvacrol methyl ether | 0.05 %–1.5 %     |
| Thymol                 | 37.0 %–55.0 %    |
| Carvacrol              | 0.5 %–5.5 %      |

(diphenyl)siloxane (film thickness 0.25  $\mu\text{m}$ ). Quantification is made by the normalization procedure of the peak areas yielding the percentage contents of ten components. The lower and the upper limits of the ten components are listed in Table 27.7. For better identification a gas chromatogram of thyme oil is given (Fig. 27.3).

### 27.6.3 Authenticity and adulteration

Adulteration of the herbal drugs of thyme and wild thyme is rare and can be demonstrated by macroscopic and microscopic analysis according to the pharmacopoeial

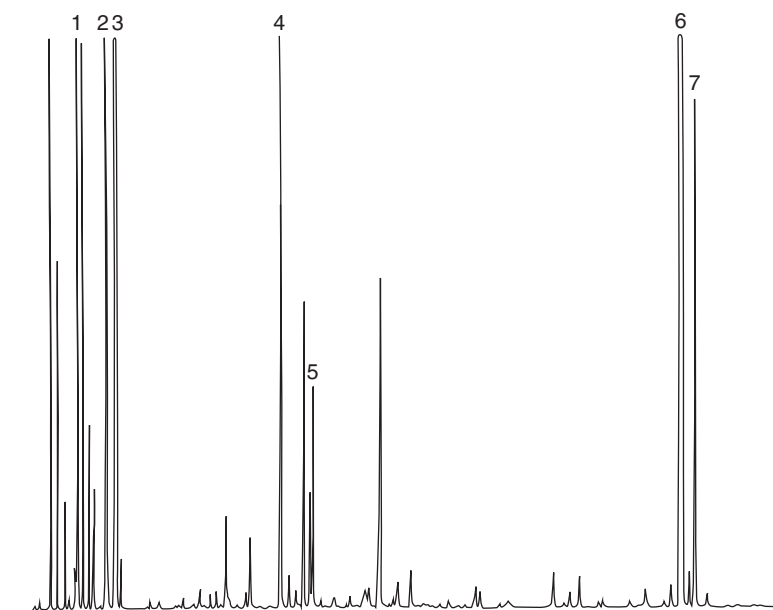


**Fig. 27.3** Gas chromatogramme of thyme oil according to the European Pharmacopoeia 4.1, 2002. 1.  $\beta$ -myrcene 2.  $\gamma$ -terpinene 3. *p*-cymene 4. linalool 5. terpinen-4-ol 6. thymol 7. carvacrol

standards. The situation of thyme oil is worse because adulteration is practised even today. In the past, thyme oil was frequently adulterated by the addition of synthetic thymol and carvacrol or of ‘thymene’, a cheap by-product mixture obtained from ajowan oil (ex *Trachyspermum copticum* (L.) Link) after removal of thymol. Adulteration is evident when thyme oil can be found on the market at low prices. GLC with capillary columns, special polar and non-polar stationary phases and GLC/mass spectrometry (MS) have led to a more accurate and detailed analysis of the oil composition and have resulted in a more reliable determination of the authenticity and purity of an essential oil.

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## Turmeric

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**Abstract:** Turmeric of commerce is the dried rhizome of the plant *Curcuma domestica* Val. syn. *C. longa* L. This chapter presents the history, origin, botany, classification, biotechnology, production and post-harvest processing of turmeric in addition to its chemical composition, cultivation and the novel approach of gene mining. Value-added products and quality aspects as well as adulteration are also covered. The chapter finishes by looking at the uses of turmeric in the food industry and its functional properties in relation to medical uses, both traditional and modern.

**Key words:** Adulterants, biotechnology, *Curcuma longa*, chemistry, curcuminoids, functional properties, production, religious uses, value added products.

### 28.1 Introduction

Turmeric of commerce is the dried rhizome of the plant *Curcuma domestica* Val. syn. *C. longa* L. Turmeric is mentioned in the 'Atharva Veda' of 1000–1500 BCE, a holy treatise of the Hindus, as 'haldi' or 'haridrar' (AV/1/22/4) (Shah, 1977). Ethnobotanical evidence indicates that it has been used in India since very ancient times. It is believed that the crop spread out from India to distant Asian countries under the influence of the Hindu religion. According to Marco Polo (1280), the spread of turmeric to China took place in CE 700 cf (Ridley, 1912). Burkill (1966) believed that the crop spread to West Africa in the thirteenth and to East Africa in the seventeenth centuries, respectively. It was introduced to Jamaica in 1783 (Velayudhan *et al.*, 1999). Although turmeric is now grown in India, Pakistan, Malaysia, Myanmar, Vietnam, Thailand, Philippines, Japan, China, Korea, Sri Lanka, Nepal, South Pacific Islands, East and West Africa, Malagasi, Caribbean islands and Central America, India is the major producer and exporter of turmeric at present. Turmeric is used in curry powder, chicken bouillon, sauces, gravies, dry seasonings, baking mixes, processed cheese, pickles, relishes, breading, soups, beverages and confections (Peter, 1999). In addition, it is used in medicine, at religious functions and as a biopesticide (Sasikumar, 2005).



### 28.1.1 Origin and classification

The genus *Curcuma* belongs to the family *Zingiberaceae* and contains 49 genera and 1400 species. The genus originated in the Indo-Malayan region (Ravindran *et al.*, 2007). There are about 80 species in the genus, globally (Sasikumar, 2005). About 40 species including *C. longa* are indigenous to India, indicating the Indian origin (Velayudhan *et al.*, 1999). In addition to *C. longa*, *C. xanthorrhiza* Roxb., *C. malabarica* Vel., etc. are also minor sources of curcumin colour. Velayudhan *et al.*, (1999) recognized six taxonomic varieties within *C. longa* based on numerical taxonomic analysis, namely *C. longa* var. *typica*, *C. longa* var. *atypica*, *C. longa* var. *camphora*, *C. longa* var. *spiralifolia*, *C. longa* var. *musacifolia* and *C. longa* var. *platifolia*. Most of the *C. longa* found in India belongs to *C. longa* var. *typica* or *atypica*.

### 28.1.2 Description

Turmeric is an erect perennial herb, grown as an annual crop. The above-ground morphology of the plant is mainly represented by an erect aerial stem bearing leaves and inflorescence. There may be two to five aerial stems (tillers) per plant. The height of the aerial stem varies from 90–100 cm depending on the variety. Leaf number ranges from 7–12. In fact, it is the leaf sheath which forms the aerial stem. The leaf sheath is usually green in colour. Lamina may be lanceolate or elliptic in shape, thin with acuminate tip. The colour of lamina is usually green above and pale green below, with a length of about 30–40 cm and width 8–12 cm. The inflorescence is a cylindrical, fleshy, central spike of 10–15 cm length, arising through the aerial stem. Flowers are subtended by bracts in the spike. The bracts are adnate for less than half of their length and are elliptic, lanceolate and acute. The upper bracts are white in colour while the lower bracts are green. One to four flowers are borne in the axil of the bract, opening one at a time. About 30 flowers are produced in a spike (Nazeema and Rema Menon, 1994). The calyx is short, usually toothed and split nearly halfway down on one side. The corolla is tubular, thin and whitish with a yellow tip. Usually the uppermost and lowermost bracts will be sterile. Seed set is observed in turmeric and seeds are viable. Seeds are produced in capsules and there will be from one to numerous sunken capsules in an inflorescence depending on the flowers fertilized (Nazeema and Rema Menon, 1994). At the base of the aerial stem below the ground, rhizomes are formed consisting of mother rhizome(s), primary, secondary and even tertiary fingers, the whole forming a compact clump. Rhizomes grow symbodically and are of orange–brown, pale yellow or reddish yellow colour. *C. longa* is predominantly triploid with a somatic chromosome number of 63 ( $2n = 3x = 63$ ). (Nair and Sasikumar, 2009).

### 28.1.3 Chemical composition

Thomas (2009) studied the chemical and biochemical profile of some of the traded turmeric from India such as ‘Alleppey Finger’, ‘Rajapuri’, ‘Wynadan’ along with the popular varieties, namely ‘Prabah’, ‘Prathibha’ and ‘Alleppey Supreme’ (Table 28.1). The nutritional profile of turmeric can be found in Table 28.2. Turmeric is valued mainly for its principal colouring pigment, curcumin, besides other nutritive constituents like potassium (Peter, 1999) (Table 28.2). Curcumin, which has the

**Table 28.1** Chemical and biochemical parameters some Indian turmeric

| Sl. No | Parameter              | Turmeric     |              |                    |                   |              |              |
|--------|------------------------|--------------|--------------|--------------------|-------------------|--------------|--------------|
|        |                        | ‘Prabha’     | ‘Prathibha’  | ‘Alleppey Supreme’ | ‘Alleppey Finger’ | ‘Rajapuri’   | ‘Wynadan’    |
| 1      | Moisture (%)           | 9.88 ± 0.29  | 9.83 ± 0.23  | 8.91 ± 0.25        | 8.33 ± 0.22       | 7.86 ± 0.21  | 9.24 ± 0.05  |
| 2      | Oleoresin (%)          | 14.75 ± 0.26 | 14.99 ± 0.12 | 15.0 ± 0.13        | 11.1 ± 0.50       | 8.57 ± 0.24  | 7.30 ± 0.60  |
| 3      | Essential oil (%)      | 4.50 ± 0.16  | 4.50 ± 0.22  | 4.00 ± 0.33        | 5.60 ± 0.45       | 3.0 ± 0.03   | 3.0 ± 0.05   |
| 4      | Starch (%)             | 53.47 ± 0.85 | 48.38 ± 0.31 | 52.49 ± 0.58       | 41.26 ± 0.09      | 43.92 ± 0.42 | 40.23 ± 0.06 |
| 5      | Carbohydrate (%)       | 57.54 ± 0.07 | 51.54 ± 0.32 | 55.85 ± 0.12       | 47.97 ± 0.54      | 45.20 ± 0.13 | 43.13 ± 0.55 |
| 6      | Reducing sugar (%)     | 1.56 ± 0.03  | 1.05 ± 0.04  | 1.46 ± 0.01        | 1.86 ± 0.05       | 1.53 ± 0.04  | 1.22 ± 0.02  |
| 7      | Phenols (%)            | 2.80 ± 0.01  | 1.43 ± 0.01  | 1.64 ± 0.01        | 2.50 ± 0.05       | 1.78 ± 0.05  | 2.62 ± 0.04  |
| 8      | Amino acids (%)        | 1.25 ± 0.11  | 1.17 ± 0.02  | 1.16 ± 0.04        | 4.24 ± 0.15       | 1.50 ± 0.25  | 1.06 ± 0.02  |
| 9      | Protein (%)            | 1.83 ± 0.05  | 2.05 ± 0.04  | 1.87 ± 0.25        | 1.50 ± 0.05       | 1.05 ± 0.32  | 1.16 ± 0.25  |
| 10     | Crude fiber (%)        | 3.20 ± 0.23  | 3.60 ± 0.02  | 4.00 ± 0.45        | 2.80 ± 0.25       | 3.80 ± 0.25  | 4.00 ± 0.68  |
| 11     | Ash (%)                | 6.77 ± 0.01  | 5.70 ± 0.04  | 6.62 ± 0.02        | 6.45 ± 0.04       | 6.87 ± 0.05  | 6.38 ± 0.03  |
| 12     | Acid insoluble ash (%) | 0.56 ± 0.02  | 0.52 ± 0.04  | 0.68 ± 0.08        | 0.62 ± 0.07       | 0.62 ± 0.01  | 0.45 ± 0.01  |

*Source:* Thomas (2009).

**Table 28.2** Nutritional composition of turmeric

| Constituent        | Quantity per 100g |
|--------------------|-------------------|
| Water (g)          | 6.0               |
| Food energy (Kcal) | 390               |
| Protein (g)        | 8.5               |
| Fat (g)            | 8.9               |
| Carbohydrate (g)   | 69.9              |
| Ash (g)            | 6.8               |
| Calcium (g)        | 0.2               |
| Phosphorous (mg)   | 260               |
| Sodium (mg)        | 30                |
| Potassium (mg)     | 2000              |
| Iron (g)           | 47.5              |
| Thiamine (mg)      | 0.09              |
| Riboflavin (mg)    | 0.19              |
| Niacin (mg)        | 4.8               |
| Ascorbic acid (mg) | 50                |

Source: Peter (1999).

molecular formula  $C_{21}H_{20}O_6$ , is the major constituent that imparts the yellow colour to turmeric. The curcumin content in different turmeric varieties varies from 2–7 % (spectrophotometric estimation). Besides curcumin there are a few other related pigments which impart the yellow colour, which, together with curcumin, are named curcuminoids. Curcumin 1,7-*bis* (4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione], demethoxy curcumin [4-hydroxy-cinnamoyl (4-hydroxy-3-methoxycinnamoyl) methane and *bis*-demethoxy curcumin [bis-(4-hydroxy-cinnamoyl)methane] together make the colouring pigment in the turmeric rhizomes (Fig. 28.1). There are not many reports on the curcuminoid profile of traded turmeric varieties barring the reports by Jayaprakasa *et al.*, (2002) and Paramasivam *et al.*, (2009). Jayaprakasa *et al.*, (2002) analysed the curcuminoid profile of the traded varieties like ‘Salem’ (9.18 %), ‘Erode’ (9.11 %) and ‘Balasore’ (7.10 %) using HPLC, whereas Paramasivam *et al.* (2009) described a high-pressure thin-layer chromatography (HPTLC) method for the determination of curcuminoids of *C. longa* germplasm from India. The result suggested that two Indian turmeric varieties, viz. ‘Nimbarg’ and ‘Kalimpong’, have higher amounts of total curcuminoids, 6.18 and 5.37 %, respectively. Recently, Thomas (2009) studied the curcuminoid profile of six popular/traded turmeric varieties, namely ‘Prabha’, ‘Prathibha’, ‘Alleppey Supreme’, ‘Alleppey Finger Turmeric’, ‘Rajapuri’ and ‘Wynadan’, and observed that, among the varieties, ‘Prathibha’ contains the highest amount of curcumin I and curcumin III (Table 28.3). The essential oil of turmeric contains a variety of molecules credited with very many pharmacological properties. In the traded Indian turmeric varieties, the major compounds identified are turmerone (26.20–31.55 %), curlone (18.64–20.48 %) and *ar*-turmerone (19.11–23.94 %) (Thomas, 2009). In ‘Alleppey Finger Turmeric’ and ‘Prathibha’, turmerone was identified as the major essential oil component, using gas chromatography–mass spectroscopy (GC–MS), at levels of 31.55 % and 30.60 %, respectively (Table 28.4) (Thomas, 2009).

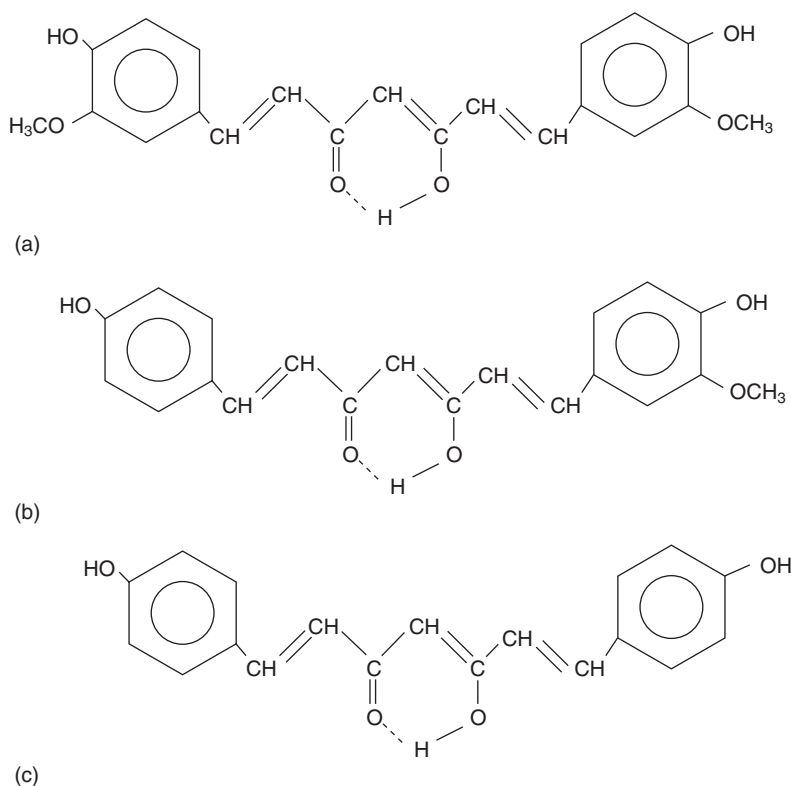


**Fig. 28.1** (a) Curcumin, (b) demethoxy curcumin and (c) *bis*-demethoxy curcumin.

**Table 28.3** Percentage of curcumin I, curcumin II, curcumin III and total curcuminoids in some traded turmeric and improved varieties

| Sl. No. | Turmeric                   | Curcumin I (%) | Curcumin II (%) | Curcumin III (%) | Total curcuminoid (%) |
|---------|----------------------------|----------------|-----------------|------------------|-----------------------|
| 1       | 'Prabha'                   | 1.64           | 1.27            | 0.76             | 3.67                  |
| 2       | 'Prathibha'                | 3.34           | 0.82            | 1.30             | 5.46                  |
| 3       | 'Alleppey Supreme'         | 1.65           | 1.35            | 1.15             | 4.15                  |
| 4       | 'Alleppey Finger Turmeric' | 2.76           | 1.19            | 0.87             | 4.82                  |
| 5       | 'Rajapuri'                 | 1.36           | 0.47            | 0.65             | 2.48                  |
| 6       | 'Wynadan'                  | 1.31           | 0.90            | 0.19             | 2.40                  |

Source: Thomas (2009).

## 28.2 Production of turmeric

India is the major producer and exporter of turmeric in the world. In India, turmeric is grown on over 174 000 ha with an annual production of 843 000 tonnes (2005–6). India exported 51 500 t of turmeric during 2006–7 to as many as 67 countries, the major importers being Bangladesh, Iran, Japan, Pakistan, South Africa, Sri Lanka, the USA, the UK and the UAE. Turmeric is exported as turmeric dry, turmeric fresh,

**Table 28.4** Composition of essential oils of dried rhizomes of some Indian turmeric

| Peak No | Compound                    | RI   | Turmeric |             |                    |                   |            |           |
|---------|-----------------------------|------|----------|-------------|--------------------|-------------------|------------|-----------|
|         |                             |      | ‘Prabha’ | ‘Prathibha’ | ‘Alleppey Supreme’ | ‘Alleppey Finger’ | ‘Rajapuri’ | ‘Wynadan’ |
| 1       | $\alpha$ -Pinene            | 935  | 0.13     | 0.14        | 0.16               | 0.4               | 0.33       | 0.2       |
| 2       | $\alpha$ -Phellandrene      | 1006 | 4.06     | 3.52        | 3.97               | 1.71              | 0.83       | 0.64      |
| 3       | limonene                    | 1021 | 0.1      | 0.1         | 0.11               | 0.13              | 0.15       | 0.14      |
| 4       | 1,8-Cineole                 | 1033 | 2.54     | 2.54        | 2.55               | 1.05              | 0.5        | 0.57      |
| 5       | $\alpha$ -Terpinolene       | 1090 | 0.22     | 0.32        | 0.12               | 0                 | 0          | 0         |
| 6       | $\alpha$ -Terpineol         | 1191 | 0.26     | 0.29        | 0.27               | 0.21              | 0          | 0         |
| 7       | $\beta$ -Caryophyllene      | 1419 | 0.39     | 0.32        | 0.37               | 0.66              | 0.33       | 0.67      |
| 8       | trans $\beta$ -farnesene    | 1464 | 0.14     | 0.15        | 0.14               | 0                 | 0          | 0.13      |
| 9       | $\alpha$ -Zingiberene       | 1469 | 1.76     | 2.26        | 2.02               | 3.43              | 2.45       | 1.64      |
| 10      | <i>ar</i> -Curcumene        | 1483 | 1.39     | 1.15        | 1.32               | 1.2               | 0          | 1.92      |
| 11      | $\beta$ -Selinene           | 1485 | 0.38     | 0.35        | 0.3                | 0.33              | 0.28       | 0.34      |
| 12      | $\beta$ -Bisabolene         | 1510 | 0.11     | 0.14        | 0.41               | 0                 | 0.5        | 0         |
| 13      | $\beta$ -Sesquiphellandrene | 1525 | 2.11     | 2.23        | 2.21               | 4.12              | 2.57       | 2.14      |
| 14      | elemol                      | 1555 | 0.14     | 0           | 0.15               | 0                 | 0          | 0         |
| 15      | Turmerone                   | 1632 | 26.2     | 30.60       | 28.66              | 31.55             | 27.74      | 20.45     |
| 16      | Curlone                     | 1635 | 19.61    | 20.48       | 19.83              | 18.64             | 19.54      | 20.25     |
| 17      | <i>ar</i> -Turmerone        | 1641 | 23.94    | 19.11       | 20.54              | 18.64             | 19.54      | 20.25     |
|         | Total                       |      | 83.48    | 83.7        | 83.13              | 82.07             | 74.76      | 69.34     |

Source: Thomas (2009).

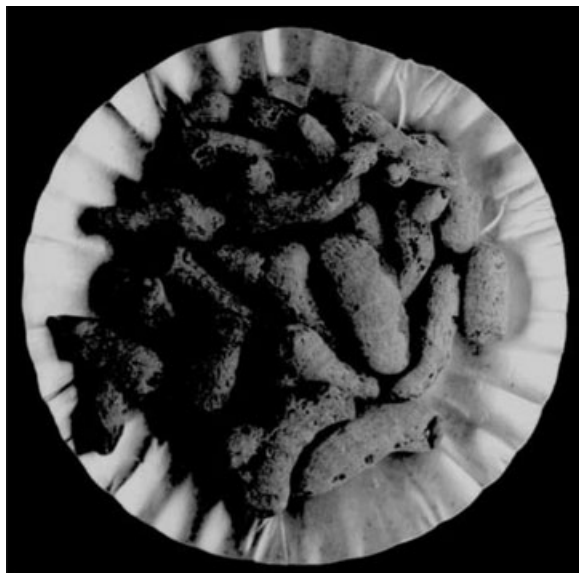


**Fig. 28.2** Pathibha, processed turmeric.

turmeric powder, turmeric oleoresin and turmeric oil. Turmeric is used mainly as fine ground turmeric in cooking in the west while those in the developing countries buy turmeric mostly in whole or split or powder form. There are about 60 turmeric cultivars (land varieties) available in India, some of the important local cultivars being 'Duggirala', 'Tekurpet', 'Sugandham', 'Amalapuram', 'Lakadong', 'Alleppey', 'Rajapuri', 'Mydukur', 'Wynad local', 'Salem', etc. Cultivars like 'Alleppey', 'Wynad local', 'Lakadong', 'Edapalayam', 'Thodupuzha', etc. are rich in curcumin content (>5 %). In addition to these land varieties, there are about 26 improved varieties in India. The important improved turmeric varieties are 'Prabha', 'Prathibha' (Fig. 28.2), 'Sudarsana', 'Suguna', 'Co-1', 'Sugandham', 'BSR 1', 'Rajendra sonia', 'Roma', 'Kedaram', 'Alleppey Supreme', 'Varna', 'Kanti', 'Sona', 'Sobha', 'Ranga', etc. (Sasikumar *et al.*, 1996). Maturity of these varieties is at 7–9 months. The yield (fresh) of the improved varieties is 20–40 ha<sup>-1</sup>.

### 28.2.1 Biotechnology and gene mining

Biotechnological work in turmeric has been centred around protocol standardization (culture media and conditions, explants), *in vitro* screening studies, *in vitro* conservation, *in vitro* mutation induction, *in vitro* microrhizome production, *in vitro* pollination besides genetic fidelity studies of micropropagated plants, genetic transformation and molecular marker-based genetic diversity analysis. A perusal of the reported works on turmeric tissue culture reveals that Murashige and Skoog MS medium supplemented with benzylaminopurine (BA) in combination with other phytohormones is the best medium for *in vitro* propagation of turmeric. A significant effect of thiadiazuron (TDZ) is also reported in some cases. Molecular marker-based diversity studies in turmeric in most cases conform the true to type nature of the *in vitro* derived plantlets (Nadagauda and Mascarenhas, 1986;

Balachandran *et al.*, 1990; Nayak, 2000; Ranjith *et al.*, 2001; Salvi *et al.*, 2001; Gayatri *et al.*, 2005; Praveen, 2005; Parthasarathy and Sasikumar, 2006; Shirgurkar *et al.*, 2006; Syamkumar and Sasikumar, 2006; Syamkumar, 2007). Microsatellite markers from genomic DNA and ESTs are currently being used to assess genetic diversity of turmeric cultivars over the RAPD and ISSR markers (Sigrist *et al.*, 2010; Siju *et al.*, 2010).

Gene mining in turmeric is a relatively novel approach. An exciting and promising approach coupling state-of-the-art metabolomic analyses and bioinformatic tools to identify apparent 'metabolic modules' in turmeric is described by Xie *et al.*, (2009). A metabolic module is defined as a group of co-regulated metabolites, and this approach elegantly represents a basic innovative and practical attempt to understand and predict metabolic pathways using detailed bioinformatics data mining following careful and well-documented GC-MS and liquid chromatography-mass spectroscopy (LC-MS) analyses. Katsuyama *et al.*, (2009) proposed a novel pathway for curcuminoid biosynthesis in turmeric implicating two type III polyketide enzymes, namely diketide-CoA synthase (DCS) and curcumin synthase (CURS). Isolation of genes regulating/controlling these key enzymes would be the next step in gene mining of turmeric.

### 28.2.2 Post-harvest processing

Harvested turmeric is washed well to remove the adhering soil, roots are removed and the fingers and mothers are separated. Mother and finger rhizomes are boiled separately for about 40–60 minutes under slightly alkaline condition (100 g of sodium bicarbonate or sodium carbonate in 100 l of water) in copper, galvanized iron (GI) or earthen vessels and sun-dried on bamboo mat or clean drying floor for 10–15 days so as to bring down the moisture content to 10–12 %. Another method of curing is by taking cleaned mother and finger rhizomes (approx. 50 kg) separately in perforated troughs of convenient size made of galvanized iron GI sheet with extended parallel handles. The troughs containing the fingers are immersed in water using a paddle. The alkaline solution is then poured into the pan so as to immerse the rhizomes, which are then boiled until they become soft and then dried. The dry recovery of cured turmeric varies between 15 and 30 % depending on variety, location and cultural practices. Dried turmeric is subjected to polishing, either manually or mechanically in power operated drums (Purseglove *et al.*, 1981). A weight loss of about 5–8 % is expected due to full polishing. Polished rhizomes are made attractive by artificially colouring them with turmeric powder. During polishing itself, turmeric is added to the drum either as powder or as emulsion.

Rama Rao *et al.* (1975) described an indigenous method of storing turmeric. The cured product is stored in suitable pits dug on a raised site. The bottom and sides of the pits are lined thickly with dried grass or similar material. After filling up the pits with cured turmeric, they are covered with mat or grass and finally with earth. The produce can be stored for one year like this. At Sangli, India, farmers usually store turmeric like this in pits dug in the field. Dealers usually store the cured turmeric in fresh jute bags or in sound, clean, dry, heat-sealed polythene bags in dry, cool, warehouses (Purseglove *et al.*, 1981).

After harvest, fresh turmeric is kept in gunny bags or baskets or heaped open in well-ventilated sheds.



### 28.2.3 Ground turmeric

A sophisticated grinding process is not needed for ground turmeric, since there is not much loss of quality during grinding. Usually clean, dry, stone-hard fingers are powdered through the use of hammer mills followed by disc-type attrition mills to obtain 60–80 mesh powder. Accessory equipment for pre-cleaning includes an aspiration system (which removes the light extraneous matter), de-stoners and magnetic separators for fine iron contamination, vacuum fumigators and the noise-reducing fixtures, dust-collection systems, mechanical or closed circuit pneumatic conveying system, blending and automatic packaging system now employed by most big spice grinders for optimizing the output and for assuring hygienic and flavour quality. The smaller spice manufacturers in the West and Asia use simple cleaning and grinding equipment and partly mechanized packaging systems (Govindarajan, 1980). Turmeric powder is packed in bulk in containers such as fibre hard drums, multiwall bags and tin containers suitably lined or coated to prevent moisture absorption, loss of flavour and colour. For the retail trade, the unit packages are in flexible packagings such as low- and high-density polyethylene, polyvinyl chloride, glassine or in glass packages. Storage studies conducted on turmeric powder using different packaging materials have shown that aluminium foil laminate or double pouch of glassine or low-density polyethylene offered good protection for the stored product for about six months without loss of quality and colour (Balasubramanian *et al.*, 1979).

### 28.2.4 Turmeric oleoresin

Turmeric oleoresin is being used increasingly by the processed food industries in the West to impart colour and aroma. Oleoresin is a mixture of compounds, namely curcumin, volatile oil and other active ingredients, non-volatile fatty and resinous material extractable by solvents, used singly, in sequence or in combination. Turmeric oleoresin is orange–red in colour and consists of an upper oily layer and a lower crystalline layer (Krishnamurthy *et al.*, 1976). For commercial use, it is usually mixed with a non-volatile edible solvent such as vegetable oil, propylene glycol or poly oxyethylene sorbitan fatty acid esters in order to disperse the extracted material and to render it free-flowing and ‘soluble’ (Purseglove *et al.*, 1981). Turmeric oleoresin is obtained by solvent extraction of the ground spice. Acetone is a good solvent for oleoresin extraction. Soxhlet apparatus or cold percolation is used for extraction. Curcumin, the principal colouring matter, forms about one-third of a good-quality oleoresin. Yield of oleoresin varies from 7–15 % depending on varieties. Govindarajan (1980) has given the detailed steps for industrial extraction of turmeric oleoresin.

### 28.2.5 Turmeric oil

Turmeric contains 3–5 % volatile oil, which is obtained by steam distillation of turmeric powder, for about 8–10 h. Turmeric leaves also yield oil. Turmeric oil is pale yellow in colour with peppery and aromatic odour. The oil contains about 60 % turmeron, 25 % zingiberene and small quantities of *d*-phellandrene, *d*-sabinene, cineole and forneol. Turmeric leaf oil, obtained from distillation of dry leaves, has application in the aroma therapy, perfumery, cosmetics and soap industries

(Sasikumar, 2005). Among the various constituents of the oil, sesquiterpenes, *ar-turmerone* and *turmerone* comprise 50 % of the oil (Purseglove *et al.*, 1981).

### 28.2.6 Curry powder

Turmeric powder is the major component (about 40–50 %) of curry powder. Curry powder is a spice mixture used for seasoning dishes containing vegetables, meat, fish, eggs or vegetable plus meat or fish (i.e. curry) in the orient. In the West also, curry powder is used for seasoning dishes. India has been the principal exporter of curry powder to many countries like the UK, Australia, Fiji, etc. Turmeric powder provides colour and background aroma to the curry powder. Govindarajan (1980) has given typical curry powder composition, quality standards, packaging details, etc.

## 28.3 Quality specifications

Cured turmeric is sorted as fingers, round, split or non-specified and marketed under its varietal name, which is usually based on the place of production such as 'Alleppey', 'Erode', 'Duggirala', 'Nizamabad', 'Rajapuri', 'Cuddappah', 'Salem', 'Wynadan', etc., from India. The Indian 'Agmark' standards include separate gradings for different varieties. 'Special', 'good' and 'fair' are some of the grade specifications. Govindarajan (1980) outlines specifications for turmeric (whole and ground) as follows.

1. Whole turmeric refers to the primary (bulbs, rounds) and secondary (fingers) rhizomes, harvested at full maturity, cured, dried to about 10 % moisture level and polished. Whole turmeric may or may not be coloured.
2. Ground turmeric is the powder which results when the cured rhizomes, cleaned and dried, are ground without any added matter.
3. Whole or powdered turmeric should have the characteristic fresh aroma and taste of turmeric and be free from foreign aromas such as mustiness. They must also be free from living insects and moulds and practically free from dead insects, insect fragments and rodent contamination visible to the naked eye or visible at a specified magnification.
4. Turmeric fingers should not be less than 15 mm in length. They should be hard, smooth and the core colour should be lemon yellow or bright yellow with only admissible levels of small pieces and bulbs (Table 28.5).
5. Whole turmeric should not contain more than 2 % by weight extraneous matter (lower limit for superior grade). The admissible level of defective rhizome allowed indifferent varieties of turmeric is given in Table 28.5.
6. The limits for chemical characteristics specified for turmeric powder are presented in Table 28.6.

The American Spice Trade Association (ASTA) cleanliness specification effective from 21 May 1997 for turmeric allows only a maximum of three dead whole insects; 5 mg lb<sup>-1</sup> mammalian or other excreta, 3 % by wt. mould, 2.5 % by wt insect defiled or infested material and 0.5 % by wt. extraneous foreign matter in turmeric

**Table 28.5** Indian specifications for turmeric grade

| Grade                     | Pieces<br>(max. wt. %) | Foreign matter<br>(max. wt. %) | Defectives<br>(max. wt. %) | Bulks<br>(max. wt. %) | Characteristics                                                                                                                                                |
|---------------------------|------------------------|--------------------------------|----------------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Fingers (general)</b>  |                        |                                |                            |                       |                                                                                                                                                                |
| Special                   | 2.0                    | 1.0                            | 0.5                        | 2.0                   | Finger-like shape, breaks with a metallic twang; well set and close grained; perfectly dry, free from weevil damage, over-boiling, etc.                        |
| Good                      | 3.0                    | 1.5                            | 1.0                        | 3.0                   |                                                                                                                                                                |
| Fair                      | 5.0                    | 2.0                            | 1.5                        | 5.0                   |                                                                                                                                                                |
| <b>Fingers (Alleppey)</b> |                        |                                |                            |                       |                                                                                                                                                                |
| Good                      | 5.0                    | 1.0                            | 3.0                        | 4.0                   | As above                                                                                                                                                       |
| Fair                      | 7.0                    | 1.5                            | 5.0                        | 5.0                   |                                                                                                                                                                |
| <b>Fingers (Rajapuri)</b> |                        |                                |                            |                       |                                                                                                                                                                |
| Special                   | 3.0                    | 1.0                            | 3.0                        | 2.0                   | As above, admixture of other turmeric varieties are allowed at a maximum of 2.5 and 10 % in 3 grades, respectively.                                            |
| Good                      | 5.0                    | 1.5                            | 5.0                        | 3.0                   |                                                                                                                                                                |
| Fair                      | 7.0                    | 2.0                            | 7.0                        | 5.0                   |                                                                                                                                                                |
| <b>Bulbs (rounds)</b>     |                        |                                |                            |                       |                                                                                                                                                                |
| Special                   | —                      | 1.0                            | 1.0                        | —                     | Be well developed, smooth round and free from rootlets<br>The ‘Rajapuri’ type has higher allowance of 3.0, 5.0 and 7.0 % defectives in 3 grades, respectively. |
| Good                      | —                      | 1.5                            | 3.0                        | —                     |                                                                                                                                                                |
| Fair                      | —                      | 2.0                            | 5.0                        | —                     |                                                                                                                                                                |

Source: Govindarajan (1980).

(Sivadasan, 1998). Thomas (2009) studied the physical quality parameters of some of the traded turmeric and popular varieties from India and found that they met the standards specified for the commodity by the regulatory agencies in India (Table 28.7).

### 28.3.1 Curcuminoids

In the pure form, curcuminoids separate as an orange–yellow crystalline powder, insoluble in water, slightly soluble in ether, soluble in alcohol and in glacial acetic acid. Verghese (1999) is of the opinion that the melting point of curcumin is an unworthy quality parameter and need not be mentioned in any specifications, as many different melting points are reported by many workers for curcuminoids. Curcumin exhibits strong absorption between 419 and 430 nm in organic solvents

Table 28.6 Analytical specification for turmeric (whole and ground)

| Sample       | Moisture<br>max.<br>(% wt.) | Ash                      |                                | Starch<br>max.<br>(% wt.) | Crude<br>fibre max.<br>(% wt.) | Vol. oil<br>max.<br>(% wt.) | Colour as<br>curcumin<br>min. (% wt.) | Lead max.<br>ppm | Chromate<br>test |
|--------------|-----------------------------|--------------------------|--------------------------------|---------------------------|--------------------------------|-----------------------------|---------------------------------------|------------------|------------------|
|              |                             | Total<br>max.<br>(% wt.) | Acid<br>insol. max.<br>(% wt.) |                           |                                |                             |                                       |                  |                  |
| Whole BP     | 8-10                        | 6-9                      | -                              | -                         | 4-6                            | 2-5                         | -                                     | -                | -                |
| US           | 9                           | 7                        | 0.5                            | -                         | 6                              | 4                           | 5                                     | -                | -                |
| DDR          | -                           | 7                        | -                              | -                         | -                              | 2.6                         | 3-4                                   | -                | -                |
| Powder India | 10                          | 7                        | 1.5                            | 60.0                      | -                              | -                           | -                                     | 1.5              | Negative         |
| WHO          | 10                          | 7                        | 1.5                            | -                         | -                              | -                           | -                                     | 3                | Negative         |

*Note:* The chromate test is negative if there is no violet colour developed when dilute acid soluble ash from 2g of sample (4-5 ml) is reacted with 1 ml of 0.2 % alcoholic solution of diphenyl carbazide.

*Source:* Govindarajan (1980).

**Table 28.7** Physical quality parameters of turmeric from India

| Sl. No | Parameter                          | Turmeric     |              |                    |                   |              |               |
|--------|------------------------------------|--------------|--------------|--------------------|-------------------|--------------|---------------|
|        |                                    | ‘Prabha’     | ‘Prathibha’  | ‘Alleppey Supreme’ | ‘Alleppey Finger’ | ‘Rajapuri’   | ‘Wynadan’     |
| 1      | Length of rhizomes (cm)            | 5.12 ± 0.12  | 5.42 ± 0.22  | 4.99 ± 0.18        | 5.11 ± 0.24       | 4.67 ± 0.23  | 5.10 ± 0.25   |
| 2      | Circumference of the rhizomes (cm) | 2.40 ± 0.06  | 2.22 ± 0.04  | 2.27 ± 0.06        | 2.30 ± 0.05       | 2.73 ± 0.01  | 2.25 ± 0.02   |
| 3      | Weight of rhizomes(g)              | 15.20 ± 0.04 | 16.50 ± 0.02 | 15.36 ± 0.02       | 16.0 ± 0.25       | 17.50 ± 0.12 | 14.60 ± 0.22  |
| 4      | Colour intensity (hue-lightness)   | 27-5 to 27-8 | 27-7 to 27-9 | 27-6 to 27-8       | 27-6 to 27-8      | 27-5 to 27-6 | 27-6 to 27-10 |

Source: Thomas (2009).

and on this property revolves the spectrophotometric methods of the ASTA (1968) and Essential Oil Association (EOA) (1965), although the HPLC method is now available (Tonnesen and Karlson, 1983). The EOA stresses the fact that turmeric extracts are evaluated strictly by colour and this is best expressed in terms of colour value (cv), which is equivalent to ten times the specific extinction coefficient in ethanol at 422 nm (Verghese, 1999).

Verghese (1999) further reported that the specific extinction coefficient in ethanol of curcumin at 420–430 nm varies between 1528 and 1586, of demethoxy curcumin at 420–430 nm between 1513 and 1580 and of *bis*-demethoxy curcumin at 419–430 nm between 1565 and 1682. By repeated crystallization from ethanol, the dye yielded specific extinction coefficient 1596 at 425 nm in ethanol (Verghese, 1999). Coupling this observation and the values already reported in the literature, a specific extinction coefficient of 1600 was recommended as a reasonable yardstick for assaying curcumin, and this fits the HPLC data excellently (Verghese, 1999). However, for most routine, quality control work, it is sufficient to measure the extinction of an alcohol extract at the absorption maximum at 420–455 nm, taking the precautions of using neutral alcohol and avoiding exposure to direct sunlight, and to calculate the curcumin content by using the molecular absorption value (Govindarajan, 1980).

### 28.3.2 Adulteration

Whole, dried or fresh turmeric is usually free from adulteration. However, turmeric powder may be adulterated with chemical and biological adulterants. The non-biological adulterants in turmeric powder include coal tar and artificial colours such as metanil yellow, orange II and lead chromate which are detected by colorimetric, chromatographic or spectrophotometric techniques (PFA, 2003; Tripathi *et al.*, 2004, 2007). The presence of chalk powder and yellow soap stone in turmeric powder can be detected by simple chemical reaction (PFA, 2003). Biological adulterants include foreign starch (tapioca, arrowroot, cereal flour) and husks. Specific tests are now available to detect each of the above adulterants in ground turmeric (Govindarajan, 1980). Adulterated turmeric powder will have a low curcumin content. Depending upon the adulterant used, the curcumin content of the samples varies from 0.37–2.07 % (Balasubramanian *et al.*, 1979). Turmeric powder is also frequently adulterated with rhizomes of cheaply available related species (Singhal *et al.*, 1997), especially with those containing the colouring pigment curcumin. If the adulterants are related cheap *Curcuma* species such as *C. xanthorrhiza*, the curcumin content of the turmeric powder will not vary (Sasikumar *et al.*, 2005). The related *Curcuma* species which are of real significance in adulteration are *C. xanthorrhiza* Rosc. or 'yellow shotti' syn. *C. malabarica* Vel. *et al.* (Dhanya and Sasikumar, 2010). Sasikumar *et al.*, (2005) also used RAPD markers for adulterant detection in traded turmeric powder. RAPD profiles of genuine turmeric (*C. longa*) and the adulterant *C. xanthorrhiza* were compared with three branded market samples of turmeric powder to identify the adulterant specific bands. The method could detect the admixing of *C. xanthorrhiza* powder in all the three market samples of turmeric powders tested. Recently, Dhanya (2009) developed SCAR markers to detect presence of *C. xanthorrhiza* adulteration in commercial samples of turmeric powder. Using these

markers presence of *C. xanthorrhiza* could be detected in four out of six market samples analysed.

## 28.4 Functional properties and uses of turmeric

### 28.4.1 Uses in the food industry

Turmeric powder is used in mustard paste and curry powder as both its colour and aroma are important in these products. In Asian countries, whole, dry or fresh turmeric, or turmeric powder with other spices like chillies, coriander, pepper, cumin, etc. are used for making vegetable and meat dishes and soup-like dishes. Turmeric powder mixed with sesame, coconut or groundnut oil is used for pickling mango, lime, gooseberry, garlic, etc. (Govindarajan, 1980).

Turmeric oleoresin is used mainly in brine pickles (Eiserle, 1966; Cripps, 1967) and to some extent in mayonnaise and relish formulations, non-alcoholic beverages such as orangeades and lemonades, gelatines, breadings for frozen fish sticks, potato croquettes, butter and cheese, in the form of powder or granules for garnishing, and even in ice creams (Perotti, 1975). In all these cases, its function is predominantly to colour the product and it merely replaces the synthetic colours such as tartrazine, formerly used (Govindarajan, 1980).

The Colours in Food Regulations came into force in the UK in 1996. Part III, Schedule 5 of this Regulation specifies the limits for curcumin in various food items (Table 28.8) (Henry, 1998). Curcumin is included in the list of colours with a restricted use because of the fact that it has been allocated only a temporary, low ADI (acceptable daily intake) value. The ADI value indicates the amount of a food additive that can be taken daily in the diet without risk, expressed as mg kg<sup>-1</sup> bodyweight (Henry, 1998). The Joint Expert Committee on Food Additives (JECFA) has allotted curcumin a temporary ADI value of 0–1.0 mg kg<sup>-1</sup> bodyweight day<sup>-1</sup>. Curcumin is specifically permitted as a colour in the EU although many countries simply list it without a specification for its colour strength. Pure 95 % curcumin, as it is usually obtained, is not an ideal product for direct use by the food industry since it is insoluble in water and has poor solubility in other solvents. Hence in many countries curcumin is dissolved in a mixture of food-grade solvent and permitted emulsifier such as Polysorbate 80 to convert it into a form that can be conveniently applied. In this form, the product contains about 10 % curcumin. Curcumin gives a bright yellow colour even at low doses. The usual dose level of curcumin is in the range of 5–200 ppm. Numerous blends are available commercially to suit the colour of the product (Henry, 1998). Vanilla ice cream, for example, is coloured with a combination of curcumin (200 ppm) and norbixin (12 ppm). Similarly in yoghurt 5 ppm curcumin will give an acceptable colour. For cakes and biscuits the required colour is achieved using a blend of curcumin (10–15 ppm) and annatto (10 ppm). Turmeric oleoresins, although permitted universally as a spice oleoresin, are not a permitted colour in the EU (Henry, 1998). Turmeric powder, extracts and curcumin exhibit antioxidant properties as observed by the induction period and oxygen absorption of coconut, groundnut, safflower, sesame, mustard, cotton seed oil and ghee at 95–220 °C for periods up to 144 h. In foods, the antioxidant property of turmeric was effective in preventing peroxide developments (Khanna, 1999).



**Table 28.8** Limits specified for curcumin in various food items ('Colours in Food Regulation Act 1995' Schedule 5, Part III)

| Food                                                                                                                                                                       | Maximum level |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Non-alcoholic flavoured drinks                                                                                                                                             | 100 mg/l      |
| Candied fruits and vegetables, mostarda di frutta                                                                                                                          | 200 mg/kg     |
| Preserves of red fruits                                                                                                                                                    | 200 mg/kg     |
| Confectionery                                                                                                                                                              | 300 mg/kg     |
| Decorations and coatings                                                                                                                                                   | 500 mg/kg     |
| Fine bakery wares (e.g. viennoiserie, biscuits, cakes and wafers)                                                                                                          | 200 mg/kg     |
| Edible ices                                                                                                                                                                | 150 mg/kg     |
| Flavoured processed cheese                                                                                                                                                 | 100 mg/kg     |
| Desserts including flavoured milk products                                                                                                                                 | 150 mg/kg     |
| Sauces, seasonings (for example, curry powder, tandoori pickles, relishes, chutney and piccalilli)                                                                         | 500 mg/kg     |
| Mustard                                                                                                                                                                    | 300 mg/kg     |
| Fish paste and crustacean paste                                                                                                                                            | 100 mg/kg     |
| Pre-cooked crustaceans                                                                                                                                                     | 250 mg/kg     |
| Salmon substitutes                                                                                                                                                         | 500 mg/kg     |
| Surimi                                                                                                                                                                     | 500 mg/kg     |
| Fish roe                                                                                                                                                                   | 300 mg/kg     |
| Smoked fish                                                                                                                                                                | 100 mg/kg     |
| 'Snacks': dry, savory potato, cereal or starch-based snack products: extruded or expanded savoury snack products                                                           | 200 mg/kg     |
| Edible cheese rind and edible casings                                                                                                                                      | quantum satis |
| Complete formulae for weight control intended to replace total daily food intake or an individual meal                                                                     | 50 mg/kg      |
| Complete formulae and nutritional supplements for use under medical supervision                                                                                            | 50 mg/kg      |
| Liquid food supplements/dietary integrators                                                                                                                                | 100 mg/l      |
| Solid food supplements/dietary integrators                                                                                                                                 | 300 mg/kg     |
| Soups                                                                                                                                                                      | 50 mg/kg      |
| Meat and fish analogues based on vegetable proteins                                                                                                                        | 100 mg/kg     |
| Spirituos beverages (including products less than 15 % alcohol by volume), except any mentioned in Schedule 2 or 3                                                         | 200 mg/l      |
| Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails as mentioned in Regulation (EEC) No. 1601/91, except any mentioned in Schedule 2 or 3 | 200 mg/l      |
| Fruit wines (still or sparkling), cider (except cidre bouche) and perry aromatized fruit wines, cider and perry                                                            | 200 mg/l      |

Source: Henry (1998).

The fate of curcumin *in vivo* is yet to be understood thoroughly. Studies by oral administration of curcumin to rats indicated that curcumin is metabolized to a certain extent in the liver and that curcumin and its metabolites are excreted via bile and faeces (Tonnesen, 1986).

#### 28.4.2 Uses in folk medicine

Turmeric is used in folk medicine to treat a variety of ailments in animals and humans (Sasikumar, 2005). Topical application of turmeric paste is recommended against vertigo, sprains, cuts injuries, swellings, skin infections, bites of insects/

scorpions/snakes, pimples and diabetic wounds. Oral administration of turmeric is said to be effective against flatulence, indigestion, diarrhoea, biliary and hepatic disorders and anorexia. Inhaling of fumes of burned dry turmeric is a common practice against sinusitis, coryza, etc. in rural India. Turmeric powder was used to be smeared on the severed umbilical chord of newborn babies in the Indian countryside as an antiseptic. In traditional veterinary practices too turmeric is important in treating scabies of cattle and dogs, skin infections of domestic animals, worm infections and mastitis of cattle, among other conditions.

#### 28.4.3 Religious uses

Turmeric has been associated with the Hindu religion since very ancient times. It was used by the pre-Aryan Indians in religious worship and tantric or magic rites. In India, turmeric is even today associated with the worship of 'Kali' (Mother Goddess) and 'Naga' (the serpent deity). Turmeric plus lime, known as 'kumkum' (a natural vermillion), is distributed to devotees from temples to be placed on their forehead very auspiciously. Married Hindu women apply 'kumkum' longitudinally on the forehead to denote their marital status. Turmeric is also used in Hindu weddings; it is very important and auspicious either to smear on the invitation card or to make the 'Mangala sutra' sacred (Sasikumar, 2005).

#### 28.4.4 Functional properties

Many reviews are available on medicinal uses of turmeric (Kirtikar and Basu, 1948; Anon., 1950; Srimal, 1993; Verghese, 1999; Khanna, 1999; Sasikumar, 2005). Curcuminoids, turmeric powder, ether/chloroform /water and alcohol extracts of turmeric, *ar*-turmerone, turmerin, sodium curcumin and turmeric volatile oil are all credited with various biological activities (Khanna, 1999; Sasikumar, 2005). Turmeric is thought to have the following biological activities: anti-inflammatory, hypocholesteremic, choleraic, antimicrobial, antirheumatic, antibacterial, antiviral, cytotoxic, spasmolytic, antidiabetic, antivenomous, antiseptic, antitoxic, anti-HIV, anti-Alzheimer's (Govindarajan, 1980; Tonnesen, 1986; Velayudhan *et al.*, 1999; Sasikumar, 2005). Turmeric is also credited with anticancer properties (Kuttan *et al.*, 1985; Rao *et al.*, 1995).

Curcuminoids are also regarded as having antioxidative, antidiabetic and anti-inflammatory effects and have been used as a chemo-preventive agent for a wide variety of cancers, including those of the colon, breast, prostate, oesophagus, lung and mouth. They have also been used for inhibition of atherosclerosis and inhibition of viral and bacterial growth (Srinivasan, 2005; Sandur *et al.*, 2007). It is also reported that the proportion of curcuminoids plays a considerable role in the protective activity of turmeric. The concept of the 'Curcumin C3 complex', an extract that contains individual curcuminoids at specific concentration limits, is an offshoot of this finding (Verghese, 1999).

The yellow pigment curcumin and demethoxyylated curcumins found in both turmeric and ginger are known to possess potent antioxidant activity (Kikuzaki *et al.*, 1994; Kikuzaki and Nakatani, 1993). Curcumin suppressed the oxidation of methyl linoleate in organic homogeneous solution and aqueous emulsions, soybean

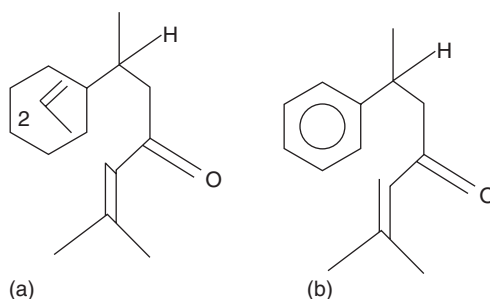


**Fig. 28.3** (a) Turmerone, (b) *ar*-Turmerone.

phosphatidylcholine liposomal membranes and rat liver homogenate induced by free radicals (Noguchi *et al.*, 1994). A mechanism for production of the dimer is proposed and its relation to curcumin's antioxidant activity is discussed by Masuda *et al.*, (1999). The results indicated that the dimer is a radical-terminated product formed during the initial stage of the process.

*In vitro* and *in vivo* studies have established the effectiveness of curcumin, volatile oil or total extracts of turmeric against many organisms such as *Micrococcus pyogenus* var. *aureus*, *Staphylococcus* spp., *Paramaecium caudatum*, *Trichophyton gypseum*, *Mycobacterium tuberculosis*, *Salmonella typhi*, *Vibrio cholerae*, *Corynebacterium diphtheria*, *Aspergillus niger*, etc. (Khanna, 1999). Turmerone and *ar*-turmerone (Fig. 28.3) in the essential oil exhibit antimicrobial /antibacterial/insect-repellent activities (Tripathi *et al.*, 2002; Garg and Jain, 2003; Singh *et al.*, 2003). Aqueous extract, fresh juice and essential oil of turmeric are also credited with biopesticidal properties (Kapoor, 1998; Saju *et al.*, 1998; Bora and Jaya Samuel, 1999). *In vitro* and *in vivo* studies have established the efficacy of turmeric constituents against various plant pathogens such as *Ralstonia solanacearum*, *Xanthomonas oryzae* pv. *oryzae*, *Helminthosporium sacchari*, *illetotrichum gloesporoides*, *Rhizoctonia solani*, etc. Turmeric oil is also effective as a mosquito repellent, housefly deterrent and in aphid vector control (Saju *et al.*, 1998; Khanna, 1999).

## 28.5 Future trends

As turmeric is gaining in importance as a cure for a variety of ailments, future research on the crop/commodity will centre on this aspect as well as on its use as a spice. Isolation of the genes regulating curcumin and turmerin biosynthesis, biochemical profiling of the commercial turmeric varieties for the antioxidants turmerin and curcuminoids, development of genotypes rich in curcumin and study on the influences of genotype x environment interactions on curcuminoid content and profile would be rewarding.

## 28.6 References

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## Vanilla

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**Abstract:** This chapter discusses relevant information on vanilla (*Vanilla planifolia* H. C. Andrews), looking first at its historical background and plant description. The chemical compounds found in vanilla of different species and geographical origin and responsible for the characteristic vanilla flavour are described, along with the cultivation, harvesting and post-harvest processing. The chapter also looks at the main products and functional properties of vanilla and at its uses as a flavouring material, in pharmaceuticals and in cosmetics. Quality issues and adulteration are described and the chapter closes by looking at conservation issues and alternative methods for natural vanillin production.

**Key words:** *Vanilla planifolia*, vanilla cultivation, vanilla curing, vanillin functional properties, vanillin biotechnological production.

### 29.1 Introduction and description

There is probably no other spice in the world that strongly evokes the sweet memories of childhood and nurture than vanilla – ice cream to cool off in the scorching heat of summer, milk to nourish young and agile bodies and warm chocolate to perk up mornings with friends and loved ones. Consider how bland these delights would be without the aroma of vanilla! From the time it was offered in the form of a flavoured concoction by a gentle people to a Spanish conquistador to the current quest for a novel biotechnological means to produce its major flavouring constituents, vanilla has continually provided gastronomic pleasure to generations of people through the centuries.

The following discussion provides an overview of the relevant information on vanilla from a brief historical account, world production and trade to its horticulture, processing, biodiversity and conservation. There is also focus on product quality assessment, methods of adulteration and its detection, and research endeavours related to the natural production of its major flavour component, vanillin, outside the cured bean. The last section predicts the future outlook for vanilla *vis-à-vis* the growing demand for natural food additives and organic products.



### 29.1.1 Historical background

Vanilla is considered to be the greatest contribution of the Americas to the world of flavours. The following historical account of vanilla discovery and geographical spread is condensed from Correll (1953). Vanilla was introduced to the Old World when the Aztec emperor Montezuma of Mexico in 1520 welcomed the Spanish conquistador Hernando Cortes with '*chocolatl*', a drink concocted from powdered cacao and flavoured with ground vanilla beans called '*tlilxochitl*' (black pod). After the discovery of the secret ingredient, vanilla was brought to Spain, where the Spaniards used the bean, which they termed as '*vaynilla*' (little sheath, in reference to the fruit appearance) as a flavouring for the manufacture of chocolate. Considerable interest in the plant soon followed. Valuable information related to the plant's description, nomenclature and horticulture was gathered and plant samples were sent to various botanical gardens, including those in Paris and Antwerp. Subsequently, vanilla was introduced to Réunion in 1793, Java in 1819, India in 1835, the island of Tahiti in 1848 and the Seychelles in 1866. More recent studies elucidating the nature of the origin and pattern of world dispersal and introduction of vanilla can be sourced from Bory *et al.* (2008a,b), Lubinsky *et al.* (2008) and Bouetard *et al.* (2010).

Prior to 1841, initial efforts to cultivate vanilla outside its native Mexican home failed because of one major constraint: lack of natural vectors to pollinate the flowers. The solution to this problem came in 1836 when Charles Moren of Liège was able to produce vanilla beans by hand pollination. Large-scale cultivation of vanilla, however, was only realized years later, in 1841, upon the development of a practical method of artificial pollination of the plant by Edmond Albius, a former slave in Réunion. By 1886, vanilla cultivation was even greater in the Mascarene Islands (Réunion, Mauritius and Rodriguez) and Java than in Mexico. Some of these initial producers have continued to play significant roles in the world production of vanilla up to the present day.

### 29.1.2 Plant description

The vanilla of international commerce is derived from *Vanilla planifolia* H. C. Andrews (syn: *V. fragrans* (Salisb.) Ames). Vanilla, a member of the orchid family, is a climbing monocot, possessing a stout, succulent stem and short-petioled, oblong-lanceolate leaves about 20 cm long (Fig. 29.1). The inflorescence is characteristically raceme with 20 or more flowers. The flowers, about 6 cm long and 2.5 cm wide, are either yellowish, wholly green or white within with oblanceolate sepals and petals (Fig. 29.2). The fruit, popularly termed as 'beans' or 'pod' in the vanilla market, is botanically a capsule, nearly cylindrical and about 20 cm long (Fig. 29.3) (Bailey and Bailey, 1976).

Other species of vanilla of secondary importance in the vanilla trade include the West Indian vanilla, *V. pompona* Schiede (synonym: *V. grandiflora* Lindl.) and vanilla Tahiti, *V. tahitensis* J. W. Moore. Purseglove *et al.* (1981) and Straver (1999) provided some important distinctions among the three species. West Indian vanilla is a native of Central America, northern South America and the Lesser Antilles. It differs from *V. planifolia* by having larger leaves, more fleshy and larger flowers and the presence of a tuft of imbricate scales, instead of hairs, in the centre of the lip



**Fig. 29.1** Vanilla, a vine, is a member of the orchid family.



**Fig. 29.2** Vanilla flowers are yellowish, wholly green or white within.



**Fig. 29.3** The fruit of vanilla is botanically a capsule, but is called a 'bean' or 'pod' in the international market.

disc. On the other hand, *V. tahitensis* is indigenous to Tahiti. It is less robust than *V. planifolia* with more slender stems and narrower leaves. Both minor species yield shorter and thicker capsules and an inferior bean product.

A comprehensive comparative anatomical study of the stem, leaves and roots of several species of vanilla, including *V. planifolia* and *V. pompona*, can be obtained from Stern and Judd (1999).

### 29.1.3 Flavour constituents

The flavour famously associated with vanilla results from a complex and varied mixture of chemical compounds. About 170 volatile constituents, most of which occur below 1 ppm, have been reported in vanilla by Klimes and Lamparsky (1976). Vanillin (Fig. 29.4a) serves as the major flavour backbone, occurring in levels from 1.52–2.42% of bean dry weight (Cowley, 1973). Other major components are *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, *p*-hydroxybenzyl alcohol and vanillyl alcohol (Fig. 29.4b–f) (Anwar, 1963; Smith, 1964; Herrmann and Stockli, 1982).

The type and levels of the major flavouring components vary depending upon the species and geographical source (Table 29.1). Tahiti vanilla stands out among the different types of beans for exhibiting higher levels of *p*-hydroxybenzoic acid.

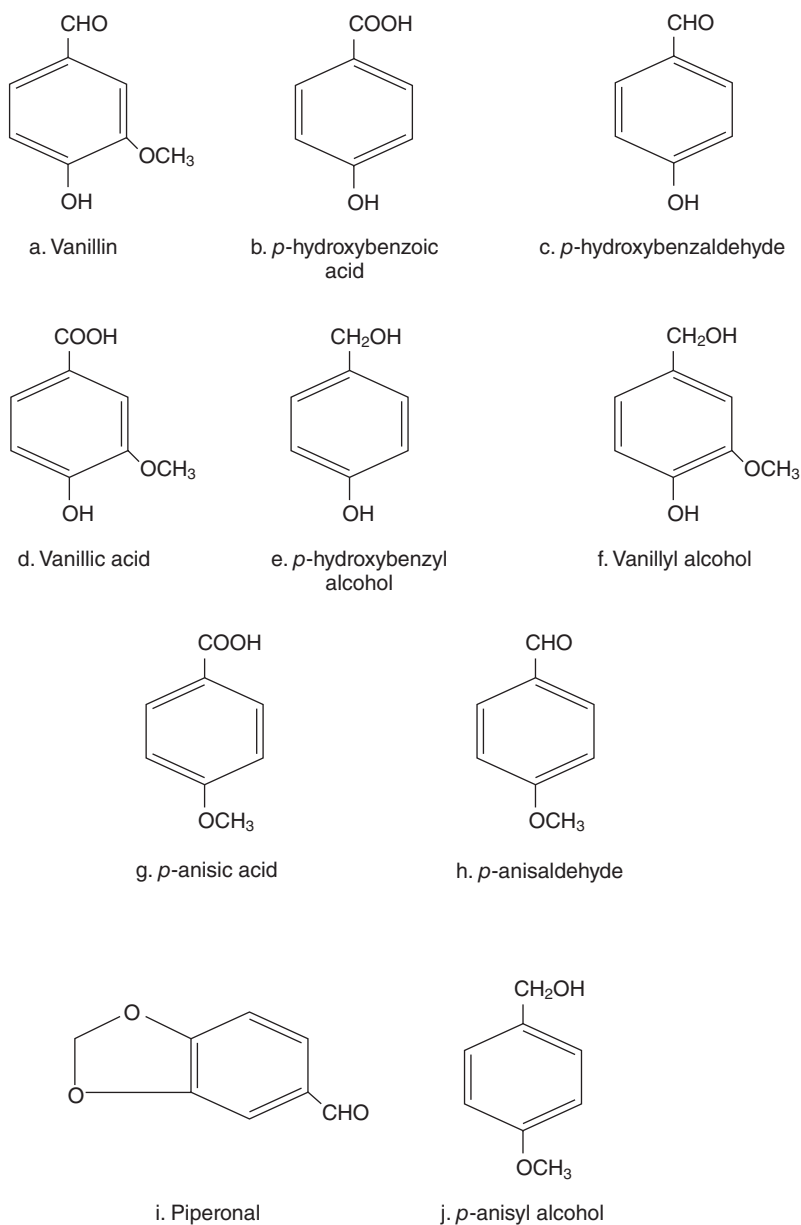


**Fig. 29.4** Chemical structures of the major flavouring constituents of vanilla.

Other components present in *V. tahitensis* that are not detected in *V. planifolia* are *p*-anisic acid, *p*-anisaldehyde and piperonal (heliotropin) (Fig. 29.4g–i) (Ranadive, 1992). Vanillons (*V. pompona*, Guadeloupe vanilla) contains vanillin, *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxybenzaldehyde, *p*-anisic acid, *p*-anisaldehyde and *p*-anisyl alcohol (Fig. 29.4j), but not piperonal (Ehlers and Pfister, 1997).

The hydrocarbon profile of the lipidic fraction, which also contributes to flavour, of different types of beans has also been investigated by Ramaroson-Raonizafinimanana *et al.* (1997). Hydrocarbon content varies between 0.2 and 0.6 %.

**Table 29.1** Reported values of major flavour constituents of cured vanilla beans from various geographical sources

| Source         | Vanillin | Vanillic acid | <i>p</i> -Hydroxybenzaldehyde<br>(mg 100 ml <sup>-1</sup> ) | <i>p</i> -Hydroxybenzoic<br>acid |
|----------------|----------|---------------|-------------------------------------------------------------|----------------------------------|
| Mexico         | 18–100   | 8–23          | 5–7                                                         | 2–7                              |
| Madagascar     | 47–216   | 12–23         | 6–13.7                                                      | 2–5.6                            |
| Comores        | 115–154  | 8–13          | 9–13                                                        | 2–7                              |
| Java/Indonesia | 63–142   | 7.7–11        | 7–10                                                        | 2–4                              |
| Uganda         | 47–186   | 5–10          | 5–8                                                         | 1–6                              |
| Tonga          | 197–320  | 7.6           | 10                                                          | 2.1                              |
| West Indies    | 20–136   | 15–22         | 2–6                                                         | 2–4                              |
| Costa Rica     | 135–161  | 12            | 14                                                          | 5.2                              |
| Jamaica        | 216–265  | 4.2           | 8.4                                                         | –                                |
| Tahiti         | 54–120   | 4–5           | 4–13                                                        | 16–32.8                          |

Sources: Smith (1964), Archer (1989), Ranadive (1992).

A total of 25 *n*-alkanes, 17 branched alkanes and 12 alkenes have been identified. Distinction between types of vanilla is also evident. *Vanilla fragrans* from Réunion is rich in *n*-alkanes (46 %) and *n*-1-alkenes (26 %), while *V. tahitensis* from Tahiti contains predominantly branched alkanes (47 % for 3-methylalkanes and 33 % for 5-ethylalkanes). Also present in the lipophilic fraction before saponification in the two vanilla species are three new  $\gamma$ -pyrones: 2-(10-nonadecenyl)-2,3-dihydro-6-methyl-4*H*-pyran-4-one; 2-(12-heneicosenyl)-2,3-dihydro-6-methyl-4*H*-pyran-4-one and 2-(14-tricosenyl)-2,3-dihydro-6-methyl-4*H*-pyran-4-one (Ramaroson-Raonizafinimanana *et al.*, 1999).  $\gamma$ -Pyrones are intermediates in the synthesis of biologically important compounds. A review of other flavour components as a function of vanilla species can be found in Richard (1991).

Werkhoff and Guntert (1997) characterized for the first time in Bourbon vanilla beans 15 esters that are derived from cyclic and acyclic terpene alcohols and aromatic acids. Among those isolated, pentyl salicylate and citronellyl isobutyrate are considered new natural compounds.

In a recent study by Perez-Silva *et al.* (2006) involving gas chromatography–mass spectroscopy (GC-MS) and GC-olfactometry analysis of aroma compounds, 65 volatiles were identified from extracts of cured vanilla beans. These compounds included 25 acids, 15 phenolic compounds, 10 alcohols, four aldehydes, four heterocyclic compounds, four esters, two hydrocarbons and one ketone. Vanillin, vanillic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid were the major compounds in the pentane/ether (1:1 v/v) aroma extract with vanillin representing 85 % of the volatile compounds at a concentration of 19118 ppm.

In 2009, Schwarz and Hofmann identified seven velvety mouth-coating molecules in cured vanilla beans by sequential application of solvent extraction, gel permeation chromatography and high-performance liquid chromatography (HPLC) in combination with taste dilution analyses followed by LC-MS and 1D/2D NMR (nuclear magnetic resonance) experiments. The identified compounds were american A, (1-*O*-vanilloyl)-(6-*O*-feruloyl)- $\beta$ -D-glucopyranoside, 4',6'-dihydroxy-3',5'-dimethoxy-[1,1'-biphenyl]-3-carboxaldehyde, 5-(4-hydroxybenzyl)vanillin, divanillin, 4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde and

4-(4-hydroxybenzyl)-2-methoxyphenol. Except for divanillin, these compounds were not reported previously in vanilla beans.

Several studies dealing with the quantitative determination of vanillin and related compounds of vanilla flavour have been reported (Jagerdeo *et al.*, 2000; Li *et al.*, 2004; Sharma *et al.*, 2006, 2007; Sinha *et al.*, 2007, 2008; Jadhav *et al.*, 2009; Kishor Kumar *et al.*, 2010).

Vanilla also contains resins, gums, amino acids and other organic acids, all which contribute to the distinct flavour characteristics of the cured beans. An enumeration and discussion of these constituents is provided by Purselove *et al.* (1981).

#### 29.1.4 Production and trade

In the international market, cured vanilla beans are conveniently classified according to their geographical source or origin (Correll, 1953). The principal groupings are: Mexican beans – those coming only from Mexico; Bourbon beans – formerly referring only to those produced from the island of Réunion (then named Bourbon) but currently inclusive of all beans from Madagascar and the Mascarene, Comoro and Seychelles islands; Tahiti beans – grown in the French group of the Society Islands; and Java beans – derived from Indonesia.

From 1990–2000, average world production of vanilla beans amounted to 4466 tonnes harvested over a total of 38485 ha (FAO, 2011). Indonesia contributed about 38 % of this world produce, slightly higher than Madagascar at around 34 %, while Mexico and Comoro Islands had less than 10 % share. The annual world import of cured vanilla beans averaged US\$545 m. In the same decade, the USA imported the biggest mean volume of cured beans at 1448 tonnes worth US\$52 m, followed by France at 374 tonnes and Germany at 318 tonnes. From 1990–5, the price of vanilla bean imported by the USA ranged from US\$42–74 kg<sup>-1</sup> averaging at US\$64 kg<sup>-1</sup> (USA Vanilla Bean Imports Statistics, 2002).

From 2000–8, the top three producers of vanilla were Indonesia, Madagascar and China, with Indonesia attaining the highest production of 3700 tonnes. During this period, the USA remained the top importing country with imports ranging from 1117–2260 tonnes, except in 2004 when it ranked second to Singapore at a recorded total imports of only 692 tonnes. As usual, Madagascar provided the largest volume of vanilla in the world market with exports reaching 3085 tonnes in 2007.

In 1988, marketing estimates by McCormick & Co., Inc. revealed that vanilla extract was largely used by the industrial sector (75 % of the total supply), followed by the retail sector (20 %) and the food service sector (5 %) (Gillette and Hoffman, 1992). Of the total industrial application, 30 % was utilized in ice cream preparations, 17 % in soft beverages, 11 % in alcoholic beverages, 10 % in yoghurt and the remaining 7 % in bakery items, confectioneries, cereals and tobacco products.

## 29.2 Cultivation of vanilla

### 29.2.1 Climate and soil requirements

Vanilla is a tropical crop that thrives best in warm and moist climate. Natural growth is obtained at latitudes 15° and 20° north and south of the equator (Lionnet, 1958).

The optimum temperature ranges from 21–32 °C, with a mean value of 27 °C, while the precipitation required falls between 2000 and 2500 mm annually (Purseglove *et al.*, 1981). A dry period of about two months is needed to restrict vegetative growth and induce flowering; rainfall in the remaining ten months should be evenly distributed (Correll, 1953). Sunlight ranging from 600–800  $\mu\text{Em}^{-2} \text{s}^{-1}$  favours maximum productivity; below this level, it promotes vegetative growth (Puthur, 2005). Vanilla does well from sea level up to 700 m in altitude, and has been found to exist even at 1000 m in Mexico (Correll, 1953; Straver, 1999). Vanilla established on gently sloping terrain with good drainage is reputed to produce better crops and to be more resistant to fungal infection (Lionnet, 1958). It grows best in light, porous and friable soils, preferably of volcanic origin, with a pH of 6–7 (Correll, 1953; Straver, 1999).

### 29.2.2 Propagation

#### *Use of cuttings*

Vanilla is commercially propagated by stem cuttings. If source is not a constraint, cuttings 2–3.5 m long are preferred since they will flower in 1–2 years, as opposed to 30 cm cuttings which will bear flowers and fruit in 3–4 years (Correll, 1953; Purseglove *et al.*, 1981). The latter, however, is known to produce more vigorous crops that will last longer (Lionnet, 1958). Long cuttings are planted directly in the field, while short cuttings are usually started in a nursery. Cuttings for storage or transport can be carefully wrapped in banana or abaca leafsheaths and will root in 20 days under shade (David, 1950). Most recently, application of *Trichoderma harzianum* before inserting the cuttings in the planting holes shortens the days to rooting to 17 days (Murthy *et al.*, 2010). Similar response, however, is noted with the growth regulator indolebutyric acid.

#### *Use of in vitro techniques*

Vanilla is also successfully propagated using *in vitro* techniques. Multiple shoots can be regenerated either directly from the shoot tips, nodal explants with axillary buds and *in vitro* fragmented explants (Kononowicz and Janick, 1984; Philip and Nainar, 1986; George and Ravishankar, 1997; Geetha and Shetty, 2000; Giridhar *et al.*, 2001; Giridhar and Ravishankar, 2004; Kalimuthu *et al.*, 2006; Abebe *et al.*, 2009; Gonzalez-Arnao *et al.*, 2009) or indirectly from callus derived from the seed (Palama *et al.*, 2010) and leaf explants (Janarthanam and Seshadri, 2008). Maximum shoot proliferation was reported when BAP-supplemented MS medium is used in combination with NAA (George and Ravishankar, 1997; Janarthanam and Sheshadri, 2008), NAA and silver nitrate (Giridhar *et al.*, 2001), coconut water (Kalimuthu *et al.*, 2006) and kinetin (Abebe *et al.*, 2009). Addition of TDZ and coconut water to MS medium can also induce shoot proliferation (Giridhar and Ravishankar, 2004).

#### *Use of seeds*

Propagation by seeds is confined to breeding work. Under natural conditions vanilla seeds, which contain a limited amount of food reserve, do not germinate unless in association with a mycorrhizal fungus (Purseglove, 1985; Porras-Alfaro and Bayman, 2007). They can, however, germinate *in vitro* in a well-defined culture medium for-



mulated by Knudson (1950). The specific histochemical changes during the *in vitro* germination and seedling growth of vanilla have been described by Philip and Nainar (1988).

### 29.2.3 Support/shade trees

Vanilla, being a shade-loving, climbing vine, needs a structure that will support its vertical growth and provide some canopy to filter intense sunlight. A living support cum shade tree suits this purpose. In the choice of the support trees, the following criteria should be considered (David, 1950; Correll, 1953; Lionnet, 1958):

- Leaves are small enough to permit chequered sunlight (about one-third to one-half of full sun exposure) to filter through.
- Branches are wide spreading and sufficiently low (*ca* 1.5–2 m from the ground) for hanging the vines.
- Growth is rapid in full sunlight.
- Propagation is easy either by seeds or cuttings.
- Sufficient protection from sun and strong wind is provided.
- Must help improve soil nutrition (e.g. legumes for N fixation).
- Should not become entirely defoliated.

The members of the Leguminosae family such as *madre de cacao* (*Gliricidia sepium* (Jacq.) Kunth ex Walp.), *ipil-ipil* (*Leucaena leucocephala* (Lamk.) de Wit.) and Indian coral tree (*Erythrina orientalis* (L.) Murr.) were found to be the best shade/support trees for vanilla (David, 1950).

### 29.2.4 Field establishment

In establishing a vanilla plantation, areas that are prone to stagnation of water (e.g. flat lands with poor drainage) or subject to soil erosion (e.g. steep contours) should be avoided. An ideal site is a gently sloping hill with sufficient drainage (Correll, 1953).

In open sites, support trees are planted ahead of the scheduled time for planting the vanilla vines, preferably 6 months to 1 year, to provide a sufficient period for root establishment and development of a spreading canopy (David, 1950; Straver, 1999). Various planting distances have been used in several countries. With wide spacing, the plantation can be easily managed and incidences of diseases are observed to be low, although the economic benefit is also lower. On the other extreme, too high a plant density makes vanilla very susceptible to fungal attack, as was observed in Seychelles, Réunion and Indonesia (Correll, 1953; Lionnet, 1958). Field operations are also more difficult. A moderate spacing of 1.5 m × 2 m or 2 m × 2 m giving 3300 or 2500 vines to a hectare, respectively, is recommended to overcome these problems (Sen, 1985).

Planting holes are dug about 30 cm from the support tree and the basal three nodes (without leaves) of the cuttings are buried into the soil and then covered with rich humus or well-decayed leaves. The remaining exposed vine is tied gently to the support tree with a piece of abaca or plastic twine. Vines set up in this way become fully established in less than 5 months (David, 1950). Only one cutting is usually placed per support tree. Planting is done at the onset of the rainy season.

### 29.2.5 Fertilizer application

Chemical analysis reveals considerable amount of inorganic nutrients taken up by the different organs of vanilla (Tjahjadi, 1987). The highest amount of nutrient found in the stem is calcium, in the leaf, magnesium, and in the fruit, potassium. The fruit contains the highest level of all the nutrients examined, except for calcium and magnesium. This result suggests the need to consider proper nutrition in this crop. It is recommended that 40–60 g N, 20–30 g P<sub>2</sub>O<sub>5</sub> and 60–100 g K<sub>2</sub>O be supplied to each vanilla vine per year (Anandaraj *et al.*, 2001). Spraying of 1 % solution of complete fertilizer (17 : 17 : 17) once a month enhances growth and flowering.

Organic fertilizers such as guano and bone meal are found to be beneficial to vanilla (Purseglove *et al.*, 1981). The use of fresh farmyard manure is reported to increase the risk of infection due to diseases (Lionnet, 1958). Composting before soil application of the animal manure, together with other crop wastes such as rice straw, circumvents this problem. Loppings, especially the leaves, of leguminous support trees are a very good source of green manure.

### 29.2.6 Pruning and training

Both the vanilla vine and the support trees are judiciously pruned to optimize vegetative growth, flowering and fruit development. At the onset of the rainy season, heavy lopping of the support tree branches is usually practised to increase the light intensity reaching vanilla and make it less susceptible to fungal attack.

When vanilla attains a sufficient length, the stem is allowed to hang or bend over the support branches until it is about 30 cm above the ground (Lionnet, 1958). The tip is then pruned to induce the growth of lateral branches below it. The hanging branch is known as a 'porteur'. Any shoot coming out of the porteur is cut off when 7–10 cm long, while those from the rest of the plant before the bend are allowed to develop. These will become the porteurs of the following cropping season. This bending and pruning technique favours flowering. In mature vines, about five or six porteurs are maintained. After a season of harvest the porteurs are cut off.

### 29.2.7 Mulching

Soil cultivation is generally not practised or done very lightly in vanilla since it is a surface feeder (Purseglove *et al.*, 1981). More attention, rather, is given to mulching. Leaves and loppings from support trees as well as weeding wastes can provide the necessary soil cover. In the Seychelles, coconut husks are applied in overlapping rows about 45 cm from the base of vanilla and decayed plant matter is incorporated in the space between (Lionnet, 1958). Coconut husks do not only conserve soil moisture but also provide a potassium-rich organic matter.

### 29.2.8 Diseases and pests

Correll (1953) and Purseglove *et al.* (1981) identified and described the various diseases of vanilla in several producing countries. The most serious and widespread is anthracnose, which is caused by *Calospora vanillae* Massee. Particularly observed in the Comoro and Seychelles Islands, the West Indies, Tahiti, Mascarene and

Colombia, the disease damages the stem apex, leaves and aerial roots as well as the fruits. Wilting and abscission of the affected organs occur in severe cases of infection. Root rot by *Fusarium batatis* Wollenw. var. *vanillae* is another important fungal disease of vanilla, which is widespread in Puerto Rico and in Indonesia (Sen, 1985). It is characterized by browning and death of underground roots with a concomitant shrivelling and drying out of the shoot. Recent work of Pinaria *et al.* (2010) reports the occurrence of stem rot disease caused by the pathogen *F. oxysporum* f. sp. *vanillae* in Indonesia. The disease is characterized by discolouration and necrosis of the infected tissues. In Kerala India, brown spot disease caused by *Cylindrocladium quinquiseptatum* Boedijn & Reitsma is reported to attack vanilla plants (Bhai and Anandaraj, 2006; Bhai and Dhanesh, 2008). The infection is associated with water-soaked spots on the beans which later developed into characteristic brownish sunken lesions.

The following conditions need to be avoided to check the onset and spread of these fungal diseases: excessive moisture, insufficient drainage, too much shade and high-density planting. In Thailand, biological control using *Emericella nidulans* is reported by Talubnak and Soyong (2010) to inhibit mycelial growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose disease in vanilla. The use of essential oils to inhibit the spore germination of *F. oxysporum* causing root rot disease of vanilla in Yunnan, China was also investigated (He, 2007). In particular, cinnamon oil at 200 ppm concentration showed 100 % inhibition.

Other debilitating diseases of vanilla which have recently been observed are viral diseases. Several types have been identified. The cucumber mosaic virus (CMV) was first reported by Farreyrol *et al.* (2001) in vanilla. In French Polynesia, CMV induces severe stunting of *V. tahitensis* with marked stem and leaf deformation. CMV is also present in *V. fragrans* samples from Réunion, although with less severe symptoms compared with those identified for *V. tahitensis*. The differences in symptomatology are attributed either to virus strain variation or differential species tolerance. In India, CMV also caused stunting of *V. planifolia* plants (Madhubala *et al.*, 2005).

Pearson *et al.* (1993) described the distribution and incidence of vanilla viruses in the following South Pacific countries: Cook Islands, Fiji, Niue, Tonga and Vanuatu. Cymbidium mosaic potexvirus (CyMV) and odontoglossum ringspot tobamovirus (ORSV), which seems to do little harm, are found in all countries surveyed in both *V. fragrans* and *V. tahitensis*. Of bigger concern, however, are potyviruses, which cause severe infection. These include: vanilla necrosis potyvirus (VNV) in Fiji, Tonga and Vanuatu detected in *V. fragrans*, and vanilla mosaic virus (VaMV) present in *V. fragrans* from the Cook Islands, Fiji and Vanuatu and *V. tahitensis* from the Cook Islands and French Polynesia. A more recent work reveals that the VaMV isolates from *V. tahitensis* from the Cook Islands and French Polynesia are strains of *Dasheen mosaic virus* that exclusively infect vanilla (Farreyrol *et al.*, 2006). Other potyviruses that react with neither VNV or VaMV antisera and rhabdovirus-like particles have also been detected in Fiji and Vanuatu. Symptoms of potyvirus infection include leaf distortion, sunken chlorotic patches, stem necrosis and vine die-back. Control measures include rouging, avoiding the use of cuttings from plantings where the symptoms are present and provision of shade houses edged with insect-proof netting (Richard *et al.*, 2009). There is also the potential of using mild virus strains for cross-protection (Liefing *et al.*, 1992).

CyMV and ORSV were also present in *V. tahitensis* growing in the Society Islands of French Polynesia, but the incidence of damage was very low (Wisler *et al.*, 1987). In India, occurrence of CyMV infecting *V. planifolia* was first reported by Bhat *et al.* in 2006. VaMV has a limited host range compared with VNV (Wang and Pearson, 1992). On the other hand, comparison of the gene for the coat protein of VNV using Western blot analysis and enzyme-linked immunosorbent assay (ELISA) reveals its serological similarity with watermelon mosaic 2 potyvirus (WM2V) (Wang *et al.*, 1993).

More recently, Grisoni *et al.* (2004, 2006) reported the findings of their long-term virus surveys in intensively cultivated vanilla plots in French Polynesia. Aside from the VaMV mentioned above, the other potyviruses listed were watermelon mosaic virus (WMV), bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV), cowpea aphid-borne mosaic virus (CABMV), ornithogalum mosaic virus (OrMV) and wisteria vein mosaic virus (WVMV).

Results of insect pest surveillance studies in Karnataka, India confirm the occurrence of nine insect pest species in vanilla growing areas (Prakash *et al.*, 2002). Among these pests, vanilla bug (*Halyomorpha* sp. nr. *Picus* (Fabricius) (Hemiptera: Pentatomidae) has been reported to cause severe economic loss. Damages associated with the pests include sucking of plant sap and feeding particularly on the tender parts of the vine. In the same year, vanilla vine weevil (*Sipalus* sp. [Dryophthoridae: Coleoptera]) was first reported attacking vanilla plants in Kerala, India (Varadarasan *et al.*, 2002). Both the adult insect and the grub feed on the plant resulting in necrosis and rotting of the affected portion.

### 29.2.9 Flowering and pollination

Vanilla flowers only once a year, staggered over an average of 2–3 months, depending upon the variation in local climate. In Mexico, flowering is observed from April–May; in Madagascar and the Comoro Islands, between November and January (Correll, 1953). In Indonesia, this occurs between July and August (Sen, 1985); in India from December–February (Anandaraj *et al.*, 2001; Sreekrishna Bhat and Sudharshan, 2002), while in the Philippines, flowering begins as early as March and lasts until June (David, 1950).

In its natural habitat in Mexico and other parts of Central America, vanilla flowers are pollinated by bees (*Melipona* sp.) and hummingbirds (Correll, 1953). The rate of natural pollination is low, only about 1 % (Fouche and Coumans, 1992). Outside its native home, fruit set in vanilla is accomplished through hand pollination.

The vanilla flower is so constructed that effective transfer of pollen to the stigma is prevented. The stamen cap enclosing the pollinia (mass of pollen) and the flap-like structure called the rostellum covering the stigma both act as physical barriers to the process of pollination (Correll, 1953; Purseglove *et al.*, 1981). In artificial pollination, the stamen cap is removed and the rostellum is pushed up with the aid of a bamboo stick or any similar object the size of a toothpick. The pollinia and stigma are then brought into contact with each other by hand manipulation. A simple description, complete with illustrations, of four different types of hand-pollinating vanilla flowers is provided by Fouche and Coumans (1992). If pollination is success-

ful, the flowers remain and wither on the rachis; otherwise, they abscise in 2–3 days. Under expert hands, from 1000–2000 flowers can be pollinated per day (Purseglove *et al.*, 1981).

Vanilla flowers last only for a day. Sivaraman Nair and Mathew (1969) observed that flower opening in vanilla occurs between 10.30 p.m. and 1 a.m., and is completed by 6 p.m. Pollination is accomplished with 98–100 % success if undertaken between 6 a.m. and 6 p.m. on the day of flower opening. Pollen viability deteriorates markedly after anthesis, with only 10–15 % fruit set when 2- to 3-day-old pollens are used.

Plant growth regulators have also been tested as an alternative to laborious hand pollination. 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) at 100–500 ppm, and gibberellic acid (GA3) at 20–100 ppm promote fruit set if applied on or before anthesis. The treated beans, however, either drop before reaching maturity or are only one-quarter to one-third the size of hand-pollinated beans.

### 29.2.10 Fruiting

In Réunion, it takes about 6 months for the fruits to mature; in Mexico, Indonesia and the Philippines, about 9 months (David, 1950; Correll, 1953; Sen, 1985). About 50–150 fruits are allowed to develop and mature per vine (Purseglove, 1985).

## 29.3 Harvesting and post-production activities

Vanilla fruits are gathered when they are fully mature but before they are too ripe. When picked immaturity, the fruits do not develop the requisite full-bodied aroma and proper colour during processing and are more prone to fungal infection (Correll, 1953). When harvested at the over-ripe stage, the fruits tend to split and lose some of their aroma. The following serves as harvest indices for vanilla (David, 1950; Purseglove, 1985):

- The thickest portion of the fruit (the ‘blossom end’) takes on a pale yellow colour.
- Overall pod colour changes from dark green to light green.
- The fruits lose their lustre and become somewhat dull.
- Two distinct lines appear from one end of the fruit to the other.

Since flowering is staggered, harvesting is likewise extended over a period of time. One kilogram of cured beans is derived from about 6 kg of green pods. Yield of cured beans ranges from 300–800 kg ha<sup>-1</sup> yr<sup>-1</sup> (Purseglove *et al.*, 1981; Anandaraj *et al.*, 2001).

### 29.3.1 Post-production activities

#### *Curing*

After harvesting, the pods of vanilla need to be cured to develop the characteristic natural flavour associated with the product. Curing can be defined as the sum total changes that occur during the primary processing of a given raw material to a desired finished product, which is ready for market (Jones and Vicente, 1949a). Curing stops the various natural vegetative processes in the harvested beans and

promotes the metabolic reactions involved in the generation of the aromatic flavouring constituents in the cured material (Arana, 1944). It can be broadly classified into two: (i) changes that involve a simple loss of water, achieved through drying, and (ii) changes that involve chemical transformation, which is usually accompanied by hydrolytic and oxidative changes with or without the aid of enzymes. In vanilla, the latter changes are more critical.

Vanillin, the main flavouring chemical of vanilla, is present only in trace amounts in the green mature beans; upon curing, however, vanillin content increases (Arana, 1943). The chemical compound from which vanillin is derived occurs in the uncured pods in the form of a glucoside called glucovanillin (Arana, 1945). During the curing process, this glucoside is hydrolyzed to form vanillin and glucose through the action of a  $\beta$ -glucosidase. The activity of this enzyme changes with the maturity of the vanilla beans, being negligible in the green beans and highest in the split, blossom-end yellow beans. Spatially, all of the enzyme is located in the fleshy portion or thick wall of the pods, where most of the glucovanillin is also concentrated (Arana, 1943). Along the bean length, 40 % of glucovanillin has been detected in the blossom end, another 40 % in the middle and the remaining 20 % in the stem end. These findings, however, are in contrast to the reported work of Odoux *et al.* (2003) on localization of  $\beta$ -glucosidase and glucovanillin in vanilla beans. Both the enzyme and the substrate are primarily located in the placental laminae and, to a lesser extent, in the papillae. The outer fleshy portion of the bean is devoid of these compounds. Other flavour constituents, such as *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde and vanillic acid, are also present in the green beans in their glycosidic forms and are released through enzymatic hydrolysis during curing (Ranadive, 1992). Aside from these compounds, Dignum *et al.* (2004) reported the presence of other glucosides of vanillyl alcohol, *p*-cresol, creosol, bis[4-( $\beta$ -D-glucopyranosyloxy)-benzyl]-2-isopropyltartrate and bis[4-( $\beta$ -D-glucopyranosyloxy)-benzyl]-2-(2-butyl)tartrate in the green beans of vanilla. Older pods have higher content of glucovanillin, vanillin, *p*-hydroxybenzaldehyde glucoside, *p*-hydroxybenzaldehyde and sucrose, while younger pods have more bis[4-( $\beta$ -D-glucopyranosyloxy)-benzyl]-2-isopropyltartrate, bis[4-( $\beta$ -D-glucopyranosyloxy)-benzyl]-2-(2-butyl)tartrate, glucose, malic acid and homocitric acid (Palama *et al.*, 2009).

The splitting of vanillin from the glucoside is initiated during the early part of the curing process, but the full development of flavour and aroma occurs only after a considerable period of pod preparation and conditioning (Arana, 1943; Jones and Vicente, 1948). Treatment of cured beans with  $\beta$ -glucosides enhances vanillin content, suggesting incomplete hydrolysis, probably as a result of (i) insufficient amount of native enzyme, (ii) inadequate enzyme–substrate interaction or (iii) inactivation of enzymes by oxidized phenols liberated during curing (Ranadive, 1992). In a study conducted by Waliszewski *et al.* (2007), hydration of cured vanilla beans with 5 % ethanol for 48 h prior to enzymatic pre-treatment using stable cellulolytic preparations doubled the vanillin content in the ethanolic extract. Chemical changes other than enzymatic hydrolysis may also contribute a great deal to the quality of cured vanilla. Balls and Arana (1941) suggested the possible role of a peroxidase system in the oxidation of vanillin to quinine compounds. These substances possess more complex structure with presumably different aroma that can add to the total flavour of the cured product. Wild-Altamirano (1969) reported that proteinase activity



declines with pod growth while the activities of glucosidase, peroxidase and polyphenoloxidase increase with pod age, being maximum near or at ripening. The trend in enzyme activities is indicative of the potential role of the various products derived from catalyzed reactions in the full development of the characteristic flavour and aroma of cured beans.

In general, vanilla curing follows four successive steps: (i) killing or wilting, (ii) 'sweating', (iii) drying and (iv) conditioning. Killing or wilting is the initial step in inhibiting the natural changes in vanilla beans. It is achieved through various techniques, depending upon the producing country (Arana, 1945; Theodose, 1973). In Mexico and Indonesia, the most popular method is sun wilting. In this method, the beans, which are contained on racks covered with dark woollen blankets, are simply heated under the sun. Wilting with the use of an oven maintained at 60 °C is alternatively practised in Mexico. In Madagascar, Réunion and Comores, beans are killed by dipping in hot water for a few minutes (scalding technique) (Fig. 29.5). On the island of Guadeloupe, beans are gently scratched on the surface with the use of a pin embedded in a cork ring prior to sun exposure. Wilting by freezing has been developed in Puerto Rico for experimental purposes only. In this technique, the beans are refrigerated until frozen and then thawed naturally at room temperature. Some of the advantages and disadvantages of these types of wilting are presented in Table 29.2.

The successive steps after killing are more or less similar for the different countries exporting vanilla. 'Sweating' or heating is done to develop the proper texture and flexibility. This is accomplished through either of two means: (i) daily sun exposure for about 6 h, with the beans covered with woollen blankets for the remainder of the day (Fig. 29.6) or (ii) incubation in ovens at 45 °C at high relative humidity (Arana, 1944, 1945). The significant change in colour of the bean to chocolate brown is manifested at this stage (Balls and Arana, 1941). Sweating is terminated when beans become pliable. The next step is slow drying, which is normally carried out at room temperature (Fig. 29.7). Drying is needed to lower the moisture content of the beans to a desirable level, usually 15–30 % (Jones and Vicente, 1948). Finally, in conditioning, the product is kept in closed containers at room temperature for



**Fig. 29.5** Implements used in the processing of beans using the hot water treatment.



**Table 29.2** Advantages and disadvantages of different methods of wilting vanilla beans

| Method of wilting | Advantages                                                                                                                                                    | Disadvantages                                                                                                                 |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| Sun wilting       | Method is simple<br>Does not require additional equipment                                                                                                     | High degree of bean splitting<br>Beans mould easily                                                                           |
| Oven wilting      | Short period of time for sweating and drying<br>Fewer split beans<br>High vanillin content                                                                    | High percentage of mouldy beans<br>Medium phenol value                                                                        |
| Hot-water wilting | Few mouldy beans and medium degree of splitting<br>Easiest and most satisfactory for the inexperienced curer                                                  | Longer period of drying<br>Low vanillin content and phenol value                                                              |
| Scratching        | Short period of time for sweating and drying<br>Low vanillin and phenol values<br>Low degree of splitting                                                     | High susceptibility to mould<br>Poor flexibility of the beans in the stem end<br>Dependent on the skill and care of the curer |
| Freezing          | Practically no mould<br>Sophisticated aroma<br>Beans are picked at the best stage of maturity and kept in the refrigerator until enough beans are accumulated | Medium values for phenol, vanillin content and percentage splitting                                                           |

Source: Arana (1944, 1945).

several months to allow the complete development of aroma (Fig. 29.8). In this last stage, beans are frequently examined for the presence of moulds. At the minimum, conditioning lasts for 3 months (Arana, 1944).

An improved curing process using drying tunnels has been developed in Madagascar (Theodose, 1973). This method relies on hot air, instead of heating by the sun, and produces homogeneous, good-quality beans in large quantities (40 tonnes dry vanilla in one season). Gillette and Hoffman (1992) present a very good comparison of the curing process associated with the different types of vanilla beans.

The nature of curing procedures adopted affects the quality of cured beans. Aside from the influence of method of wilting, Arana (1945) pointed out that non-uniformity in drying, sweating and drying under the sun, use of dirty blankets and improper ventilation in curing rooms, all contribute to the susceptibility of beans to mould infection, which in turn lowers the quality of the product. He further noted that the moisture content of cured beans should be properly controlled to obtain the full development of the vanilla aroma. The aroma of cured beans with 50–54 % moisture is characteristically fermented; those with 24–27 % moisture, sophisticated and well developed; while those with 31–34 % moisture, just desirable.

A more recent study by Krishnakumar *et al.* (2007) investigates the effect of storing fresh beans, killing temperature and duration of killing on the quality of cured beans. Storage of freshly harvested beans under room condition for 3 days prior to killing yielded the highest vanillin content of 2.51 %. Dipping the beans in hot water maintained either at 63 °C for 5 minutes or 65 °C for 3 minutes and



**Fig. 29.6** Sweating of vanilla beans.



**Fig. 29.7** Drying of cured beans in open shelves.



**Fig. 29.8** Dried beans are placed in plastic bags and conditioned for several months.

immediately wrapping in woollen cloth are essential for successful killing and sweating, respectively.

Factors other than those related to curing protocol are also known to influence the quality of the final product. Vanilla beans that ripen early in the harvesting season yield higher quality cured beans than those gathered at mid-season or late in the season (Jones and Vicente, 1949b). The best cured material comes from pods harvested when the blossom-end section is yellow. When picked prior to this stage, beans give an undeveloped vanilla flavour; when beyond, a full but undesirable flavour is obtained (Broderick, 1955a). Immature beans when processed are also readily attacked by fungi (Arana, 1945).

Recent reports show the potential of using exogenous enzymes and tissue disruption by freezing and thawing on fresh green vanilla pods to increase the yield of vanillin (Madhava Naidu *et al.*, 2009; Perera and Owen, 2010), thereby eliminating the tedious and time-consuming process of curing. Vanillin content of 4.2 % was obtained when tea leaf enzyme extract was used (Madhava Naidu *et al.*, 2009) while a high of 7.0 % on a dry weight basis was successfully achieved with the addition of exogenous cellulase, pectinase and  $\beta$ -glucosidase enzymes and cellular damaging techniques on green Tongan vanilla beans (Perera and Owen, 2010). Similarly, the use of biotic elicitors during accelerated curing of vanilla beans generates more than a three-fold increase in vanillin content compared to conventional curing (Sreedhar *et al.*, 2009).

### *Grading*

The grading and classification of cured vanilla beans vary depending upon the producing countries. Mexican beans, for example, are usually graded (from the highest to the lowest quality product) as 'prime', 'good to prime', 'good', 'fair' and 'ordinary', while Bourbon beans are graded as 'prime', 'firsts', 'seconds', 'thirds', 'fourths' and 'foxy splits' (Merory, 1960). Classification is commonly based on: bean integrity (either whole, broken or split), bean length, appearance (particularly colour and surface blemishes), moisture content and aroma quality (Arana, 1945; Heath and Reineccius, 1986).

### *Packaging*

After sorting, the beans are tied into bundles, usually 70–130, weighing between 150 and 500 g (Heath and Reineccius, 1986). These are then packed into cardboard or tin boxes lined with waxed paper. The beans are now ready for shipment.

## **29.4 Main products and functional properties of vanilla**

There is probably no other spice material or aromatic plant comparable to vanilla in terms of wide scope of application. The use of vanilla is generally grouped into three: as a ubiquitous flavouring material, as a critical intermediary in a host of pharmaceutical products and as a subtle component of perfumes. As a flavouring agent, vanilla is a popular and most preferred ingredient in the preparation of ice cream, milk, beverages, candies, confectioneries and various bakery items. In the pharmaceutical and chemical industries, vanillin serves as an important intermediate in the manufacture of: L-dopa (the anti-Parkinsonian drug), methyl dopa (a compound with anti-hypertensive and tranquillizing properties), papaverine (treatment of heart problem), trimethoprim (anti-bacterial agent), hydrazones (2,4-D-like herbicide) and antifoaming agent (in lubricating oils) (Rosenbaum, 1974; Hocking, 1997).

Vanilla in perfumery was initially incorporated to complement the scent provided by tonka extract. It became a perfume ingredient to reckon with when François Coty, who is often regarded as the first of the great perfumers of modern times, used it in 'L'Aimant' (Groom, 1992). Vanilla subsequently became the principal note of about 23 % of all quality perfumes, e.g. 'Amouge', 'Bois de Isles', 'Jicky', 'Habanita'.

The cured beans are further processed to produce the various vanilla products. This is commonly accomplished in the importing countries. The different products developed from vanilla are described below.

### **29.4.1 Vanilla extract**

The major product derived from cured vanilla is an alcoholic essence, which is commercially known as vanilla extract. The vanilla flavour is obtained through solvent extraction with the use of the best grade of ethanol. Generally, the basic process in the preparation of vanilla extract involves (i) the reduction of the bean size using a comminuting machine and (ii) the subsequent alcohol extraction of the macerated

**Table 29.3** Nutrient composition of vanilla extract (with 34.4 % ethyl alcohol)

| Nutrient                               | Value per 100 g of edible portion |
|----------------------------------------|-----------------------------------|
| <b>Proximates</b>                      |                                   |
| Water (g)                              | 52.58                             |
| Energy (kcal)                          | 288                               |
| Protein (g)                            | 0.06                              |
| Total lipid (fat) (g)                  | 0.06                              |
| Ash (g)                                | 0.26                              |
| Carbohydrate, by difference (g)        | 12.65                             |
| <b>Minerals</b>                        |                                   |
| Ca (mg)                                | 11                                |
| Fe (mg)                                | 0.12                              |
| Mg (mg)                                | 12                                |
| P (mg)                                 | 6                                 |
| K (mg)                                 | 148                               |
| Na (mg)                                | 9                                 |
| Zn (mg)                                | 0.11                              |
| Cu (mg)                                | 0.072                             |
| Mn (mg)                                | 0.230                             |
| <b>Vitamins</b>                        |                                   |
| Thiamin (mg)                           | 0.011                             |
| Riboflavin (mg)                        | 0.095                             |
| Niacin (mg)                            | 0.425                             |
| Panthothenic acid (mg)                 | 0.035                             |
| Vitamin B <sub>6</sub> (mg)            | 0.026                             |
| <b>Lipids</b>                          |                                   |
| Fatty acids, total saturated (g)       | 0.010                             |
| Fatty acids, monounsaturated (g)       | 0.010                             |
| Fatty acids, total polyunsaturated (g) | 0.004                             |

*Source:* USDA National Nutrient Database for Standard Reference (2002). [www.nal.usda.gov](http://www.nal.usda.gov).

beans through a series of percolation techniques (Arana, 1945; Heath and Reinecius, 1986). For best results Merory (1956, 1960) recommends the following protocol. Three consecutive extractions are done with varying amounts of the menstruum – a maximum of 65 % ethanol for the first extraction, 35 % for the second and about 15 % for the third. Each of these takes place for a minimum of 5 days. Extraction is done in a continuous slow flow, the percolate collected in fractions and later blended to yield the final product. The extract is filtered or centrifuged and the alcohol content is adjusted to meet market specifications. Vanilla extract is then stored in stainless steel or glass containers. If the extract is aged for a period of about 3–6 months, the delicate and subtle aroma for which vanilla is famous is fully realized.

The nutrient composition of a typical vanilla extract is listed in Table 29.3. Several factors influence the quality of vanilla extract. These include: (i) method of curing,

(ii) blending of different quality beans, (iii) degree of maceration, (iv) method of extraction, (v) level of alcohol in the menstruum and (vi) appropriate period of ageing (Broderick, 1955b; Merory, 1960; Heath and Reineccius, 1986).

#### 29.4.2 Vanilla oleoresin

Vanilla oleoresin is a dark brown, semi-fluid extract produced from solvent extraction of macerated beans. It differs from vanilla extract in that the solvent used is completely removed by evaporation under vacuum and the finer top-notes of the vanilla aroma are lost or modified by heat treatment (Heath and Reineccius, 1986). Vanilla oleoresin can also be obtained using CO<sub>2</sub> under supercritical conditions, producing products considerably superior to those obtained by conventional extraction with organic solvents (Schuetz *et al.*, 1984). Yield of oleoresin is from 29.9–64.8 % of bean dry weight (Cowley, 1973).

#### 29.4.3 Vanilla sugar

Also known as powdered vanilla, vanilla sugar is prepared by mixing ground cured beans or their oleoresin with sugar (Arana, 1945). Minimum sugar content is 30 % (Heath and Reineccius, 1986).

#### 29.4.4 Vanilla absolute

Preferred in perfumery products, absolute vanilla is obtained by selective solvent extraction, using initially a non-polar solvent such as benzene followed by a polar solvent such as ethanol (Heath and Reineccius, 1986).

#### 29.4.5 Functional properties

Vanillin exhibits antimicrobial activity against bacteria, moulds and yeasts. The list of these microorganisms and their corresponding minimal inhibitory concentration to vanillin is presented in Table 29.4.

The potential medical importance of vanillin is suggested by the following studies. Vanillin has been found to possess antimutagenic effects in mice (Imanishi *et al.*, 1990), bacteria (Ohta *et al.*, 1988; Shaughnessy *et al.*, 2006) and human cells (King *et al.*, 2007). In yeast, however, it is shown to be co-mutagenic and co-recombinogenic (Fahrig, 1996). Vanillin offers protection against x-ray and UV radiation-induced chromosomal change in V79 Chinese hamster lung cells (Keshava *et al.*, 1998). More recently, vanillin showed potent radioactive effects to DNA under *in vitro*, *ex vivo* and *in vivo* conditions (Maurya *et al.*, 2007). Vanillin also possesses antimetastatic potential by decreasing invasiveness of cancer cells in mice (Lirpramongkol *et al.*, 2005). As a potent anti-invasive agent, vanillin suppresses the matrix metalloproteinase-9 enzymatic activity via NF-κB signalling pathway (Liang *et al.*, 2009). The hypotriglyceridemic (Srinivasan *et al.*, 2008) and anti-inflammatory (Wu *et al.*, 2009) effects of vanillin in rats were also reported. Recently, ethyl vanillin, another major phenolic constituent of vanilla, showed anti-angiogenic, anti-inflammatory and antinociceptive properties (Jung *et al.*, 2010).

**Table 29.4** Susceptibility (expressed as minimal inhibitory concentration, MIC) of various microorganisms to vanillin

| Microorganism                                                                                                                       | MIC           | Media and culture conditions                                                    | Reference                               |
|-------------------------------------------------------------------------------------------------------------------------------------|---------------|---------------------------------------------------------------------------------|-----------------------------------------|
| <i>Aspergillus flavus</i><br><i>A. niger</i><br><i>A. ochraceus</i><br><i>A. parasiticus</i>                                        | 1000–2000 ppm | Fruit-based agars (apple, banana, mango, papaya, pineapple);<br>pH 3.5;<br>25°C | Lopez-Malo <i>et al.</i> (1995)         |
| <i>Aspergillus flavus</i><br><i>A. niger</i><br><i>A. ochraceus</i><br><i>A. parasiticus</i>                                        | 350–1200 ppm  | Potato dextrose agar;<br>pH 3.0–4.0;<br>10–30°C temp.                           | Lopez-Malo <i>et al.</i> (1997)         |
| <i>A. flavus</i>                                                                                                                    | 1300 ppm      | Potato dextrose agar;<br>pH 3.5–4.5;<br>25°C temp.                              | Lopez-Malo <i>et al.</i> (2002), (2005) |
| <i>Saccharomyces cerevisiae</i>                                                                                                     | 21 mM         | YEPD;                                                                           | Fitzgerald <i>et al.</i> (2003)         |
| <i>Zygosaccharomyces baillii</i>                                                                                                    | 20 mM         | pH 4.0;                                                                         |                                         |
| <i>Z. rouxii</i>                                                                                                                    | 13 mM         | 25°C temp.                                                                      |                                         |
| <i>Saccharomyces cerevisiae</i>                                                                                                     | 20 mM         | Apple juice (pH 3.5) and<br>Peach-flavoured soft drink (pH 3.1);<br>25°C temp.  | Fitzgerald <i>et al.</i> (2004)         |
| <i>Candida parapsilosis</i>                                                                                                         | 10 mM         |                                                                                 |                                         |
| <i>S. cerevisiae</i>                                                                                                                | 5 mM          | 8°C temp.                                                                       | Corte <i>et al.</i> (2004)              |
| <i>C. parapsilosis</i>                                                                                                              | 1 mM          |                                                                                 |                                         |
| <i>Listeria innocua</i>                                                                                                             | 3000 ppm      | Apple juice;<br>pH 3.3;<br>30°C temp.                                           |                                         |
| Spoilage microbes                                                                                                                   | 1000–2000 ppm | Wild blueberry yogurt;<br>4°C temp.                                             | Penney <i>et al.</i> (2004)             |
| <i>Pantoea agglomerans</i><br><i>Aeromonas enteropelogenes</i><br><i>Micro-coccus lylae</i><br><i>Sphingobacterium spiritovorun</i> | 10–13.3 mM    | Fresh-cut mango slices;<br>pH 5.0;<br>5°C and 10°C temp.                        | Ngarmsak <i>et al.</i> (2006)           |
| <i>Alternaria</i> sp.<br><i>Aspergillus</i> sp.<br><i>Penicillium</i> sp.<br><i>Fusarium</i> sp.                                    | 12.5–13.3 mM  |                                                                                 |                                         |
| Unidentified yeasts                                                                                                                 | 5–6.7 mM      |                                                                                 |                                         |
| <i>Listeria monocytogenes</i><br><i>Escherichia coli</i> 0157:H7                                                                    | 40 mM         | Apple juice;<br>pH 3.5 – 4.5;<br>4°C and 15°C temp.                             | Moon <i>et al.</i> (2006)               |



**Table 29.4** Susceptibility (expressed as minimal inhibitory concentration, MIC) of various microorganisms to vanillin—cont'd

| Microorganism                                                                                                                                                                                                                                                                                | MIC          | Media and culture conditions                                        | Reference                       |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|---------------------------------------------------------------------|---------------------------------|
| <i>Escherichia coli</i><br><i>Pseudomonas aeruginosa</i><br><i>Enterobacter aerogenes</i><br><i>Salmonella enterica</i> subsp.<br><i>enterica</i> serovar Newport<br><i>Candida albicans</i><br><i>Lactobacillus casei</i><br><i>Penicillium expansum</i><br><i>Saccharomyces cerevisiae</i> | 6–18 mM      | Fresh-cut apples;<br>4°C temp.                                      | Rupasinghe <i>et al.</i> (2006) |
| <i>Listeria innocua</i>                                                                                                                                                                                                                                                                      | 500–1100 ppm | Thermally-processed orange juice;<br>pH 3.5 ± 0.1;<br>57–61°C temp. | Char <i>et al.</i> (2009)       |
| <i>Listeria innocua</i>                                                                                                                                                                                                                                                                      | 900–1100 ppm | Orange juice;<br>pH 3.5 ± 0.1;<br>52°C and 57°C temp.               | Char <i>et al.</i> (2010)       |
| <i>Listeria monocytogenes</i>                                                                                                                                                                                                                                                                | 2800 ppm     | Tripticase soy broth;<br>35°C temp.                                 | Cava-Roda <i>et al.</i> (2010)  |
| <i>Escherichia coli</i> 0157:H7                                                                                                                                                                                                                                                              | 2400 ppm     | pH 6                                                                |                                 |
| <i>L. monocytogenes</i>                                                                                                                                                                                                                                                                      | 3000 ppm     | Tripticase soy broth;<br>35°C temp.<br>pH 7                         |                                 |
| <i>E. coli</i> 0157:H7                                                                                                                                                                                                                                                                       | 2800 ppm     |                                                                     |                                 |
| <i>L. monocytogenes</i>                                                                                                                                                                                                                                                                      | 2500 ppm     | Semi-skim milk<br>7°C                                               |                                 |
| <i>E. coli</i> 0157:H7                                                                                                                                                                                                                                                                       | 1000 ppm     |                                                                     |                                 |
| <i>L. monocytogenes</i>                                                                                                                                                                                                                                                                      | 4000 ppm     | Semi-skim milk<br>35°C                                              |                                 |
| <i>E. coli</i> 0157:H7                                                                                                                                                                                                                                                                       | 3000 ppm     |                                                                     |                                 |

Vanillin also functions as an antioxidant. At concentrations normally added to food preparations, it offers significant protection against protein oxidation and lipid peroxidation induced by photosensitization in rat liver mitochondria (Kamat *et al.*, 2000). This study shows the potential of using this popular flavouring chemical to inhibit oxidative damage to membranes in mammalian tissues. Mahal *et al.* (2001) reported the radical-scavenging properties of vanillin. In another study involving thermal treatment of vanillin, Mourtzinis *et al.* (2009) proved that heat-treated vanillin was more effective as a food constituent in terms of radical-scavenging and antimicrobial activities due to the conversion of vanillin to vanillic acid during heating. Most recently, addition of 0.4 % vanilla extract gave higher antioxidant activity and lowered the peroxide value in biscuits (Anuradha *et al.*, 2010). Aside from vanillin, other components of vanilla extract show potential as antioxidants. For instance, 4-hydroxy-3-methoxybenzyl alcohol and 4-hydroxybenzyl alcohol exhibited antioxidant activity of 65 % and 45 % by  $\beta$ -carotene-linoleate method and 90 % and 50 % by DPPH method, respectively (Shyamala *et al.*, 2007).

Tai *et al.* (2010) found inconsistencies in the results of studies on the antioxidant activity of vanillin. To resolve the issue, multiple assays such as DPPH, galvinoxyl radical, ABTS<sup>+</sup>, ORAC and oxidative haemolysis inhibition were used in evaluating the antioxidant activity of vanillin.

Sun *et al.* (2001) reported the bioactivity of five aromatic compounds extracted from the leaves and stems of *V. fragrans* against mosquito (*Culex pipiens*) larvae. Among the isolated compounds, 4-butoxymethylphenol was the most toxic, exhibiting 100 % mortality at 0.2 mg ml<sup>-1</sup> within only 3 h of treatment. This was followed by 4-ethoxymethylphenol, which was also the most abundant component. Vanillin, when given at 2 mg ml<sup>-1</sup> for 10 h exhibited more than 90 % mortality. The least toxic of the phenolic derivatives was 3,4-dihydroxyphenylacetic acid. This compound, together with 4-hydroxy-2-methoxycinnamaldehyde, was isolated from vanilla for the first time. 4-Butoxymethylphenol has not been reported to occur in natural form.

## 29.5 Quality issues and adulteration

The quality of cured vanilla beans is the result of confluent factors that run the whole gamut of raw material production to curing. The agro-climatic conditions during cultivation, coupled with various degrees of sophistication or non-sophistication of methods employed in the preparation of harvested beans, can make the difference in meeting market standards. Physical attributes such as those enumerated in Section 29.3.1 provide the initial criteria by which to judge the cured bean and assign it to a particular grade. The quality of vanilla extract can be determined through chemical analysis, and Winton's analytical values have been employed in this regard (Merory, 1960). The concentration of vanillin is an important criterion, although organoleptic quality does not entirely depend on it. Various flavour notes, described as characteristically woody, prune, resinous, leathery, floral and fruity aromatics, also need to be considered (Gillette and Hoffman, 1992). Bourbon vanilla serves as the standard by which to measure the chemical and sensory quality of other types of vanilla. Imitation vanilla extract spiked with vanillin is less desirable than the natural extract because the critical flavour notes are wanting (Fig. 29.9). Extracts of Indonesian beans are not only low in vanillin but also possess a smoky/tobacco flavour and aroma (Fig. 29.10), while Mexican beans yield extracts with woody and smoky/tobacco notes (Fig. 29.11). In the USA, the Food and Drug Administration explicitly provides standard specifications for vanilla products. As an example, the amount of bean present with a given moisture content and level of alcohol in the mixture define vanilla extract quality (Table 29.5). It should be emphasized here that no amount of modern technology of processing can improve the quality of an already poor bean at harvest. Improper curing and handling, on the other hand, is sure to lead to quality deterioration of vanilla produced under excellent cultivation practices.

### 29.5.1 Substitutes, adulterants and additives

#### *Plant material*

Correll (1953) identified the following plants that have been used as raw material substitute or adulterant for vanilla. The most commonly employed is the fruit of

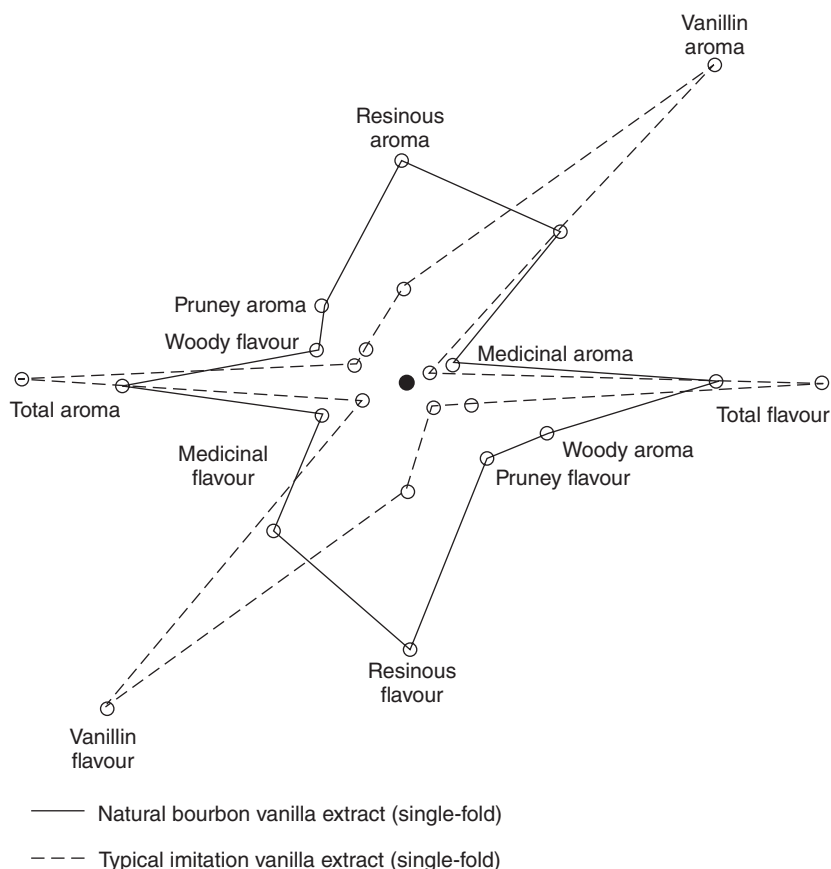


**Fig. 29.9** Aroma and flavour sensory profiles of natural and imitation vanilla extracts. (Source: Gillette M. H. and Hoffman P. G. 1992. 'Vanilla extract', in Hui Y. H. (ed.), *Encyclopedia of Food Science and Technology*, Vol 4, New York, John Wiley and Sons, Inc. Reprinted with permission of John Wiley & Sons, Inc.)

tonka or snuff bean (*Dipteryx odorata* (Aubl.) Willd.), a leguminous plant native to northern South America and Trinidad. Other plants include: another species of tonka (*D. oppositifolia* Willd.); vanillin (*V. pompona*), a less common wild vanilla; the long cylindrical pods of little vanilla (*Selenipedium chica* Reichb. f.); leaves of the orchid *Angraecum fragrans* Thou. and *Orchis fusca* Jaq.; ladies' tresses (*Spiranthes cernua* (L.) L.C. Rich var. *odorata* (Nutt.) Correll); 'vanilla-plant' (*Trilisa odoratissima* (Walt.) Cass.); little orchid 'herb vanilla' (*Nigritella angustifolia* Rich.) and common sweet clovers (*Melilotus* spp.).

#### Chemical substances

Because of high demand, limited supply of quality beans and increased cost of production, natural vanilla extracts have been most often adulterated with inexpensive, synthetic vanillin. This fraudulent alternative becomes very attractive in light of the expected profitable return: 1 kg of Madagascar beans yields about 8.4 l vanilla extract, while 1 kg of synthetic vanillin, combined with other botanical extracts,

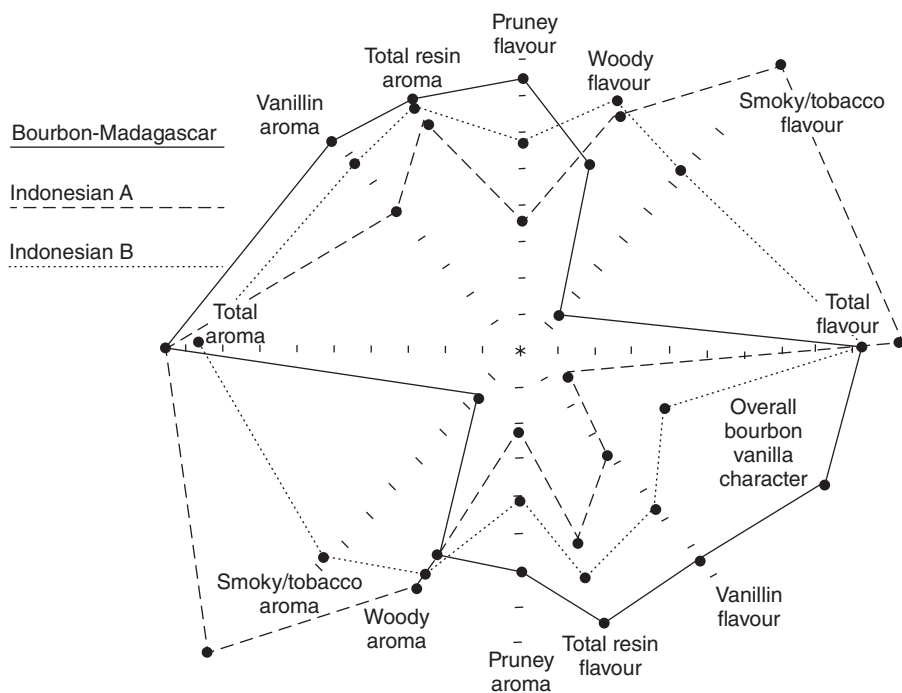


**Fig. 29.10** Aroma and flavour sensory profiles of Bourbon and Indonesian pure vanilla extracts. (Source: Gillette M. H. and Hoffman P. G. 1992. 'Vanilla extract', in Hui Y. H. (ed.), *Encyclopedia of Food Science and Technology*, Vol 4, New York, John Wiley and Sons, Inc. Reprinted with permission of John Wiley & Sons, Inc.)

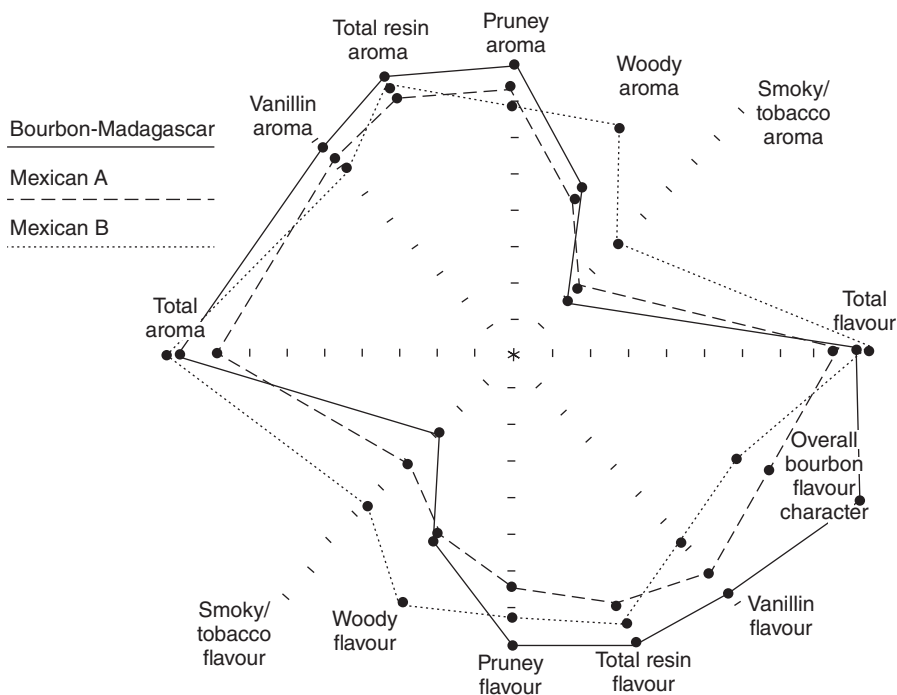


**Fig. 29.11** Aroma and flavour sensory profiles of Bourbon and Mexican pure vanilla extracts. (Source: Gillette M. H. and Hoffman P. G. 1992. 'Vanilla extract', in Hui Y. H. (ed.), *Encyclopedia of Food Science and Technology*, Vol 4, New York, John Wiley and Sons, Inc. Reprinted with permission of John Wiley & Sons, Inc.)

**Table 29.5** US standard specifications for vanilla and vanilla extract

## 21CFR169.3

## Sec. 169.3 Definitions

- a) The term vanilla beans means the properly cured and dried pods of *Vanilla planifolia* Andrews and of *Vanilla tahitensis* Moore.
- b) The term unit weight of beans means, in the case of vanilla beans containing not more than 25 percent moisture, 13.35 ounces of such beans; and, in the case of vanilla beans containing more than 25 percent moisture, it means the weight of such beans equivalent in content of moisture-free vanilla-bean solids to 13.35 ounces of vanilla beans containing 25 percent moisture.
- c) The term unit of vanilla constituent means the total sapid and odorous principles extractable from one unit weight of vanilla beans, as defined in paragraph (b) of this section, by an aqueous alcohol solution in which the content of ethyl alcohol by volume amounts to not less than 35 percent.

## 21CFR169.175

## Sec. 169.175 Vanilla extract

- a) Vanilla extract is the solution in aqueous ethyl alcohol of the sapid and odorous principles extractable from vanilla beans. In vanilla extract the content of ethyl alcohol is not less than 35 percent by volume and the content of vanilla constituent, as defined in Sec. 169.3 (c), is not less than one unit per gallon. The vanilla constituent may be extracted directly from vanilla beans or it may be added in the form of concentrated extract or concentrated vanilla flavoring or vanilla flavoring concentrated to the semi-solid form called vanilla oleo-resin. Vanilla extract may contain one or more of the following optional ingredients:
  - (1) Glycerin
  - (2) Propylene glycol
  - (3) Sugar (including invert sugar)
  - (4) Dextrose
  - (5) Corn sirup (including dried corn sirup)

Source: FDA (2002).

produces about 499 l artificial vanilla flavouring, retailing for over 50 times as much as the natural product (Breedlove, 1988)! Synthetic vanillin is chemically produced via different routes with eugenol, guaiacol, safrole or lignin as the starting compound. A detailed discussion of these synthetic pathways is provided by Faith *et al.* (1957), Diddams and Krum (1970), Rosenbaum (1974) and Hocking (1997). Another most common additive is ethyl vanillin, 3-ethoxy-4-hydroxybenzaldehyde. It is synthetically produced from safrole and is about three to four times more powerful as a flavouring agent than vanillin (Heath and Reineccius, 1986). A maximum of 10 % of vanillin is replaceable by this compound without obvious objectionable note. Other compounds utilized are: veratraldehyde (methyl vanillin), piperonal (heliotropin), vanitrope (propenyl guaethol) and coumarin (Kahan and Fitelson, 1964). Coumarin, because of its high level of toxicity, has been banned for use in food in the USA (Rosengarten, 1969).

### 29.5.2 Detection of adulteration

Some of the techniques utilized to test the purity of vanilla extracts are the determination of lead number, which is directly related to the quantity of organic acids (AOAC, 1975), paper chromatography (Jorysch, 1959; Stahl *et al.*, 1960; Fitelson,

1960a, b, 1963, 1964; Kahan and Fitelson, 1964), gradient elution method using an ion exchange resin (Sullivan *et al.*, 1960; Fitelson, 1964) and thin-layer chromatography (Kahan and Fitelson, 1964). These methods, however, are subject to low sensitivity, high experimental error, lack of reproducibility and, most importantly, are too laborious and time-consuming for normal routine work. Ensminger (1953, 1956) and Feeny (1964) reported the application of a UV photometric procedure to reveal the type of flavouring constituents in vanilla extract; this method, however, does not differentiate vanillin from ethyl vanillin without preliminary separation. Gas-liquid chromatography (GLC) essentially eliminates this problem, aside from revealing the presence of other types of contaminants (Martin *et al.*, 1964, 1975; Johansen, 1965). Determination of the ratio of vanillin levels to the concentration of the nutrients potassium, phosphorus and nitrogen provides the so-called 'identification ratios', which not only separate authentic vanilla extracts from their imitation but also give indication of the strength of the vanilla extract (Martin *et al.*, 1975). Relatively higher identification ratios provide strong evidence for adulteration. The technique suffers from a need to input a significant amount of time and the regular analysis of the ratios for authentic standards. More sensitive assays for adulteration are accomplished by the application of reverse phase liquid chromatography (Guarino and Brown, 1985) and HPLC (Herrmann and Stockli, 1982; Archer, 1989).

A recent technique involves the application of the stable isotope ratio analysis mass spectrometry (SIRA-MS), which has been employed in deciphering the original source material for flavouring food products (Cordella *et al.*, 2002). The method measures and compares the ratio of abundances of stable isotopes such as carbon ( $^{13}\text{C}/^{12}\text{C}$ ) between natural and synthetic vanillin. In principle, the isotopic fractionation of carbon through photosynthesis has been shown to depend upon the type of  $\text{CO}_2$  assimilation in the plant. Plants that follow the Calvin cycle discriminate the most against  $^{13}\text{C}$ , while those that follow the Hatch-Slack cycle discriminate the least. In between are those characteristic of the Crassulacean acid metabolism (CAM) pathway, which is exhibited in vanilla (Osmond *et al.*, 1973). Vanillin extracted from vanilla beans has been shown to be enriched in  $^{13}\text{C}$  compared with synthetic vanillin produced from lignin, eugenol and guaiacol (Bricout *et al.*, 1974; Hoffman and Salb, 1979; Martin *et al.*, 1981; Culp and Noakes, 1992).

The SIRA method for carbon was further refined by Krueger and Krueger (1983, 1985) to detect synthetic vanillin whose isotopic compositions have been adjusted to resemble natural vanillin. Aside from carbon, the stable isotope of hydrogen (deuterium/hydrogen) has also been examined and found useful in discriminating between authentic and fraudulent samples of vanilla extract. Natural vanillin is more depleted in deuterium content compared with its synthetic counterpart (Culp and Noakes, 1992). Further refinement of the technique to detect isotopically manipulated vanilla is reported by Remaud *et al.* (1997) with the application of site-specific natural isotopic fractionation analysed by nuclear magnetic resonance (SNIF-NMR). This method is applied not only to vanillin but also to *p*-hydroxybenzaldehyde.

For a compilation of the official methods of analysis of vanilla extract by the Association of Official Analytical Chemists (AOAC), the reader is referred to the work of Krueger (1995).

## 29.6 Conservation and alternative methods for natural vanillin production

### 29.6.1 Biodiversity and genetic conservation

The need to conserve vanilla cannot be over-emphasized: many of its species are rare and endangered, the primary gene pool is limited and there is a threat of genetic erosion due to habitat destruction and over-collection (Minoo *et al.*, 2006a; Grisoni *et al.*, 2007). A comprehensive review of the current status of biodiversity and preservation of vanilla focusing on its taxonomy and phylogeny, reproductive biology and preservation of genetic resources is provided by Bory *et al.* (2008a). This paper also cites existing germplasm collection sites and programmes worldwide such as those found in the Centro Agronomico Tropical de Investigacion y Enseñanza of Turrialba, Costa Rica; the Indian Institute of Spices Research in Calicut, India; Establissement Vanille de Tahiti in French Polynesia; Secretary of the Pacific Community in Fiji and the International Cooperation Centre in Agronomic Research for Development (CIRAD) in Reunion Island. Long term *in vitro* conservation of vanilla using synthetic seed technology and application of osmoticum to slow down growth has also been successfully reported (Minoo *et al.*, 2006a).

Recent researches on the elucidation of genetic variation and patterns of introduction and dispersion of vanilla have utilized molecular tools such as the use of randomly amplified polymorphic DNA (RAPD) (Besse *et al.*, 2004; Minoo *et al.*, 2006b, 2007; Schlüter *et al.*, 2007) and amplified fragment length polymorphism (AFLP) profiles (Minoo *et al.*, 2006b; Bory *et al.*, 2008b; Lubinsky *et al.*, 2008). These undertakings point to the narrow genetic variation in vanilla. More recently, Bouetard and co-workers (2010) provided evidence for the transoceanic dispersion of vanilla using plastid DNA phylogenetic analysis. There is limited current research work on enhancing genetic variation in vanilla (Minoo *et al.*, 2007, 2008). The narrow genetic base of vanilla serves as a stumbling block in the development of vanilla plants not only with high yield and more diverse aromatic and flavour composition but also resistant to critical diseases that could wipe out the commonly vegetatively propagated plantations.

### 29.6.2 Improving production of natural vanillin

The vanilla flavouring market is dominated by synthetic vanillin: it makes up more than 90 % of the US market and 50 % of the French market (the lowest national share) (RAP Market Information Bulletin, 2002). The reason for such a huge market share is two-fold. First, considering flavour strength, 100 g of synthetic vanillin approximately yields the same flavouring power as 13 l of natural vanilla extract. Second, the price of synthetic vanillin is only about one-hundredth of vanillin derived from cured beans. The high cost of the natural flavouring extract has been attributed to the exacting requirements of the plant and the finished product in relation to agro-climatic conditions, cultivation, pollination, harvesting and curing. Ramachandra Rao and Ravishankar (2000) estimated that, to produce 1 kg of vanillin, 500 kg of beans (based on 20 g kg<sup>-1</sup> vanillin yield, dry weight basis) derived from about 40000 pollinated flowers will be needed.

Within the context of food legislation, vanillin chemically synthesized from lignin, guaiacol, safrole or eugenol is labelled as synthetic vanillin, even if the precursor



input is from a natural source (e.g. eugenol, safrole) (Rabenhorst and Hopp, 2000). Products sold as natural vanillin in the international market command a higher premium because of the presence of other flavour components contributing to the overall organoleptic quality of the material. Vanillin production through chemical synthesis suffers from the following disadvantages: presence of impurities/contaminants, large consumption of chemical products and energy, production of ecologically toxic waste products (as in the case of lignin degradation), and production of isomers which are difficult to separate (e.g. isovanillin/vanillin from safrole) (Hocking, 1997; Mane and Zucca, 1998). Inevitably, alternative methods have been sought and tested to try to eliminate the above constraints.

#### *Tissue culture technique*

The potential of organ and cell culture to produce natural vanilla flavouring components outside the cured bean has been explored. Using ferulic acid as a precursor, Westcott *et al.* (1994) employed vanilla plant aerial roots, cultured in a charcoal medium, as biocatalysts in the production of vanillin. The charcoal acts as reservoir for the root-synthesized vanillin, which is removed by selective solvent extraction. Vanillin production using this technique is five to ten times faster than the usual rate in vanilla beans, with productivities of up to 400 mg kg<sup>-1</sup> dw tissue day<sup>-1</sup>, about 40 % of the productivity in mature vanilla beans. Initial efforts to produce vanillin from plant cell culture have been unsuccessful. Feeding of precursors and inhibitors of the phenylpropanoid metabolic pathways to undifferentiated suspension cells derived from green vanilla beans induced the formation of 4-hydroxybenzoic acid, vanillic acid and syringic acid but not vanillin (Funk and Brodelius, 1990a,b,c). Under appropriate, controlled conditions, callus suspensions derived from root tips and leaf primordia of vanilla have been claimed to induce the production not only of vanillin but also of 3,4-dihydroxybenzaldehyde, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde and vanillic acid (Knuth and Sahai, 1991). Havkin-Frenkel *et al.* (1996) found that, in shoot-derived cluster culture of vanilla (suspensions growing in clumps), light was an important factor in the accumulation of some of the flavour chemicals, particularly vanillyl alcohol. Davidonis and co-workers (1996) also showed that the synthesis of vanillin from calluses, which proliferated from shoot tips of vanilla, is enhanced when ferulic acid is topically applied to them. Thus, even an undifferentiated mass of cells is capable of metabolic synthesis of vanillin.

#### *Microbial biocatalytic transformation*

Microbial catalysis of naturally occurring and abundant substrates offers another interesting alternative to the production of natural vanillin. Hagedorn and Kaphammer (1994) reviewed work on the microbial transformation of natural flavour precursors to vanillin. The list of some microorganisms and the specific substrates utilized for the microbial production of natural vanillin is presented in Table 29.6.

The basic protocol for microbial vanillin formation is fairly straightforward (Labuda *et al.*, 1992; Gross *et al.*, 1993; Rabenhorst and Hopp, 2000; Muheim *et al.*, 2001). In general, the selected microbe, either bacterium, yeast or fungus, is initially grown in a culture medium, commonly supplemented with carbon and nitrogen sources, inorganic salts and, in some cases, trace elements and vitamins to optimize

**Table 29.6** Some microorganisms utilized in the bioconversion of selected substrates to vanillin

| Microorganism                                         | Substrate                                      | Reference                       |
|-------------------------------------------------------|------------------------------------------------|---------------------------------|
| <i>Aspergillus niger</i> ATCC11414                    | Coniferyl alcohol,                             | Labuda <i>et al.</i> (1992)     |
| <i>Corynebacterium glutamicum</i> ATCC 13032          | Eugenol, ferulic Acid, 4-vinylguaiaicol        |                                 |
| <i>Pseudomonas putida</i> ATCC 55180                  |                                                |                                 |
| <i>Rhodotorula glutinis</i> ATCC 74056                |                                                |                                 |
| <i>Pseudomonas</i> sp. TK-2101                        | Eugenol                                        | Washisu <i>et al.</i> (1993)    |
| <i>Pycnopus cinnabarinus</i> CNCM No. I-937 and I-938 | Ferulic acid, vanillic acid                    | Gross <i>et al.</i> (1993)      |
| <i>Pseudomonas fluorescens</i> AN103                  | Ferulic acid                                   | Narbad and Gasson (1998)        |
| <i>Streptomyces setonii</i> ATCC 391169               | Ferulic acid                                   | Muheim and Lerch (1999)         |
| <i>Pycnopus cinnabarinus</i> MUCL 39533               | Ferulic acid                                   | Oddou <i>et al.</i> (1999)      |
| <i>Amycolatopsis</i> sp. DSM 9991 and 9992            | Ferulic acid                                   | Rabenhorst and Hopp (2000)      |
| <i>Nocardia</i> sp. NRRL 5646                         | Vanillic acid                                  | Li and Rosazza (2000)           |
| <i>Bacillus subtilis</i> sp.                          | Isoeugenol                                     | Shimoni <i>et al.</i> (2000)    |
| <i>Streptomyces setonii</i> ATCC 39116                | Ferulic acid                                   | Muheim <i>et al.</i> (2001)     |
| <i>Escherichia coli</i> JM 109(pBB1)                  | Ferulate                                       | Converti <i>et al.</i> (2003)   |
| <i>Bacillus fusiformis</i> CGMCC 1347                 | Isoeugenol                                     | Zhao <i>et al.</i> (2005, 2006) |
| <i>Oenococcus oeni</i> ICEB 8413                      | Ferulic acid                                   | Bloem <i>et al.</i> (2007)      |
| <i>Pseudomonas chlororaphis</i> CDAE5                 | Isoeugenol                                     | Kasana <i>et al.</i> (2007)     |
| <i>Aspergillus niger</i> CGMCC 0774                   | Rice bran oil                                  | Zheng <i>et al.</i> (2007)      |
| <i>Panerochaete chrysosporium</i>                     | Green coconut husk in solid state fermentation | Barbosa <i>et al.</i> (2008)    |
| <i>Schizosaccharomyces pombe</i>                      | glucose                                        | Hansen <i>et al.</i> (2009)     |
| <i>Saccharomyces cerevisiae</i>                       |                                                |                                 |
| <i>Escherichia coli</i> JM 109/pBB1                   | Corn cob alkaline hydrolyzate                  | Torres <i>et al.</i> (2009)     |
| <i>Staphylococcus aureus</i>                          | Ferulic acid                                   | Sarangi <i>et al.</i> (2010)    |

growth. When the desired biomass is attained, the appropriate substrate is added, and the loss of the precursor and the corresponding appearance of vanillin is regularly monitored using HPLC. Once the optimum level of vanillin is formed, it is isolated using distillation, solvent extraction or chromatography. Vanillin can then be further purified using standard recrystallization techniques.

Vanillin yield from microbial transformation varies depending upon the adopted protocol, starting substrate used and the microorganism employed. Using a 10 l fermenter, the amount of vanillin synthesized from ferulic acid by *Amycolatopsis* is 11.5 g l<sup>-1</sup> or 78 % of theory based on converted precursor (Rabenhorst and Hopp, 2000). With *Pycnopus*, molar yield conversion of vanillic acid to vanillin is 35.1 %; much lower conversion is obtained from ferulic acid: 20.5 % (Gross *et al.*, 1993).

#### *Use of isolated and purified enzymes*

Enzymes isolated and purified from microorganisms or of plant or animal origin have also been employed to catalyse the biological conversion of starting chemical compounds to vanillin. Examples of these *in vitro* vanillin production include: dioxygenase-active enzyme from *Pseudomonas* TMY 1009 strain using a styrene derivative as substrate (Yoshimoto *et al.*, 1990); lipoxygenase, naturally present in

soya bean, wheat or beet and also in pig, calf or fish liver, acting on isoeugenol (Mane and Zucca, 1998); lignin peroxidase from the white-rot fungus *Bjerkandera* sp. BOS55 converting the *o*-acetyl ester of isoeugenol into vanillyl acetate, which can then be cleaved to vanillin (Ten Have *et al.*, 1998a,b); carboxylic acid reductase, taken from *Nocardia* sp. strain NRRL 5646, biotransforming vanillic acid to vanillin (Li and Rosazza, 2000); flavoprotein vanillyl-alcohol oxidase (VAO) from *Penicillium simplicissimum* using creosol or vanillylamine as precursor (van den Heuvel *et al.*, 2001), and acylase from *Streptomyces mobaraensis* DSM 40847 that efficiently hydrolyzes capsaicin (Di Matteo, 2009).

#### *Application of genetic engineering*

The prospect of utilizing the tools of genetic engineering for the production of vanillin has also been explored. Research undertakings related to this novel approach have been reviewed by Walton *et al.* (2000) and by Ramachandra Rao and Ravishankar (2000).

Genes of microbial origin and encoding for certain enzymes have been inserted into plant cells for the purpose of modifying the biocatalytic pathway of the transgenic plant in favour of initiation or enhanced vanillin production and other related flavour metabolites. Consider, for example, the gene isolated and characterized from *Pseudomonas fluorescens* biovar V strain AN103, which utilizes ferulic acid as a sole carbon source. It encodes for an enzyme that converts feruloyl-S-CoA to vanillin and acetyl-S-CoA, which has been confirmed by heterologous expression in *Escherichia coli* (Gasson *et al.*, 1998). Subsequent study showed that the enzyme is not only active with the substrate feruloyl-S-CoA but also with 4-coumaroyl-CoA and caffeoyl-CoA and has been identified as 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL) (Mitra *et al.*, 1999). When the *HCHL* gene is expressed in tobacco plants, there is a massive accumulation of glucosides and glucose esters of 4-hydroxybenzoic acids and vanillic acid and the glucosides of hydroxybenzyl alcohol and vanillyl alcohol (Mayer *et al.*, 2001). In hairy root cultures of *Datura stramonium* L. transformed with *Agrobacterium rhizogenes*, the introduced *HCHL* gene similarly encodes most of the previously mentioned compounds (Mitra *et al.*, 2002). In both cases, the aldehyde products (which include vanillin), whether free or conjugated, were not detected, suggesting their rapid conversion to the acid and alcohol forms. Further refinement of this type of metabolic engineering work is clearly in order.

A patented work describes the use of a recombinant microbe (*E. coli* ATCC 98859) for the five-enzyme biocatalysis of a carbon source, e.g. glucose, to vanillic acid (Frost, 2002). The vanillic acid is separated from the bioconversion mixture by organic extraction and subsequently reduced to vanillin by aryl-aldehyde dehydrogenase purified from *Neurospora crassa* SY 7A. Recent work by Barghini *et al.* (2007) and Ruzzi *et al.* (2008) explores the possibility of integrating the *Pseudomonas fluorescens* BF13 genes encoding feruloyl-CoA synthetase and feruloyl hydratase/aldolase into *E. coli* JM109 chromosome to enhance the conversion of ferulic acid to vanillin. The resulting strain FR13 was shown to be more efficient in vanillin production than a JM109 derivative expressing the same genes from a low copy plasmid vector.

## 29.7 Future trends

The growing interest in natural and organic products opens up windows of opportunity for the expansion of the current vanilla market, especially in Japan, Canada, Scandinavia, the UK, Italy, Eastern Europe and the Gulf States (Ratsiazio, 1998). To sustain this trend, there is a need to develop exciting products that will fully exploit the potential of dietary and medicinal properties of natural vanilla. New food recipes have to be formulated, catering to the use of the so-called 'edible vanilla' or 'vanilla stick' (cured pods sold in retail). This development should be coupled with the improvement of cultural management practices in vanilla specifically geared towards sustainable production. Recommendations on organic methods of cultivation of vanilla can be found in Augstburger *et al.* (2000).

As *in vitro* manufacture of natural vanillin, aided with the tools of genetic engineering, becomes more of a reality, there is a great and valid concern for the possible displacement of vanilla farmers who are mostly located in the developing countries. Resolving such a social dilemma is indeed a daunting task. It should be noted, however, that the faithful organic consumer veers away from products derived from genetically modified organisms.

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