

Chapter 2

Essential Oils Chemistry

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What are Essential Oils?

Essential oils, also known as essences, volatile oils, etheric oils, or aetheroleum, are natural products formed by several volatile compounds (Sangwan et al. 2001; Baser and Demirci 2007). According to the International Standard Organization on Essential Oils (ISO 9235: 2013) and the European Pharmacopoeia (Council of Europe 2004) an essential oil is defined as the product obtained from plant raw material by hydrodistillation, steam distillation or dry distillation or by a suitable mechanical process (for *Citrus* fruits). Cold pressing without heat is usually used for *Citrus* fruit oils because their constituents are thermosensitive and unstable, converting into artifacts under heat and pressure. Moreover, essential oils are frequently associated with gums and resins that are separated by the distillation process (Baser and Demirci 2007).

The definition of an essential oil excludes other aromatic/volatile products obtained by different extractive techniques like extraction with solvents (concretes, absolutes), supercritical fluid extraction, and microwave-assisted extraction. Essential oils also differ from fixed oils or fatty oils in both chemical and physical properties. Fatty oils contain glycerides of fatty acids and leave a permanent stain on filter paper, whereas essential oils contain volatile compounds and vanish rapidly without leaving any stain.

In nature, essential oils play very important roles in plant defense and signaling processes (Harborne 1993; Bowsher et al. 2008; Taiz and Zeiger 2010). For example, essential oils are involved in plant defense against microorganisms, insects, and herbivores, attraction of pollinating insects and fruit-dispersing animals, water regulation and allelopathic interactions (Fahn 1979; Harborne 1993;

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Pichersky and Gershenzon 2002; Bakkali et al. 2008). Also, they are valuable natural products used as raw materials in many fields, such as pharmaceutical, agromomic, food, sanitary, cosmetic, and perfume industries (Buchbauer 2000).

Essential oils can be found in various plant organs (flowers, fruits, seeds, leaves, stems, and roots) being produced and stored in secretory structures that differ in morphology, structure, function, and distribution. These specialized structures minimize the risk of autotoxicity and can be found on the surface of the plant organs or within the plant tissues, being classified as external or internal secretory structures, respectively. Internal secretory structures include secretory cells (often idioblasts), secretory cavities, and secretory ducts whereas external ones include glandular trichomes, epidermal cells, and osmophores (Svoboda and Svoboda 2000). Some plant organs and tissues, such as roots, tubers, and wood, are very hard and need to be broken down to expose the oil-containing cells and cavities for extraction.

Essential oils are complex mixtures of volatile (around 100 u) to semi-volatile compounds (around 300 u), usually with a strong odor, rarely colored, soluble in organic solvents, and insoluble in water. They comprise volatile compounds of terpenoid and non-terpenoid origin, synthesized through different biosynthetic routes and with distinct primary metabolic precursors. Terpenoids biosynthesis involves both the mevalonate and non-mevalonate (deoxyxylulose phosphate) pathways, whereas phenylpropanoids are formed via the shikimate pathway (Litchenthaler 1999; Dewick 2002a; Baser and Demirci 2007; Sell 2010). Monoterpenes and sesquiterpenes are usually the main group of compounds found in essential oils. In addition, phenylpropanoids are also very frequent. Moreover, some essential oils may also contain fatty acids and their esters and, more rarely, nitrogen and sulfur derivatives (Baser and Demirci 2007; Bakkali et al. 2008).

In aromatic plants, the composition of essential oils usually varies considerably because of both intrinsic (sexual, seasonal, ontogenetic, and genetic variations) and extrinsic (ecological and environmental aspects) factors (Figueiredo et al. 2008a; Taiz and Zeiger 2010). Genetic variations may result in the expression of different metabolic pathways and, consequently, quantitative and qualitative variations in essential oil composition may occur. When significant differences are found, an intraspecific category (chemotype) is defined. Essential oil quality strongly depends on all these factors that may interfere and also limit plant yield. Analytical guidelines published by several institutions such as the European Pharmacopoeia, International Standard Organization (ISO), and World Health Organization (WHO) are available and must be followed to assure the good quality of the commercialized essential oils and of the plants from which they are obtained. In general, the industries choose the chemotypes that have most commercial interest, in order to obtain high-quality end products as well as efficient biological activities.

Quality assessments of essential oils include sensory evaluations, very common in perfumery houses; physical and chemical tests, required in standards, pharmacopoeias, and codices; and chromatosppectral techniques for oil analysis. Hyphenation of gas chromatography (GC) separation step with spectroscopic techniques is often required for accurate compound identification, gas chromatography–mass spectrometry (GC–MS) being one of the most popular hyphenated techniques for

characterization and identification of complex volatile compounds. A flame ionization detector is usually used for quantitative analysis, while a quadrupole mass detector or ion-trap detector is necessary to characterize essential oil constituents (Baser and Demirci 2007). Identification of the compounds is made by comparison of both chromatographic data (e.g., Kováts indices and linear retention indices) and mass spectra data with those of authentic samples and library reference spectra. Notwithstanding the achievements in analytical techniques, the total separation and identification of all compounds of the volatile mixture remains unattainable because of the large number of compounds, structural similarities, isomeric forms, and concentration range of the compounds present in essential oils (Gomes da Silva et al. 2008). In this way, similar retention times may occur and confirmation on two columns of different polarity is advised in order to avoid misleading identifications. Taking into account that essential oils may contain hundreds of constituents, co-elutions are inevitable and therefore new analytical strategies have been developed to maximize compound separation, namely multidimensional GC (MD-GC) and comprehensive two-dimensional GC (GCxGC) (Gomes da Silva et al. 2008). Moreover, compounds with similar mass spectra and identical retention indices make essential oil characterization a very difficult task. In these cases, other methodologies like GC in tandem with Fourier transform infrared (GC-FTIR) and nuclear magnetic resonance spectroscopy (^{13}C -NMR) should be considered (Gomes da Silva et al. 2008; Tomi et al. 1995).

Several plant families comprise well-known aromatic species, many of them included in the Generally Recognized as Safe (GRAS) list fully approved by the US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA, USA) for addition to food and beverages. The major essential oil bearing plant families include Apiaceae, a widely distributed group of annual, biennial, and perennial plants, with essential oils in tubular ducts; Asteraceae, comprising over 30,000 species of evergreen shrubs, rhizomatous herbs, tuberous perennials, and tree herbs; Cupressaceae, a group of conifers usually resinous trees and shrubs producing essential oils within woods; Lamiaceae, a very diverse group of aromatic herbs and shrubs with volatile compounds normally accumulated in glandular trichomes; Lauraceae, comprising flowering plants and a number of aromatic trees with volatiles present in cells within the bark and wood; Myrtaceae, a highly aromatic group, including several fruit species; Pinaceae, a group of high growing conifers with resinous aromatic materials with acids, turpentine, and terpenoids; Piperaceae, a small family of flowering plants; Santalaceae with only a few aromatic species of interest; and Zingiberaceae, the ginger family with several aromatic rhizomes (Hunter 2009).

Biosynthetic Pathways

In nature, two main groups of metabolites can be found: primary and secondary metabolites. Primary metabolites are universal compounds, present in all living organisms, and include proteins, carbohydrates, lipids, and nucleic acids. Secondary

metabolites are found only in some species and are classified as terpenoids, shikimates, polyketides, and alkaloids, the first two being the most relevant in essential oils (Sell 2010). Although terpenoids are more frequent and abundant in essential oils, certain species contain high quantities of shikimates, namely phenylpropanoids and when these compounds are present, they provide specific odor and flavor to the plants (Sangwan et al. 2001).

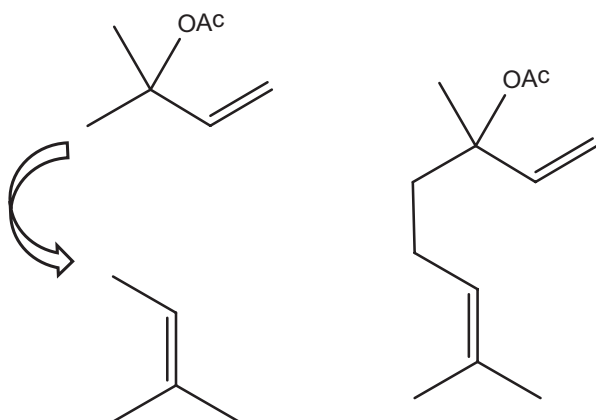
Terpenes

Terpenes result from the condensation of a pentacarbonate unit with two unsaturated bonds, isoprene (2-methyl-1,3-butadiene), and therefore are many times called isoprenoides.

The designation “terpenes” was first used by Kekulé in 1880 to name $C_{10}H_{16}$ compounds found in turpentine (Baser and Demirci 2007). In 1887, his assistant Otto Wallace formulated the “isoprene rule” suggesting that terpenes were formed by two or more isoprene units. Later, Robinson suggested that the isoprene units were connected in a head-to-tail way (Fig. 2.1). In 1950, Leopold Ruzicka replaced this rule by the “biogenetic isoprene rule” which states that a compound is an isoprenoid if it is derived biologically with or without rearrangements from an isoprenoid precursor (Little and Croteau 1999). In summary, terpenoids are derived from aliphatic precursors such as geraniol for the formation of monoterpenes, farnesol for sesquiterpenes, and geranylgeraniol for diterpenes (Baser and Demirci 2007).

Terpenes are classified into different structural and functional classes. According to the number of isoprene units in their structure, terpenes can be classified into hemiterpenes (1 unit), monoterpenes (2 units), sesquiterpenes (3 units), diterpenes (4 units), and so on. The terpenes most often found in essential oils are monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$). These compounds have many isomeric cyclic or linear structures, various degrees of unsaturations, substitutions, and oxygenated derivatives, being generally called terpenoids.

Fig. 2.1 Head-to-tail coupling of two isoprene units to form linalyl acetate



The biosynthesis of terpenes involves two universal precursors: isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP). In higher plants, IPP is biosynthesized through two pathways: the mevalonate pathway (MVA) and the non-mevalonate (mevalonate independent) or deoxyxylulose phosphate pathway, schematized in Fig. 2.2.

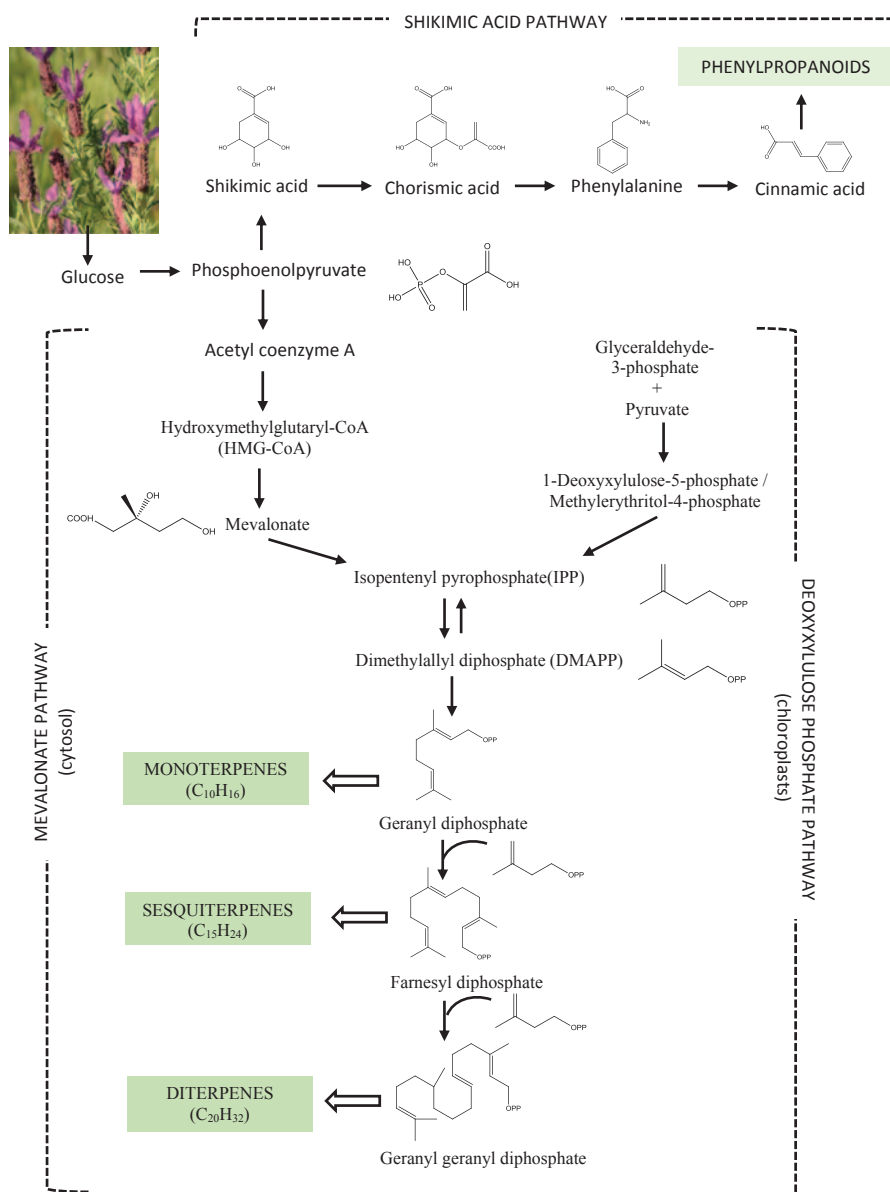


Fig. 2.2 Terpenoids and phenylpropanoids biosynthesis in plants

In the mevalonate pathway, IPP is formed through mevalonic acid that results from the condensation of 3 acetylcoenzyme-A moieties. In the non-mevalonate pathway, 2 C-metil-D-erythritol-4-phosphate (MEP) and 1-deoxy-D-xylulose-5-phosphate (DOXP) are involved, resulting from the condensation of glyceraldehyde phosphate and pyruvate (Baser and Demirci 2007). The former takes place in the cytoplasm and leads to the formation of most sesquiterpenes whereas the latter occurs in the chloroplasts, producing primarily monoterpenes and diterpenes (Bouwmeester 2006). IPP and DMAPP lead to geranyl diphosphate (GPP), the immediate precursor of monoterpenes. The condensation of GPP with IPP leads to farnesyl diphosphate (FPP), the immediate precursor of sesquiterpenes, and the condensation of FPP with IPP results in geranyl geranyl diphosphate, the precursor of phytol, other diterpenes, and carotenoids. Monoterpenes and sesquiterpenes are the main compounds found in essential oils (Bakkali et al. 2008). Heavier terpenes, such as diterpenes, may also be present but usually do not contribute to the odor of essential oils (Hunter 2009).

Phenylpropanoids

Phenylpropanoids contain one or more C_6-C_3 units, the C_6 being a benzene ring. Many of the phenylpropanoids found in essential oils are phenols or phenol ethers and in some cases, the side chain is shortened (C_1) as, for example, in methyl salicylate and vanillin (Tyler et al. 1988). Phenylpropanoids are synthesized via the shikimic acid pathway (Fig. 2.2), their main precursors being cinnamic acid and *p*-hydroxycinnamic acid, originated from the aromatic amino acids phenylalanine and tyrosine, respectively (Dewick 2002a; Sangwan et al. 2001). Shikimic acid is synthesized from erythrose 4-phosphate and phosphoenolpyruvate. Elimination of one of the ring alcohols of shikimic acid and reaction with phosphoenol pyruvate gives chorismic acid. This compound forms the phenylpropionic acid skeleton. Amination and reduction of the ketone function produces the amino acid phenylalanine while reduction and elimination leads to cinnamic acid that produces *o*- and *p*-coumaric acids. Moreover, aromatization of shikimic acid gives benzoic acid derivatives, present in several essential oils (Sell 2010).

Chemical Composition

The constituents of plant essential oils fall mainly into two distinct chemical classes: terpenoids and phenylpropanoids. Terpenoids are extremely variable, showing different carbon skeletons and a wide variety of oxygenated derivatives, including alcohols, esters, aldehydes, ketones, ethers, peroxydes, and phenols. There is a very little difference between the molecular weights of terpenes and their oxygenated products. The similarity of many of these structures reflects the difficulty of their chemical characterization. There is also the problem of stereoisomerism, whereby

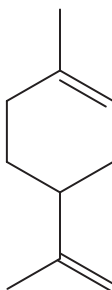
one or more groups are arranged in a mirror image style in space compared with their isomer. Both enantiomers (optically active isomers) of many terpenoids occur in nature. However, some species produce only one enantiomer whereas in other cases both are produced (racemic mixture). Examples of the most relevant essential oil constituents, namely hydrocarbon and oxygenated derivatives of monoterpenes, sesquiterpenes, and phenylpropanoids, are presented in detail. Moreover, other compounds found in essential oils such as diterpenes, sulfur- and nitrogen-containing constituents and lactones are also referred.

Monoterpenes ($C_{10}H_{16}$)

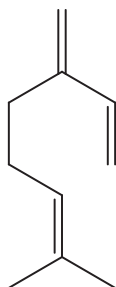
Monoterpenes can be found in nearly all essential oils and are the most representative constituents, attaining around 90% of many oils (Bakkali et al. 2008). They are formed by the attachment of two isoprene units (10 carbon atoms and at least one double bond). These compounds oxidize easily because of their rapid reaction to air and heat sources. Monocyclic monoterpene hydrocarbons are the most common in essential oils but linear (acyclic) and bicyclic compounds also occur. The main linear monoterpene hydrocarbons found in essential oils have a typical 2,6-dimethyloctane structure with three double bonds while bicyclic compounds have a second ring with three, four, or five carbons besides the hexane ring. Biochemical modifications including oxidations or rearrangements produce several other compounds, generally called monoterpeneoids. These compounds include highly functionalized chemical entities (Hunter 2009).

Hydrocarbons

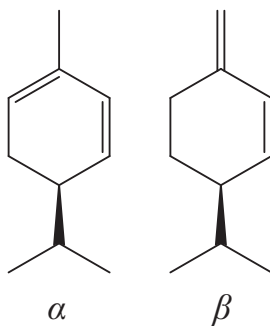
Limonene Terpene hydrocarbon widely spread among essential oils, being very abundant in citrus oils. (+)-Limonene, the enantiomer of orange and lemon peels, has a lemon-like odor making it attractive as an additive in cosmetics and foods (Kim et al. 2013). This enantiomer is the most abundant in plants. It is widely used in household and industrial cleaning solvents, as a paint stripper and botanical insecticide as well as in food flavorings (Hunter 2009). Racemic limonenes are commercially available under the name dipentene (Bauer and Garbe 2001).



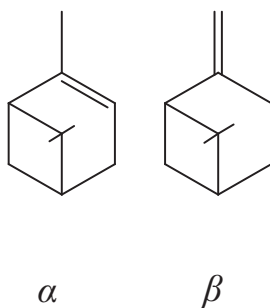
β -Myrcene Has a fresh citrus odor occurring in several essential oils like hop – *Humulus lupulus* (Vázquez-Araújo et al. 2013), thyme – *Thymus serpyllum* (Raal et al. 2004), and *Margotia gummifera* (Valente et al. 2013). This compound is very relevant in perfumery as an important intermediate in the synthesis of menthol, citral, citronellol, citronellal, geraniol, nerol, and linalool as well as vitamins A and E (Behr and Johnen 2009). The isomer α does not occur in nature.



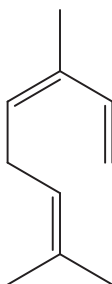
Phellandrene (–)- α -Phellandrene is primarily found in dill – *Anethum graveolens* and in *Eucalyptus dives* oils. It has a citrus odor with a slight peppery note. (–)- β -Phellandrene is characteristic of lodgepole pine – *Pinus contorta* (Sell 2010) and sea fennel – *Crithmum maritimum* oils (Ozcan et al. 2006).



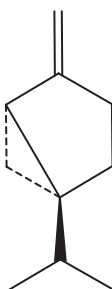
Pinene There are two structural isoforms found in nature: α - and β -pinenes. Pinenes are the most important naturally occurring hydrocarbons (Bauer and Garbe 2001). Both *l*- and/or *d*-forms as well as racemic forms may occur. As the name suggests, both isoforms are important constituents of pine resin as well as resin of many other conifers. High concentrations of these compounds are also present in a wide variety of essential oils such as ironwort – *Sideritis erythrantha* (Kose et al. 2010), sage – *Salvia rosifolia* (Ozek et al. 2010), lemon – *Citrus limon* (Vekiari et al. 2002), *Eucalyptus* sp. (Juan et al. 2011), juniper – *Juniperus communis* berries and needles (Gonny et al. 2006), and rosemary – *Rosmarinus officinalis* (Wang et al. 2012) oils. The fresh pine odor makes pinenes interesting compounds for household perfumery. α -Pinene is also used in the synthesis of other compounds like terpineol, borneol, and camphor (Bauer and Garbe 2001).



Ocimene β -Ocimene (*cis* and *trans*) is the isomer most frequently found in essential oils like *Lavandula multifida*. It has a pleasant odor highly appreciated in perfumery and has showed a potent effect on the inhibition of *Candida albicans* filamentation (Zuzarte et al. 2012b).

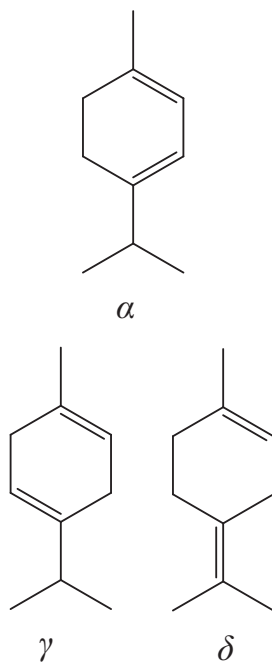


Sabinene An important compound in carrot – *Daucus carota* seed (Marzouki et al. 2010) and in *Juniperus communis* oils (Ottavioli et al. 2009) that also contributes to the spiciness of black pepper. This compound is used as a perfume additive (Zhang et al. 2014) and has been explored as a component for the next generation of aircraft fuels (Rude and Schirmer 2009).



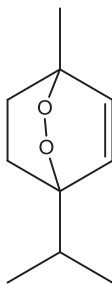
Terpinene α -Terpinene is an important compound in cardamom – *Elettaria cardamomum* oil (Abbasipour et al. 2011) and is one of the compounds responsible for the antioxidant activity of tea tree – *Melaleuca alternifolia* oil (Rudbäck et al. 2012). γ -Terpinene has a herbal citrus odor and occurs frequently in several *Thymus*

species (Jamali et al. 2012). δ -Terpinene or terpinolene is relevant in tea tree oil (Homer et al. 2000). It has a sweet piny odor with citrus characteristics and is used in household perfumes (Hunter 2009).



Oxygenated derivatives

Ascaridole A terpene peroxide with a pungent smell and taste. Present, as a major constituent, in wormseed – *Chenopodium ambrosioides* oil. Ascaridole has anthelmintic properties but is very toxic to mammals, and oils with this compound should be treated carefully since they can explode when heated or treated with acids (Harborne and Baxter 2001).



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