Chapter 1
The flavor of citrus fruit

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Introduction

Citrus is the most important cultivated fruit tree crop in terms of area and production values. It is grown commercially in more than 140 countries in tropical and subtropical regions of the world, with total annual production of over 100 million tons and providing a contribution of US$6–8 billion to the world economy (Ladaniya 2008; USDA 2012).

The genus Citrus belongs to the Rutaceae family, subfamily Aurantioidae, and originates from Southeast Asia, nearby North India, Myanmar, and China (Swingle and Reece 1967; Scora 1975). According to the classification by Swingle, the most commercially important citrus species are sweet orange (C. sinensis), sour orange (C. aurantium), mandarin (C. reticulata), grapefruit (C. paradisi), pummelo (C. grandis), lemon (C. limon), citron (C. medica), and lime (C. aurantifolia). Furthermore, phylogenetic and taxonomic studies of the genus Citrus revealed that there are only three basic “true” citrus ancestors, which are citron (C. medica), mandarin (C. reticulata) and pummelo (C. grandis), and all other Citrus species were actually evolved from crosses between these true original citrus species or other relatives (Scora 1975; Barrett and Rhodes 1976). For example, sweet orange was derived from a cross between mandarin and pummelo; grapefruit was derived from a cross between pummelo and sweet orange; and lemon was derived from a cross between citron and sour orange (Barkley et al. 2006; Li et al. 2010).

From a botanical perspective, citrus fruit is a hesperidium, i.e., a special type of berry with a leathery rind internally divided into segments (Grierson 2006a). The fruit is anatomically divided into three separate layers: the outer colored portion of the rind called the flavedo
or exocarp, which includes the cuticle, the colored epidermis cells containing chlorophyll or carotenoid pigments and the hypodermis cells consisting of the oil glands; the inner white portion of the peel called the albedo or mesocarp, which is comprised of spongy parenchymous cells; and the internal part of the fruit called flesh or endocarp, which represents the edible portion of the fruit including the juice sacs, segment membranes, and seeds (Schneider 1968; Grierson 2006a) (Fig. 1.1). From the nutritional aspect, citrus fruit provide an important beneficial source to the human diet for consumption of ascorbic acid (vitamin C) and folic acid (vitamin B₉), pectin and soluble fibers, different minerals, carotenoids, and specific flavonoids and limonoids, all phytomolecules playing a role in preventing degenerative diseases such as heart diseases and various types of cancers (Patil et al. 2006).

Citrus fruit are either grown for fresh consumption or for juice and/or peel oil manufacturing. With respect to fresh consumption, the main producing countries are China, Brazil, Spain, Mexico and the United States, while with respect to juice manufacturing, the main producing countries are Brazil and the United States (Florida), with sweet oranges being the main product followed by grapefruit and lemons (Ladaniya 2008; USDA 2012). It is worth noting that during the last few years, consumption of fresh oranges, grapefruit, lemons and limes remained constant, whereas easy-to-peel mandarins and tangerines have seen a steady and significant increase (Ladaniya 2008; USDA 2012).
Above all, citrus fruit are appreciated and consumed by billions of people around the globe because of their unique delicate and attractive flavor evolved from a blend of fruity and freshness and earthy notes. In fact, what we perceive as flavor of citrus fruit is actually the combination of basic taste, aroma, and mouth-feel sensations that are perceived simultaneously by the brain during the eating of foods (Goff and Klee 2006). The sensation of taste providing sweet, sour, bitter, salty, and umami attributes is perceived by receptors present on the tongue and in the mouth that bind soluble components in the food matrix, whereas sensation of aroma is perceived via receptors present in the olfactory bulb in the nose cavity that specifically bind thousands of different volatiles providing various kinds of floral, fruity, minty, woody, mushroom, and other odors (Schwab et al. 2008). In this chapter, we discuss the sensory quality and biochemical constituents involved in creating the unique flavor of different citrus fruit species, including oranges, mandarins, grapefruit, and lemons. The chapter focuses on describing the flavor attributes of fresh citrus fruit and essential oils, but not of processed juices. For further information regarding the effects of juice manufacturing processes, such as extraction methods, pulp separation, thermal processing, and concentration and reconstitution methods on orange volatiles, readers are referred to the excellent review by Perez-Cacho and Rouseff (2008b).

**Taste components of citrus fruit**

The taste of citrus fruit is principally governed by the levels of sugars and acids in the juice sacs and the relative ratio among them; the latter relationship is also termed the total soluble solids to titratable acidity ratio (TSS : TA), or fruit ripening ratio, and is widely used by growers as an indicator of fruit maturity. During fruit ripening, juice TSS levels gradually increase whereas acidity levels gradually decrease, resulting in a continuous rise in the relative ripening ratio of the fruit (Ramana et al. 1981; Grierson 2006b). For example, the ripening ratios of navel oranges in California increase from a low level of 6 in September to above 20 in January (Obenland et al. 2009), and the ripening ratios of “Or” mandarins in Israel increase from 9 in January to 18 in March (R. Porat, unpublished data).

Because of these dynamic changes in TSS and TA levels during citrus fruit maturation (continuous increase in TSS and decrease in acidity),
the overall taste of the fruit will vary with the ripening stage; within each cultivar, early-season fruit are more sour than late-season fruit. Therefore, to make sure that the fruit will not be harvested too early when they may be too sour for the market, maturity and grade standards were developed in each country and enforced by local plant protection and inspection services (Grierson 2006b). For example, in Florida, it is permitted to harvest tangerines only when their TSS levels are above 9% and TSS:TA is greater than 7.5, whereas in Israel, export of early-season Satsuma mandarins is allowed only when TSS levels are above 12% and juice acidity levels are below 1.3%, resulting in TSS:TA greater than 7.0 (Tietel et al. 2010a). In California, the minimum allowed TSS:TA for harvesting and marketing of Navel oranges is 8, even though it was shown that consumer acceptability was higher at a ripening ratio of 10 (Obenland et al. 2009). Obviously, harvesting non-mature sour fruit is not recommended because it might deter consumers from buying more fruit later in the season. However, it is also not recommended to harvest over-mature fruit, which will have a too high ripening ratio, since those fruit will suffer from low flavor preference scores (Grierson 1995). Therefore, each citrus species should be harvested at its optimal and preferred maturity index (between 8 and 12 for oranges), and either too high or too low ripening ratios are not desirable. Furthermore, it was proposed that a good tasty fruit should have high levels of sugars and moderate levels of acids rather than any other combination which may result in a similar ripening ratio (Kader 2008).

In addition to the conventional measurements of TSS:TA ratios to monitor the degree of fruit maturation, Jordan et al. (2001) suggested a new formula to evaluate the sweetness to sourness ratios termed BrimA, which takes into account the fact that receptors on the tongue have a different response to sugars and acids, and that small changes in acids are much more easily perceived than small changes in sugars. The BrimA index is derived by subtracting a multiple of TA from TSS, so that BrimA = TSS – k(TA), with constant k being characteristic of a fruit product. In the case of Navel oranges, a better correlation was found between flavor hedonic scores and sugar and acid concentrations using the BrimA index (with k = 3) rather than using the standard TSS:TA ratio, and that was true especially for low acid-containing fruit (Obenland et al. 2009). A better correlation between sweetness intensity determined by a trained panel and BrimA ($r^2 = 0.92$) as compared with using the TSS:TA ratio ($r^2 = 0.76$) or TSS alone ($r^2 = 0.74$) was also found by Plotto and co-workers (unpublished data).
In the following sections, we describe the biochemical components involved in creating the sweet, sour, and bitter tastes in citrus fruit.

**Sugars**

In most citrus species (apart from lemons that contain high amounts of acids and low amounts of sugars), sugars provide about 80% of the juice TSS content, and therefore TSS measurements provide a useful and simple indicator to evaluate total sugar levels (Erickson 1968). Table 1.1 provides data regarding the average TSS levels in juices of different citrus fruit. It can be seen that the highest TSS levels were observed in mandarin juice (12.0%), followed by oranges (11.6%) and grapefruit (10.5%), and the least TSS levels were recorded in sour lemons (8.6%).

More detailed analyses of sugar contents and composition revealed that the principal sugars present in citrus juices are the monosaccharides glucose and fructose and the disaccharide sucrose (Ting and Attaway 1971). In addition, several other sugars, including mannose, arabinose, xylose, and lactose, were detected in trace amounts, but since their levels are so low they probably do not have much effect on overall fruit flavor and sweetness perception (Wali and Hassan 1965; Ladaniya 2008). In some citrus juices, such as Valencia oranges and some mandarins, it was reported that sucrose, glucose, and fructose were distributed in a ratio of 2:1:1. In contrast, it was reported that lemons contain only very small amounts of sucrose (Ting and Attaway 1971; Ladaniya 2008). Note that fructose is a more potent sweetener than sucrose and glucose in the following order: fructose (1.2) > sucrose (1.0) > glucose (0.64), therefore not only the total amount of sugars but also their composition will affect the overall perceived sweetness of the fruit (Kader 2008).

**Table 1.1** Average TSS and acidity levels, and ripening ratios of different citrus fruit.

<table>
<thead>
<tr>
<th>Citrus species</th>
<th>TSS (%)</th>
<th>Acidity (%)</th>
<th>TSS to acid ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oranges</td>
<td>11.6</td>
<td>1.0</td>
<td>11.6</td>
</tr>
<tr>
<td>Mandarins</td>
<td>12.0</td>
<td>0.9</td>
<td>13.3</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>10.5</td>
<td>1.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Lemons</td>
<td>8.6</td>
<td>5.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Data were calculated from information provided by Ramana *et al.* (1981) and are means of 36 measurements of different orange varieties, 14 measurements of different mandarin varieties, 9 measurements of different grapefruit varieties, and 11 measurements of different lemon varieties.
**Acids**

Organic acids containing free carboxyl groups (COOH) are the main source providing the acidic taste of citrus fruits. The principal acid in the juice sacs of most citrus species is citric acid, which normally provides about 85–90% of total organic acid content, the rest being mostly malic and succinic acids (Erickson 1968; Ting and Attaway 1971). In mandarins and oranges, citric acid provides about 7–8% of juice TSS content, whereas in lemons it may consist of up to 60–70% of the juice TSS content (Ting and Attaway 1971; Sinclair 1984). As presented in Table 1.1, the average acidity levels in mandarin, orange, and grapefruit juices are 0.9, 1.0, and 1.6%, respectively, whereas the average acidity level in lemons is about 5.4%.

**Bitter compounds**

The numerous limonoids and flavonoids in citrus fruit may or may not be responsible for bitterness, depending on the sugar moiety attached to the aglycone structure. For example, the flavanones naringenin, hesperetin, and isosakuranetin become naringin, neohesperidin, and poncirin, respectively, when they are attached to a neohesperidose with a rhamnosyl-α-1,2-glucose (Horowitz and Gentili 1961; Tripoli et al. 2007). These glucosides are bitter as a result of the 1–2 linkage between the two sugar units (Horowitz and Gentili 1961). In contrast, their rutinosides (flavanone with a rhamnosyl-α-1,6 glucose attached) are tasteless (Tripoli et al. 2007), such as hesperidin, the main flavonoid in oranges. Hesperidin can be found at concentrations ranging from 300 to 900 mg per 100 g fresh weight in sweet orange, mandarin, and lemon, whereas the bitter naringin is the main compound in grapefruit and sour orange (concentrations ranging from 1000 to 1400 mg per 100 g fresh weight) (Nogata et al. 2006). Other bitter flavonoids include neoeriocitrin, neohesperidin, and poncirin, present at high concentrations in the peel of sour orange (Nogata et al. 2006). Further, Frydman et al. (2004) cloned the gene 1,2-rhamnosyltransferase (Cm1.2RhaT), which catalyzes the biosynthesis of the bitter neohesperidosides, from bitter pummelo, and the function of the gene was demonstrated in transgenic tobacco cells. The enzyme that catalyzes the biosynthesis of the tasteless rutinosides is 1,6-rhamnosyltransferase and was described by Lewinsohn et al. (1989). In general, flavonoids are found with the highest concentration in the fruit albedo, which may affect the taste of some juice depending on the mode of extraction.
Whereas naringin is responsible for the sensation of “immediate” or “primary” bitterness sensed when eating fresh bitter citrus fruit, such as grapefruit, pummelo, and bitter orange, limonin is responsible for the “delayed” bitterness that becomes detectable some time after juice extraction (Puri et al. 1996). The “delayed bitterness” phenomenon that occurs after juice extraction from both bitter and non-bitter citrus species results from conversion of the tasteless limonoic acid A-ring lactone to the bitter compound limonin. This reaction is catalyzed by the prevailing acidic conditions that occur after juicing (Manners 2007; Dea et al. 2010). Because of the great importance of removing bitter compounds from processed citrus juices, various technological debittering processes have been developed by the citrus juice manufacturing industry, in order to absorb or separate the bitter compounds using various types of absorbers and ion-exchange columns (Puri et al. 1996; Singh et al. 2003; Kola et al. 2010). A patent by Japanese workers described a process to decrease the limonin content using high pressure, with apparently no changes in the citrus juice compositions (Tamaki et al. 1991). The process consists in applying a pressure of 1200–4000 kg/cm$^2$ for 1–30 min to the freshly squeezed citrus juices. The authors were able to decrease the limonin content by a few parts per million (ppm) with the highest pressure at the longest time exposure. It is likely that the mode of action was by denaturation of the limonin D-ring hydrolase. However, it is unlikely that bitterness was completely removed if the limonin content was above 1 ppm (Plotto et al. 2010). It is not known to the authors whether this process is being commercially applied.

Recently, it was found that orange juice that was processed with fruit that were harvested from trees severely affected with huanglongbing (HLB or citrus greening) disease had higher content of two bitter limonoids, limonoin and nomilin (Baldwin et al. 2010), and that this juice had a bitter taste in comparison with juice made with fruit from healthy trees (Dagulo et al. 2010; Plotto et al. 2010). HLB disease is due to a bacterium, Candidatus Liberibacter asiaticus (Las), which prevents phloem flow from the root to the fruit, hence producing stress symptoms on the tree. Limonin, nomilin, and naringin have very low taste thresholds, 1, 1, and 20 ppm, respectively, and therefore their bitter taste can be sensed even at very low concentrations of just a few micrograms per milliliter of juice (Guadagni et al. 1973; Rouseff and Matthews 1984). Further, limonin and nomilin are synergistic to each other, which means that their thresholds are significantly reduced when they are present together in a juice (S. Dea et al., unpublished data).
Aroma compounds of citrus fruit

The unique and delicate aroma of citrus fruits results from the accumulation in the juice sacs and oil glands of dozens or hundreds of volatiles, which provide various fruity, floral, terpeney, citrus, green/grassy, fatty, metallic, herbal, mushroom, and so on odors. Overall, it has been reported that citrus fruit, such as mandarins and oranges, consist of more than 200 (Miyazaki et al. 2011) and even 300 volatiles (Perez-Cacho and Rouseff 2008a), respectively. Nevertheless, gas chromatography–olfactometry (GC–O) (“sniffing”) experiments, where the eluates from a gas chromatograph are smelled by one or several human subjects, showed that only a small portion of just a few dozen volatiles are present in citrus juices at levels that are above their odor thresholds, and thus actually contribute to the sensation of citrus flavor. For example, in oranges, grapefruit, and mandarins, only about 36–49 volatiles had aroma activity and essentially contributed to fruit odor (Buettner and Schieberle 1999; Perez-Cacho and Rouseff 2008a; Miyazaki et al. 2012). Further, compounds such as limonene and valencene are present in large quantities in orange juice, but do not directly contribute to citrus aroma. On the other hand, compounds that are barely detected by an instrument can be detected by the human nose and therefore contribute to the fruit aroma.

It is generally assumed that the unique odor of different citrus fruit species results from its unique composition of aroma volatiles that accumulate at different concentrations and at specific ratios among each other and, therefore, artificial reconstitution of these volatiles at the optimal concentrations within the appropriate food matrix would allow one to mimic the original flavor of the fruit (Grosch 2001). Overall, this assumption is generally true; however, in practice, many of the aroma-active compounds detected in citrus and also in other fruit are present at very low levels that are difficult to detect and quantify, making it almost impossible to create synthetically the exact combination of volatiles that is present in the natural fruit. Nevertheless, in spite of these considerable limitations, it has been reported that a combination of about 23–25 volatiles at the appropriate concentrations may be sufficient to mimic typical orange and grapefruit odors (Buettner and Schieberle 2001a; Perez-Cacho and Rouseff 2008a). Furthermore, in the case of oranges and mandarins, it was reported that only a combination of several different volatiles can create the typical fruity odor (Perez-Cacho and Rouseff 2008a; Miyazaki et al. 2012). In contrast, in grapefruit and lemons, a few “key odorants” or “character impact compounds” were detected, which provide the typical characteristic
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Terpene hydrocarbons

Terpene hydrocarbons comprise about 90–95% of the total amount of volatiles present in citrus juices and essential oils. The most abundant terpene volatile in citrus fruit is no doubt limonene, which alone accounts for over 90% of the total amount of citrus volatiles, and provides a mild, minty, citrus-like odor. Limonene is a chiral molecule and D-limonene is produced by citrus fruit; it will be referred to just as limonene hereafter. In spite of the extremely high concentration of limonene in citrus juice, which is about 400 times higher than its odor threshold, its role in conferring the flavor of citrus fruit is not fully understood. In most GC–O evaluation studies, limonene was reported to be an important citrus aroma-active compound (Hinterholzer and Schieberle 1998; Buettner and Schieberle 2001b; Schieberle et al. 2003; Arena et al. 2006; Averbeck and Schieberle 2009; Obenland et al. 2009; Tietel et al. 2011b), whereas in other studies it barely showed any aromatic activity at all. On some GC column types, such as the apolar DB-5 (5% diphenyl, 95% dimethyl polysiloxane), limonene co-elutes with 1,8-cineole, a compound with a minty or eucalyptus odor. This may explain why the odor activity of limonene is unclear. Odor and taste thresholds of limonene in a bland orange juice matrix ranged from 8 to 13 ppm, about 50 times higher than thresholds in water (Plotto et al. 2004). To determine that threshold, limonene was distilled to remove any co-eluting compounds with an anise/minty odor. As a consequence of these contradictory results, it was suggested that limonene may make an important contribution to citrus flavor by functioning as a “lifting agent” for other volatiles in a similar manner to that of ethanol in wine (Perez-Cacho and Rouseff 2008a).

Two other relatively high-abundance terpene hydrocarbons present in concentrations of between 0.5-2.5% of the total amount of citrus
volatiles are β-pinene and β-myrcene which provide woody, musty and terpene-like odors. Once again, due to their high concentrations and relatively high odor-activity values they are important contributors to create a typical citrus flavor. Other terpenes that also possess aroma activity are α-pinene, α-terpinolene, γ-terpinene, p-cymene, and others. Thresholds of terpene hydrocarbons and other aroma compounds were measured in a deodorized orange juice matrix (Plotto et al. 2004). Thresholds of hydrophobic terpene hydrocarbons were 10–200 higher in the orange juice matrix than in water, presumably due to non-covalent binding of the volatile compounds to the non-volatile soluble compounds in the juice matrix. A study by Rega et al. (2004) elegantly showed the effect of pulp and “cloud” on volatiles in the headspace and on sensory perception. Most terpene and lipophilic compounds remain in the non-soluble fraction of the juice, namely pulp and “cloud.” An example of terpene thresholds in the deodorized orange juice and comparison with thresholds in water is given in Table 1.2.

**Aldehydes**

Aldehydes are an important and large group of citrus aroma volatiles generally providing green, fresh, and citrus-like notes. However, the overall role of aldehydes in conferring the desired citrus flavor is not yet completely clear, since some aldehydes, such as the straight-chain aldehydes octanal, nonanal, and decanal, and the terpenic aldehydes neral and geranial, provide favorable fresh, green, minty, and citrus-like notes, whereas some unsaturated aliphatic aldehydes,

<table>
<thead>
<tr>
<th>Compound</th>
<th>Threshold in deodorized orange juice</th>
<th>Threshold in water*</th>
<th>(T_{oj}:T_{water}) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odor</td>
<td>Taste</td>
<td>Odor</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>2120</td>
<td>2120</td>
<td>9.5</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>38700</td>
<td>38700</td>
<td>–</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>532</td>
<td>399</td>
<td>36</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>2390</td>
<td>2650</td>
<td>–</td>
</tr>
<tr>
<td>Limonene</td>
<td>8500</td>
<td>8470</td>
<td>60</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>16600</td>
<td>9020</td>
<td>280</td>
</tr>
<tr>
<td>Linalool</td>
<td>67</td>
<td>66</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*Thresholds in water from Ahmed et al. (1978).
such as (Z)-2-nonenal, (E)-2-nonenal, (E,E)-2,4-nonadienal and (E,E)-2,4-decadienal, rather provide undesired fatty, waxy, and soapy notes (Buettner and Schieberle 2001a; Perez-Cacho and Rouseff 2008a; Selli and Kelebek 2011). Miyazaki et al. (2012) found that the abundance of aldehydes combined with the lack of esters or terpene hydrocarbons with fruity/floral aroma explained a peculiar “pumpkin” flavor in a mandarin hybrid.

**Alcohols**

The terpene alcohol linalool is a very important compound in orange juice and orange peel oil, contributing to a distinct fresh and floral flavor (Perez-Cacho and Rouseff 2008a). Geraniol is another terpene alcohol contributing floral and fruity notes. In fresh citrus fruit, an important alcohol is ethanol, which provides ethanol-like odor, and its accumulation is highly associated with the development of off-flavors in stored fruit (Cohen et al. 1990; Hagenmaier and Shaw 2002; Navarro-Tarazaga et al. 2008). Other important citrus aroma-active alcohols are aliphatic alcohols, such as 1-hexanol and 1-octanol, which provide herbal and green notes (Perez-Cacho and Rouseff 2008a; Miyazaki et al. 2012).

**Esters**

Esters make an important contribution to the typical flavor of orange juice, since some esters, including ethyl butanoate, methyl butanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and ethyl hexanoate, impart strong fruity aromatic notes (Hinterholzer and Schieberle 1998; Buettner and Schieberle 2001b). In contrast, some ethyl esters, especially ethyl acetate, accumulate in high levels following postharvest storage of waxed fruit, and were proposed to be involved in causing a sensation of off-flavors (Tietel et al. 2011c). Further, a massive accumulation of “fruity” esters during ripening or upon prolonged storage may lead to a perception of an “over-ripe” odor (Tietel et al. 2010b).

**Ketones**

Ketones are important contributors to citrus flavor, and some of them have been reported to have high odor intensity values. For example, carvone provides a fresh minty note; α-ionone and β-ionone provide sweet floral notes; 1-octen-3-one has a very low odor threshold and provides a mushroom note; β-damascenone provides a fruity note; and nootkatone is an important character impact compound providing
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a typical grapefruit-like odor (Hinterholzer and Schieberle 1998; Buettner and Schieberle 2001a,b; Perez-Cacho and Rouseff 2008; Miyazaki et al. 2012).

Other volatiles

In addition to these main classes of volatiles, citrus fruit also contain minor amounts of other types of volatiles, such as acids (acetic acid, hexanoic acid, etc.), phenols (thymol), ethers (diethyl ether, 1,8-cineole), epoxides (caryophyllene oxide), furanones [3(2H)-furanone], pyrazines (2-isopropyl-3-methoxypyrazine) and sulfur compounds (dimethyl sulfide), all of which make specific contributions to the overall aroma of citrus fruit.

Citrus genes involved in flavor production

The recent advances in citrus genomics research and the completion of the full-length sequences of the sweet orange and clementine genomes now permit the cloning, expression, and characterization of many citrus genes that are involved in the biosynthesis of flavor compounds and may be used in the future for flavor manipulation using biotechnology approaches. In this section we provide a few examples of how, by using genetic engineering strategies, it may be possible to manipulate taste and aroma attributes and consequently citrus flavor perception.

One of the most important factors governing the taste and flavor of citrus fruit is the regulation of citric acid levels in the vacuoles of juice sac cells. Until recently, the molecular mechanisms involved in the regulation of citric acid levels in citrus fruit were unknown, as no differences were found in citrate synthase activity and gene expression levels between sweet and sour citrus fruit (Sadka et al. 2001). However, in a more recent study, Aprile et al. (2011) analyzed the transcriptomes of Faris sweet lemon fruit as compared with a chimera producing sour fruit and Lisbon sour lemon fruit, and found that a citrus homolog of the Arabidopsis H⁺-ATPase proton pump AHA10 involved in the acidification of vacuoles was highly expressed in sour lemon fruit but was not expressed at all in the sweet non-acid fruit. Furthermore, the authors also noticed activation of genes related to the GABA shunt pathway in the low-acid fruit, which further explains the rapid catabolism of citric acid in low-acid fruit. Overall, identification of genes involved in the regulation of citric acid metabolism in citrus fruit may now promote future manipulation of acidity levels in citrus fruit by genetic engineering strategies.
Another important factor governing the taste of citrus fruit and juice is regulation of bitterness. As noted earlier, the gene 1,2-rhamnosyltransferase (Cm1,2RhaT), which catalyzes the biosynthesis of bitter neohesperidosides, was cloned from bitter pummelo, and possibly may be used in the future for genetic engineering of transgenic plants in which normal expression of Cm1,2RhaT during fruit development may be silenced in order to reduce bitterness (Frydman et al. 2004). Alternatively, several other genes involved in limonin biosynthesis have been cloned and characterized, and may be used in the future to inhibit limonin metabolism and biosynthesis, and thus reduce the formation of “delayed bitterness” (Hasegawa et al. 1996; Puri et al. 1996).

Regarding biotechnologic manipulation of aroma volatiles production in citrus fruit, some key studies regarding the cloning and functional characterization of genes involved in terpene biosynthesis are worth noting. In a pioneering study, 49 genes encoding terpene synthases were identified from orange, but it was found that only a few terpene synthase genes were actually expressed during fruit ripening (Dornelas and Mazzafera, 2007). In a more recent study, Rodriguez et al. (2011) downregulated the expression of the limonene synthase gene using an antisense construct in transgenic orange fruit, and found that accumulation of limonene is important for the mediation of ecological interactions between the fruit and various insects and pathogens. It is possible that a similar approach may be used in the future for manipulating flavor acceptability, and also to study the roles of particular volatiles in conferring citrus flavor.

Sesquiterpenes, including valencene, nootkatone, and sinensal, are important volatile compounds in citrus fruit. Sharon-Asa et al. (2003) isolated the Cstps1 gene encoding a sesquiterpene synthase involved in the production of the sesquiterpene valencene, and this finding opened up new opportunities for the metabolic engineering of terpene biosynthesis and flavor manipulation in citrus fruit (Chappell 2004).

The unique flavor of different citrus species

The most important citrus species in terms of fresh consumption and juice manufacturing are sweet orange [C. sinensis (L.) Osb.], mandarin (C. reticulata Blanco), grapefruit (C. paradisi Macf.), pummelo [C. grandis (L.) Osb.], lemon [C. limon (L.) Burm.] and lime (C. auran-
tifolia Swing.), and each type of fruit has its typical unique flavor. Sour oranges (C. aurantium L.) are mainly used in marmalades and for essential oil production for food flavoring. In the following sections,
we describe the specific combinations of biochemical constituents that make up the unique flavors of oranges, mandarins, grapefruit, and lemons.

The flavor of oranges

Orange is the major citrus fruit produced worldwide and is the main citrus species used for juice manufacturing, and therefore much research has been dedicated to elucidating the biochemical basis of its flavor generation. Oranges have a desired sweet–sour taste, as they consist about 11–12% TSS and 1.0–1.2% TA, resulting in a TSS:TA ratio of ~10, and are considered non-bitter. The main aroma attributes of oranges are fruity, citrus, herbaceous, spicy, and floral notes (Arena et al. 2006). In various studies, it was found that oranges contain between 15 and 42 aroma-active compounds (Hinterholzer and Schieberle 1998; Buettner and Schieberle 2001b; Arena et al. 2006; Perez-Cacho and Rouseff 2008a; Averbeck and Schieberle 2009; Selli and Kelebek 2011). According to Perez-Cacho and Rouseff (2008a), the 36 consensus orange aroma-active compounds consist of 14 aldehydes, seven esters, five terpenes, six alcohols, and four ketones. Averbeck and Schieberle (2009) reported that the volatiles with the highest odor activity values (AOV >1000) were ethyl butanoate (fruity), linalool (flowery), and octanal (citrus-like), whereas Selli and Kelebek (2011) noted that the highest odor-active compounds were limonene, linalool, and nootkatone (citrus, grapefruit note). Miyazaki et al. (2011) showed that hybrids of mandarins and oranges that had an orange flavor contained more esters and sesquiterpenes than true mandarins, and that ethyl butanoate, ethyl hexanoate, and β-damascenone were true contributors to the fruity and orange flavor of this hybrid (Miyazaki et al. 2012). By conducting reconstitution experiments, Averbeck and Schieberle (2009) showed that a mixture of 14 odorants including ethyl butanoate (fruity), linalool (flowery), octanal (citrus-like), limonene (citrus-like), (S)-ethyl 2-methylbutanoate (fruity), α-pinene (pine tree), β-myrcene (metallic, geranium-like), acetaldehyde (pungent, ethereal), decanal (green, soapy), and (E)-β-damascenone (cooked apple) could successfully mimic the overall aroma of fresh orange juice.

The flavor of mandarins

Mandarins are easy to peel and easy to consume and therefore became very important in commercial trading of fresh citrus fruit. Overall,
The flavor of citrus fruit

Mandarins are somewhat sweeter and less sour than oranges and consist of about 11–13% TSS and 0.8–1.2% TA, resulting in an average TSS : TA ratio of ~12 (Table 1.1). Like oranges, mandarins do not have any bitterness. The main aroma attributes of mandarins are fruity, floral, citrus, green/grassy, fatty, metallic/rubber, herbal, and mushroom notes (Miyazaki et al. 2012). As for oranges, there is not just one specific character impact compound imparting typical mandarin flavor, which is rather generated from a combination of several different volatiles (Schieberle et al. 2003; Tietel et al. 2011b; Miyazaki et al. 2012). By comparing and summarizing data from different GC–MS studies of mandarins, it was found that nine volatiles were detected in almost all experiments, and thus can be considered as core aroma volatiles in mandarin juice. These core volatiles were linalool (floral, citrus), α-terpineol (floral), terpinen-4-ol (woody, earthy), nonanal (piney, floral, citrus), decanal (fatty, musty), carvone (spearmint, caraway), limonene (citrus-like), α-pinene (pine-like), and β-myrcene (musty, wet soil) (Tietel et al. 2011a). Recent GC–O evaluations of five distinct mandarin hybrids allowed the identification of 49 mandarin aroma-active compounds, which included seven monoterpenes, 12 aldehydes, three esters, four alcohols, seven ketones, and a few other volatiles (Miyazaki et al. 2012). It is worth noting that 1,8-cineole (green, herb), β-myrcene (green, metallic), (E,E)-2,4-nonadienal (fatty, vegetable), hexanal (green, grassy), ethyl 2-methylbutanoate (fruity, floral), and linalool (floral) were perceived with high odor intensities in most samples. Thymol and methyl N-methylanthranilate were reported earlier to be important compounds contributing to the typical mandarin flavor (Kugler and Kovats 1963), but this has only been partially demonstrated by addition of these compounds to bland mandarin juice (Wilson and Shaw 1981). To conclude, the genetic diversity in the group of Citrus reticulata Blanco is such that there is no typical volatile production pattern, and no obvious typical mandarin or tangerine flavor (Myazaki et al. 2011).

The flavor of grapefruit

Grapefruit are less sweet and more sour than oranges and mandarins, and consist about 10–11% TSS and 1.4–1.8% TA, resulting in a low TSS : TA ratio of ~6.6 (Table 1.1). In addition, grapefruit accumulate the flavonoid neohesperidose naringin that is responsible for providing a bitter taste (Horowitz and Gentili 1961). The main aroma attributes of grapefruit are fruity, sweet, green/grassy, terpene-like, pungent, citrus, sulfurous/catty, and grapefruit-like notes (Buettner and
Schieberle 1999, 2001a). It was found that fresh grapefruit juice consists of about 25–37 odor-active compounds, and that fruity notes were provided by ethyl 2-methylpropanoate, ethyl butanoate and (S)-ethyl 2-methylbutanoate; a sweet note was provided by wine lactone; grassy smelling notes were provided by (Z)-3-hexenal and trans-4,5-epoxy-(E)-2-decenal; and typical sulfurous grapefruit-like notes were provided by the catty, blackcurrant-like compound 4-mercapto-4-methylpentan-2-one and the grapefruit-like smelling volatile 1-p-menthene-8-thiol (Buettner and Schieberle 1999, 2001a). These findings regarding the above-mentioned grapefruit odor-active compounds were confirmed by reconstitution experiments that were able to simulate the aroma of fresh grapefruit juice (Buettner and Schieberle 2001a). Also, unlike oranges and mandarins, grapefruit juice flavor consists of a few “character impact compounds,” which provide typical grapefruit-like odor: nootkatone, 1-p-menthene-8-thiol, and 4-mercapto-4-methylpentan-2-one (Macleod and Buigues 1964; Demole et al. 1982; Buettner and Schieberle 2001a). Furthermore, the grapefruit sulfurous key odorant 1-p-menthene-8-thiol was reported to be one of the most powerful flavor compounds found in nature, with a taste detection threshold lower than $1 \times 10^{-4}$ ppb, i.e., a minute amount of just $1 \times 10^{-4}$ mg in 1000 L of water (Demole et al. 1982). In addition to 1-p-menthene-8-thiol, Jabalpurwala et al. (2010) was able to identify and quantify 13 sulfur volatile compounds, some of them having aroma activity and detected by GC–O (Jabalpurwala et al. 2010).

The flavor of lemons

The flavor of lemon fruit is dominated by its high acidity levels, usually between 4.4 and 6.4%, comprised mainly of citric acid and minor amounts of malic acid (Sinclair 1984). The high acidity levels in lemons and relatively low sugar levels result in a very sour sensation with an average TSS : TA ratio of ~1.6 (Table 1.1). Therefore, the flavor attributes of lemon are first 1 sourness, with some citrus-like/fruity background notes. It was found that lemon juice consists of about 26–35 aroma volatiles, the major volatile constituents being limonene, α-terpineol, 4-terpineol, neral, geranial, neryl acetate, geranyl acetate, linalool and 2-methyl-3-buten-2-ol (Moshonas and Shaw 1972; Kane et al. 1995; Allegrone et al. 2006). In terms of identification of lemon aroma-active compounds, it was suggested that citral is a key character impact compound of lemon odor (Ikeda et al. 1962).
Accumulation of off-flavors in fresh citrus fruit during postharvest storage

In today’s global markets, fresh citrus fruit are often shipped for long distances from production to consumption sites and, therefore, need to be held in cold storage for at least several weeks during the transportation and distribution processes. For example, shipment of citrus fruit from South America (in the southern hemisphere) to European markets (in the northern hemisphere) often requires maintaining fruit quality for at least 5–6 weeks after harvest, including 4 weeks of shipment by sea, followed by ground transportation by trucks, logistic distribution within supermarket chains, and marketing at retail shops. The problem is that during this long transport and distribution period, the fruit continues to respire and undergoes various metabolic changes that affect its biochemical composition, including sugars, acids, and aroma volatiles, which affect fruit flavor and overall sensory acceptability. The actual problem of decrease in “flavor life” is somewhat less prominent in grapefruit and lemons, but is more pronounced in oranges and especially in mandarins, which are more delicate and susceptible to the development of off-flavors after harvest (Tietel et al. 2011a).

The decrease in citrus fruit flavor acceptability after harvest is caused by two main factors: (1) a decrease in acidity during prolonged storage and (2) changes in aroma volatile levels and composition leading to a decrease in perception of typical citrus flavor on the one hand, and accumulation of off-flavors on the other. The decrease in acidity levels after harvest does not cause a serious problem in grapefruit and lemons, which are sour anyway, but may cause a crucial problem in late-season oranges and especially in low-acid mandarin varieties that have initial low acidity levels and therefore any further decrease in their acidity levels will result in the perception of low-acid, bland fruit (Grierson 1995; Tietel et al. 2010b; 2011a).

Regarding the observed changes in the aroma profiles of fresh citrus fruit after harvest, it was found that some volatiles, especially terpene hydrocarbons (β-myrcene, α-pinene, and terpinolene), terpene alcohols (linalool, β-citronellol, α-terpineol), aldehydes (pentanal, octanal, decanal), and 1-octanol, all of which impart pleasant, desirable, fruity and citrus-like notes, decreased during cold storage and, therefore, might account for the observed decrease in sensation of typical citrus and fruity flavor (Tietel et al. 2010b). In contrast, the contents of other volatiles, particularly those belonging to the ethanol fermentation
metabolism and amino acid and fatty acid catabolism pathways, increased significantly during storage. Specifically, the contents of the anaerobic fermentation products, ethanol and acetaldehyde, and of various ethyl esters which are amino acid catabolism products, such as ethyl 2-methylpropanoate and ethyl 2-methylbutanoate, or fatty acid catabolism products such as ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, increased significantly during postharvest storage in different mandarin varieties (Tietel et al. 2010b; Obenland et al. 2011). Most of these compounds impart solventy, pungent, malty, ethereal, or fruity aromas, and with the decrease of typical citrus volatiles are likely to account for the increase in perception of “fermented fruit” and off-flavors. Furthermore, the increase in accumulation of the above volatiles was further supported by a genome-wide transcript profiling analysis of Mor mandarin during storage using the Affymetrix Citrus Genome Array: significant upregulation of transcripts involved in ethanol fermentation metabolism and in amino acid and fatty acid catabolism pathways were detected (Tietel et al. 2011d). These were related to the need for the intact fruit to produce energy and synthesize acetyl-CoA, which is the immediate precursor of the tricarboxylic acid (TCA) cycle (Tietel et al. 2011d).

Based on the above findings, a model was proposed that describes the biochemical mechanisms leading to increased synthesis of ethanol and of ethyl ester volatiles during postharvest storage, which purportedly leads to the development of off-flavors (Fig. 1.2). According to this model, during storage, there is a simultaneous induction of ethanol fermentation metabolism (resulting in a many-fold accumulation of ethanol) and of fatty acid and amino acid catabolism, most likely as precursors for production of acetyl-CoA, which is the direct substrate of the TCA cycle required for energy production. Accordingly, the increased levels of ethanol together with accumulation of acyl-CoAs derived from fatty acid and amino acid catabolism provide substrates for further esterification reactions catalyzed by alcohol acyltransferases (AAT’s) that lead to the formation and accumulation of ethyl esters responsible for causing over-ripe and fermented off-flavor sensations (Fig. 1.2) (Tietel et al. 2011c). Thus, whereas in the past it was thought that only ethanol fermentation products were responsible for off-flavor development in citrus fruit (Davis and Hofmann 1973; Cohen et al. 1990; Hagenmaier and Shaw 2002), the recent data suggest that high levels of ethanol that accumulate during storage further serve as substrates for subsequent downstream esterification reactions with acyl-CoAs derived from fatty acid and amino acid
The flavor of citrus fruit

Postharvest storage

Amino acid catabolism
Ethanol accumulation
Fatty acid catabolism

Ethanol + amino acid derivatives
Ethanol + fatty acid derivatives

AATs

Ethyl 2-methylpropanoate
Ethyl 2-methylbutanoate

Over-ripe odor

Ethyl acetate
Ethyl propanoate
Ethyl butanoate
Ethyl 2-butanoate
Ethyl hexanoate
Ethyl octanoate
Ethyl decanoate

Off-flavor odor

Fig. 1.2  Model describing the biochemical pathways involved in ethyl ester synthesis, responsible for the formation of over-ripe and off-flavor sensations during postharvest storage of citrus fruit. AAT, alcohol acyltransferase.

catabolism, resulting in the accumulation of ethyl ester volatiles, which impart over-ripe and undesired odors, and contribute to the sensation of off-flavors in stored fruit (Fig. 1.2).

Flavor of citrus essential oils

A chapter on citrus flavor would not be complete without a mention of citrus oils. As mentioned in the introduction, the exocarp contains the oil gland (Figs 1.1b and 1.3). Any process involving peeling and crushing the fruit will break the oil gland and entrain volatile components in the flesh or juice. In fact, the type of juice extractor will affect the amount of peel oil in an orange juice, and modify its biochemical properties and taste (Baldwin et al. 2012). Even the simple act of peeling a fruit before eating will provide some transfer of peel oil onto the fruit. A good example is that volatiles contributing to clementine flavor were
very different from those in the fresh fruit (Schieberle et al. 2003) and in the peel oil (Buettner et al. 2003): compounds with a high odor impact in fresh clementines were pyrazines and esters (Schieberle et al. 2003), whereas in peel oil they were mostly aldehydes (Buettner et al. 2003).

Commercially, most citrus oils are the result of mechanical cold pressing (or extraction), except lime oil, which is produced by directly distillation of the lime fruit (Haro-Guzmán 2002). For cold extraction, the general mechanism is by abrasion of the fruit surface in special hoppers, pressing between rollers with spikes or special screws, and the oil is washed away with a spray of water. Such systems include the “pelatrice,” Brown Oil Extractor, Polycitrus Extractor, and various others, and are described in detail in several specialized books (Guenther 1949; Di Giacomo and Di Giacomo 2002; Can Baser and Buchbauer 2010). One system widely used in orange oil production is the FMC (now JBT) peel oil recovery system (JBT Food Technologies, Lakeland, FL, USA). In that system, the fruit is crushed with its peel being shredded, thereby rupturing the oil glands. From there, the fruit juice is collected and sent to further juice production with various levels of filtering through “finishers,” and in a separate process the oil is washed away with pressurized water. As in the other systems, the oil does not come into contact with the juice, and is
recovered from the water emulsion by centrifugation (Di Giacomo and Di Giacomo 2002). Oil yields vary dramatically with the type of fruit, harvest maturity, growing conditions (soil and climate), and, of course, type of extraction process. For example, for oranges, the peel oil yield may vary from 3.0 to 7.5 kg per metric ton (MT) of fruit (Kesterson and Braddock 1975). In other words, 1 kg of fruit contains between 3.0 and 7.5 g of essential oil. Further, there can be 2.2 times as much essential oil from unripe fruit as from ripe fruit (Guenther 1949). For other citrus, peel oil yields were 2.0–4.0 kg/MT for grapefruit, 3.5–5.0 kg/MT for Persian lime and 5.0–11.5 kg/MT for lemon (Kesterson and Braddock 1975). Likewise, oil quality can vary with the extraction process, with the traditional hand pressing of the peel or “sfumatura” (slow-folding) methods giving the highest quality oil with most of the top notes. Finally, essential oils must be stored appropriately to avoid any photodegradation and oxidation (Di Giacomo and Di Giacomo 2002).

Cold-pressed oil consists mainly of terpene hydrocarbons, with limonene comprising 70–96%, depending on the fruit and extraction technique. However, oxygenated compounds such as aldehydes, esters, ketones, and alcohols are more desirable because they provide the typical aroma of the fruit from which they are extracted and the “top notes” in citrus beverages (Moyler 2002; Perez-Cacho and Rouseff 2008b). Oxygenated compounds are soluble in alcohols and, except for some aldehydes, are stable under oxidative conditions. In contrast, non-oxygenated terpene hydrocarbons contribute only slightly to the flavor. Moreover, owing to their unsaturated character, these compounds can be easily oxidized when in contact with air and light or under inappropriate storage conditions such as elevated temperature, thus damaging or altering the flavor (Guenther 1949; Perez-Cacho et al. 2008b). For example, limonene can be oxidized to carvone and carveol, which are undesirable in orange oil. Therefore, the essential oil composition can be improved by concentration and/or fractionation into desired component mixtures. These “deterpenation” processes involve solvent extractions, distillation, or chromatographic methods (Moyler 2002; Ziegler 2011).

Solvent extractions are most typically used with ethanol–water mixtures and are based on the solubility properties in hydro-alcoholic systems of the different compounds of the essential oil. The oil is mixed with a water–ethanol mixture and then allowed to settle to separate the lipophilic (terpene hydrocarbons) from hydrophilic (oxygenated compounds) phases. This process is called “washing” as it is a long-used purification technique. Oxygenated compounds
and terpene hydrocarbons are soluble in water–ethanol mixtures that contain a minimum of 46–48 and 78–79% v/v of ethanol, respectively (Licandro et al. 1990). The number of extractions (or washings) will affect the final concentration of terpene hydrocarbons; increasing the number of successive extractions will increase terpene hydrocarbon removal. As an example, Table 1.3 gives the composition of sweet orange essential oil before and after different concentration processes (Owusu-Yaw et al. 1986). This study shows that a small change in solvent concentration, together with the appropriate oil to solvent ratio, can significantly affect the removal of terpene hydrocarbons: 61.0% of limonene still remained in the essential oil after solvent extraction at 60% ethanol whereas only 2.7% was left when using 70% ethanol, with an oil to solvent ratio of 1:7.

In addition to ethanol, other solvents could be used but are less practical nowadays with stricter environmental and food regulations. Supercritical fluid extraction using carbon dioxide (CO\(_2\)) as the solvent has potential application, but it requires special equipment able to handle high pressures that may be too costly for small operators (Moyler 2002). In a study on deterpenation of bitter orange oil, CO\(_2\) extraction yielded a terpene-rich fraction with mainly limonene (88%) but also some oxygenated terpenes and an oxygenated-rich fraction containing linalool (4.7%), linalyl acetate (6.1%), α-terpineol (3.1%), and geranyl acetate (2.8%) (Chouchi et al. 1996). This technique also allows for the complete removal of the undesirable non-volatile residues such as waxes and coumarins.

Table 1.3  Compositions of concentrated sweet orange essential oil (EO) produced from distillation or solvent (ethanol) extraction. Values are percent of oil composition. Source: Adapted from Owusu-Yaw et al. (1986).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Orange EO before treatment</th>
<th>“10-fold” orange EO produced by distillation</th>
<th>Orange EO after extraction at 60 vol.% of ethanol (EO: ethanol ratio = 1:7)</th>
<th>Orange EO after extraction at 70 vol.% of ethanol (EO: ethanol ratio = 1:7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoterpenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>98.89</td>
<td>82.37</td>
<td>67.38</td>
<td>14.18</td>
</tr>
<tr>
<td>Limonene</td>
<td>96.08</td>
<td>81.09</td>
<td>60.91</td>
<td>2.74</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>0.12</td>
<td>1.61</td>
<td>0.18</td>
<td>0.32</td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldehydes</td>
<td>0.69</td>
<td>4.37</td>
<td>18.9</td>
<td>51.21</td>
</tr>
<tr>
<td>Alcohols</td>
<td>0.25</td>
<td>0.8</td>
<td>8.05</td>
<td>22.15</td>
</tr>
<tr>
<td>Esters</td>
<td>nd</td>
<td>0.05</td>
<td>0.27</td>
<td>0.47</td>
</tr>
<tr>
<td>Ketones</td>
<td>0.02</td>
<td>0.16</td>
<td>0.51</td>
<td>1.08</td>
</tr>
</tbody>
</table>

*An EO called “10-fold” is a concentrated EO produced by distillation and the final mass represents one-tenth of the initial mass.
Distillation is a process in which the differences in boiling temperatures between hydrocarbons and oxygenated compounds make it possible to separate them. Mild conditions are necessary because numerous terpene hydrocarbons are thermally sensitive and oxidize easily, and their degradation might affect the flavor of the resulting concentrated essential oil. Distillation under vacuum lowers the components’ boiling points and allows working at lower temperatures (Guenther 1972; Ziegler 2011). The citrus industry traditionally used distillation to separate hydrocarbons from the oxygenated fraction, and thus created a nomenclature for the oils that were concentrated a certain number of times. From Ziegler (2011): “if 80% of the volatile, terpene hydrocarbons containing part (distillate) was removed from the oil, a residue of 20% (concentrate) remained; thus a yield of 20% was obtained and the oil was characterized as 100/20 = 5-fold.” A single-fold oil is the original cold-pressed oil, and oils can be sold as up to 20-fold (Ziegler 2011). For detailed information on the composition and quality of citrus oils, readers are referred to the two specialized books by Dugo and Di Giacomo (2002) and Dugo and Mondello (2011).

Citrus oils are used in foods and perfumes. In foods, they are used extensively in beverages and in confectionery (candies, cookies, and ice-cream). In the beverage industry, they are widely used as flavorings in sodas and soft drinks. Orange essence oil has its own use as it is usually added back to processed orange juice, where it was lost and recovered in the manufacturing process (Colombo et al. 2002). Two specialty oils are bergamot oil from *Citrus bergamia*, used in tobacco flavoring and in the aromatization of the British Earl Grey tea, and bitter orange oil (from *Citrus aurantium* L.), mostly used in liqueurs (Colombo et al. 2002). Lemon and lime oils are used in many foods and also cosmetic products (Colombo et al. 2002). Further, because of its properties as a solvent and its biological properties, limonene from orange oil, or orange oil itself, is included in many industrial cleaners and degreasers, and are used in manufacturing processes that require metal degreasing or adhesive removal (automotive industry, printing, gas industry, textiles, etc.). Orange oil is also an ingredient in household cleaners, agrochemicals (such as insecticides), and medical and pharmaceutical products (Figueiredo 2012).

In terms of volume of oil produced in the world, most is from sweet orange (about 62,000 tons), as a by-product of orange juice processing, followed by lemon (5800 tons), and lime (1300 tons) oils (Di Giacomo 2002). The main producing countries are Brazil and the United States for orange oil, Argentina for lemon oil, and Mexico for lime oil.
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References


Dugo, G. and Di Giacomo, A. (eds.), *Citrus. The Genus Citrus*. CRC Press, 
Boca Raton, FL, pp. 114–147.

Dornelas, M.C. and Mazzafera, P.A. (2007) Genomic approach to character-
ization of the Citrus terpene synthase gene family. *Genetics and Molecular 
Biology*, 30, S832–S840.

Press, Boca Raton, FL.

lytical Techniques, Contaminants, and Biological Activity*. CRC Press, Boca 
Raton, FL.

Division of Agricultural Science, University of California, Berkley, CA, pp. 
86–126.

Figueiredo, L. (2012) The Brazilian orange oil and essences industry. Pre-

tented at the International Citrus Beverage Conference, 18–21 September 
2012, Clearwater, FL. http://conference.ifas.ufl.edu/citrus/agenda.html 
(accessed 4 September 2015).

Frydman, A., Weisshaus, O., Bar-Peled, M., Huhman, D.V., Sumner, L.W., 
rus fruit bitter flavors: isolation and functional characterization of the gene 
*Cml, 2RhaT* encoding a 1,2 rhamnosyltransferase, a key enzyme in the 


Grierson, W. (1995) Late season storage and export: how to make some money 

Florida Science Source, Longboat Key, FL, pp. 1–22.

Miller, W.M., Hall, D.J. and Grierson, G. (eds.), *Fresh Citrus Fruits*, 2nd 

Grosch, W. (2001) Evaluation of the key odorants of foods by dilution exper-

Guadagni, D.G., Maier, V.P. and Turnbaugh, J.G. (1973) Effect of some citrus 
juice constituents on taste thresholds for limonin and naringin bitterness. 
*Journal of the Science of Food and Agriculture*, 24, 1277–1288.

Families Rutaceae and Labiatae*, vol. 3. Robert E. Krieger, Huntington, 
NY, pp. 5–359.


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