Chapter 2

Literature review

The origins of nearly all citrus varieties were probably in the Himalayas region of northern India, southwestern China and the adjacent Bhutam and Burma. The first written reference to oranges appeared in Chinese charactors around 2200 B.C. Little is known about the route of citrus fruits as they spread around the world. It is believed they were spread to the Mediterranean and hence to southern Europe in the Middle Ages. The Europeans brought them across the Atlantic. Citrus was brought to the Americas by Columbus in 1493 and the first seeds planted on the main land of the Americas were brought by the expendition of Juan de Grijalva when he landed in Central America in 1518. The exact date of the introduction of citrus trees into Florida is not known. One of the oldest cultivated groves in Florida was planted sometime between 1809 and 1820 (Nagy *et al.*, 1992).

2.1 Varieties of oranges

Citrus fruits belong to the Rutaceae family and are classified as follows (Rieger, 2005):

2.1.1) *Citrus limon* Burm. f. - Lemons. Variants of this species include: rough lemon (*C. jambhiri*), sweet lemon (*C. limetta*) and Volkamer lemon (*C. volkameriana*).

2.1.2) *Citrus reticula* Blanco - Tangerine, mandarin or satsuma. Due to the success of breeding with these types (as they are often monoembryonic), many cultivars and hybrids have been produced or formed naturally, some erroneously given species status. Other species names sometimes are given in the literature include: *C. unshiu* (Satsuma), *C. deliciosa* (Willowleaf), *C. reshni* (Cleopatra), *C. nobilis* (King) and *C. temple* (Temple).

2.1.3) *Citrus medica* L. - Citron. This lemon-like fruit may be the progenitor species of modern lemons and limes. The peel is very thick and the white, spongy portion of the peel is edible. Rarely seen in the USA, it is used in Mediterranean countries, particularly Israel. 'Etrog' citron is used in the Jewish feast of the Tabernacles. 'Buddha's Hand' or 'Fingered Citron' is a striking fruit sometimes used in a religious context; outgrowths of peel tissue appear as fingers.

2.1.4) *Citrus grandis* (L.) Osb. or *C. maxima* (Burm.) Merr. - Pummelo or shaddock. This species originates from southeast Asia where it is as common as grapefruit in the USA. It is much larger and thicker-peeled than grapefruit, but has milder flavor. There are some arguments about its nomenclature.

2.1.5) *Citrus paradisi* Macf. - Grapefruit. This is clearly not a true species, but its economic importance today has granted species status. Grapefruit is thought to be a hybrid of pummelo and sweet orange that occurred naturally somewhere in the Caribbean between the time of Columbus' voyages and its introduction to Florida in 1809.

2.1.6) *Citrus aurantifolia* L. - Limes. The literature distinguishes the two main cultivars - 'Key' and 'Tahiti' - as separate species, with the latter labeled *C. latifolia* Tanaka or *Citrus X tahiti* Campbell. Cultivar and variants include: 'Rangpur' lime (*C. limonia*) and sweet limes (*C. limettioides*).

2.1.7) *Citrus sinensis* - sweet orange. This is a widely accepted name for this crop, containing 4 groups of cultivars: common oranges, blood oranges, navel oranges and acidless oranges. Examples include: 'Temple' and 'Page' oranges (tangerine hybrids), Satsuma orange (a cold hardy variant of tangerine) and Trifoliate orange (*Poncirus trifoliata*).

2.1.8) *Citrus aurantium* L. - Sour orange. This is allied with limes by some, but is a very important rootstock and ornamental. Since it often appears in the literature, it is convenient to keep this species name. Cultivars and variants include:

Bittersweet, Oklawaha, Vermillion Globe, Paraguay, Trabut var. *myrtifolia* (Myrtle), Bergamot, Daidai (Japanese), Leaf of Chinnoto and *C. taiwanica* Tanaka.

The orange consists of an outer peel which serves largely as a cover to the inner pulp or juice-bearing bodies of the fruit. Viewing these facts in more detail in Figure 2.1, the inner flesh of the ripe fruit consists of segments (carpels, locules) distributed about a soft pithy core forming the central axis of the fruit. Each segment is surrounded by a thin wall (carpellary membrane, locular wall) which is a tissue of epidermal origin. The juice-containing bodies of the mature fruit are closely compacted, club-shaped vesicles which completely fill the segments and are attached to the walls with small hair-like papillae. Multicellular in structure, the extremely thin-walled cells contain, besides juice, the color-bearing yellow chromatophores. Oil droplets embedded within the cellular tissue occur in the central part of each juice vesicle. In addition, the segment of most varieties contain seeds attached by means of placentae to the walls (carpellary membrane) where these come in contact with the pithy core of the fruit. The surrounding rind, or peel, consists of an outer colored portion (flavedo) and an inner white, spongy layer of parenchymatous cells (albedo) closely adherent to the outer walls of the segments. The epidermal layer of cells comprising the flavedo contain numerous oil vesicles and chromatophores. The oil vesicles are balloon-shaped cells which are more or less easily ruptured; oil from these

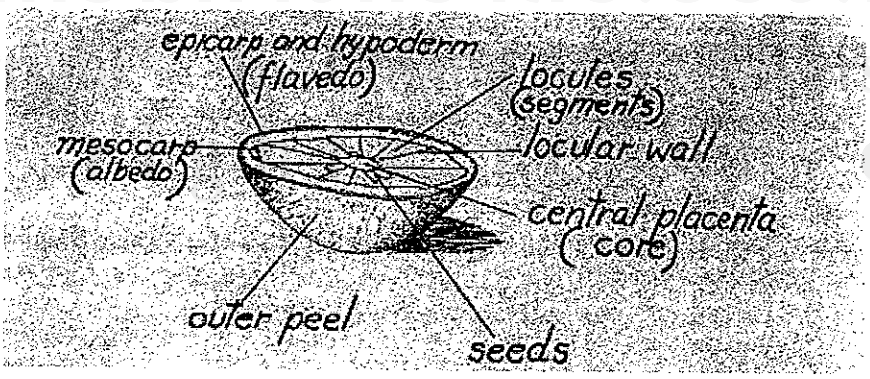


Fig. 2.1 A macroscopic structure of halved orange

cells furnishes the familiar orange oil of commerce. The chromatophores are green in young fruits and yellow in mature fruits. The cells of the spongy tissue underlying the epidermal cells are loosely arranged toward the inner portion. An extensive system of radially branching vascular bundles extends throughout this whole tissue (Tressler and Joslyn, 1971). For the dietary value of different citrus fruits, it can be seen in Table 2.1.

Table 2.1 Dietary value of citrus fruits, per 100 gram edible portion (Rieger, 2005)

	Sweet orange	**Grapefruit**	**Tangerine**	**Lemon**	**Lime**
Water (%)	88	90	87	90	86
Calories	44	40	44	27	37
Protein (%)	0.75	0.45	0.8	1.1	0.1
Fat (%)	0.2	0.1	0.3	0.3	0.3
Carbohydrates (%)	10.3	9.5	10.9	8.2	12.3
Crude Fiber (%)	0.6	0.3	1.0	0.4	0.9
	% of US RDA*				
Vitamin A	4.0	0.2 (white) 8.8 (pink)	1.9	<1.0	<1.0
Thiamin, B1	6.4	2.8	5.6	2.9	3.1
Riboflavin, B2	1.9	1.2	1.7	1.2	1.1
Niacin	2.2	1.1	1.6	0.5	1.1
Vitamin C	109	84	76	118	88
Calcium	1.2	1.1	4.5	3.2	2.4
Phosphorus	2.1	1.9	2.2	2.0	1.9

Table 2.1 (continued) Dietary value of citrus fruits, per 100 gram edible portion

	Sweet orange	Grapefruit	Tangerine	Lemon	Lime
Iron	2.5	2.0	4.0	6.0	2.6
Sodium	---	---	---	---	---
Potassium	4.2	3.4	2.3	2.9	2.1

* Percent of recommended daily allowance set by FDA, assuming a 70 kg male adult, 2700 calories per day.

For mandarins and tangerines, the fruits come from small, thorny trees that bear simple leaves and orange-like blossoms. The fruits resemble slightly flattened oranges with loose orange skin and have a fragrant aroma. Not only do the skins peel easily, but the segments also come apart with ease. Although the flesh is sweet and juicy, it does contain a high number of seeds. The word reticulata means 'netted', in reference to the fibrous strands of pith located under the loose skin. All mandarins and their hybrids are extremely good sources of Vitamin C and beta-carotene (Anonymous, 2005a).

The production of orange juice products has undergone a remarkable development in the past 25 to 30 years. Previous to the World War II orange juice production was 3 million cases a year or lower and blends of orange and grapefruit about the same, while frozen concentrated juices and tangerine juices were not existent. Since that time the volume of citrus juice product has increased until it has reached the equivalent of over 175 million cases (24 cans 18 oz) per year and has become a very important factor in the diet (Tressler and Joslyn, 1971).

2.2 Varieties of orange in the northern part of Thailand

Some mandarin varieties that are commercially grown in the northern part of Thailand include (Anonymous, 2005b):

2.2.1) Thanathon No.1. The fruit has a yellow peel with a big, thick and soft pulp. The fruit segment has a sweet and sour taste with a lot of aromatic juices.

2.2.2) Fremont. Fremont is a mandarin hybrid introduced by the California Citrus Experimentation Station. Its deep orange rind is medium thick, but easy to peel. It is a delicate fruit, with up to twelve segments, and plenty of seeds. Its bright reddish-orange flesh has a rich, sweet taste compared to that of Clementine. The Freemont, grown in Turkey, is able to sustain its abundant juiciness for more than three months after maturing on the tree.

2.2.3) Special Honey. The variety is a mandarin hybrid produced from the King Mandarin and the Willowleaf, accomplished by H. B. Frost of the University of California Research Center, in Riverside. It is a small mandarin, with a glossy, golden-orange rind and a flame orange flesh that can house up to twelve segments and many seeds. The flesh is easy to pull apart and very juicy.

2.2.4) Golden Skin. The orange fruit is small with a yellow peel. However, the flesh is easy to peel and has a sweet taste.

2.3 Benefit for the consumption of oranges and their juices

Several recent studies have suggested that citrus fruits and their juices have the following potential benefits (Anonymous, 2000):

2.3.1) A study in the *Journal of the American Medical Association* found that consuming just one extra daily serving of certain fruits and vegetables, including citrus fruits or juices, reduces the risk of stroke by about 20%. Earlier research had also suggested that flavonoids such as those in citrus can lower the risk of stroke.

2.3.2) An Israeli study linked orange juices with a reduction in oxidation of Low Density Lipoprotein (LDL) cholesterol. Oxidation increases artery-damaging deposits and thus promotes atherosclerosis.

2.3.3) A Canadian study found that orange juice could boosts High Density Lipoprotein (HDL) cholesterol. However, further studies will be needed to confirm this finding.

2.3.4) A Dutch study found that citrus fruit, probably because of its supply of the B vitamin folic acid, lowers blood levels of homo-cysteine, a substance that may increase the risk of heart disease.

2.3.5) Citrus skin contains D-limonene and coumarins, which leach into the juice. D-limonene may help detoxify potentially cancer-causing compounds while reducing the activity of proteins that trigger abnormal cell growth. Coumarins help reduce blood clotting and may also have anti-cancer capacity. Certain compounds that give citrus fruit their bitter or tart taste are also believed to have antioxidant and anti-cancer effects.

2.3.6) A study presented in the 1999 conference of the American Institute for Cancer Research showed that animals fed orange juice had a reduced incidence of colon cancer. A further study is needed whether a similar effect can happen in humans.

2.3.7) Oranges contain the following carotenoids: beta cryptoxanthin, which may help reduce the risk of certain cancers; and zeaxanthin and lutein, which may help keep eyes healthy. Besides beta carotene, pink and red grapefruit provide lycopene, another carotenoid, which may help prevent prostate cancer.

2.4 Codex standards for orange juices

The description of an orange juice based on Codex Standard is the unfermented but fermentable juice, intended for direct consumption, obtained by a mechanical process from the endocarp of sound, ripe oranges (*Citrus sinensis* (L.) Osbeck), preserved exclusively by physical means. The juice may contain up to 10% m/m of mandarin juice (*Citrus reticulata* Blanco). The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factors of the juice (Codex, 2005). The nutrition value of an orange juice can be seen in Table 2.2.

Table 2.2 Nutrition value of orange juices (Anonymous, 2005c)
(per 1 oz or 28.35 g serving)

Food components	**Amount**	**Food components**	**Amount**
Calories	14 kcal	Fiber	0.1 g
Energy	58 kj	Sugars	2.6 g
Fats	0.1 g	Cholesterol	0 mg
Carbohydrates	3.2 g	Sodium	3 mg
Protein	0.2 g	Alcohol	0 g

The essential composition and quality factors of orange juice based on Codex standard include (Codex, 2005):

1. The content of soluble orange solids in an orange juice (exclusive of added sugars) shall be not less than 10.0% m/m as determined by a refractometer at 20°C, uncorrected for acidity and read as °Brix on the International Sucrose Scales. Where the juice had been obtained using concentrated juice with the addition of water, the soluble orange juice solids content shall be not less than 11% m/m as determined by a refractometer at 20°C, uncorrected for acidity and read as °Brix on the International Sucrose Scales.

2. One or more solid sugars, as defined by the Codex Alimentarius Commission, may be added. The total quantity of added sugars shall not exceed 50 g/kg.

3. **The ethanol content** shall not exceed 3 g/kg.

4. **Only traces of volatile acids** are allowed.

5. **The essential oils content** shall not exceed 0.4 ml/kg.

6. **The product shall have the characteristic colour, aroma and flavour of orange juice.** Natural volatile orange juice components may be restored to any orange juice from which natural volatile orange juice components have been removed.

7. **The addition of concentrate to juice** is permitted. Only concentrate from orange (*C. sinensis* (L.) Osbeck) and mandarin (*C. reticulata* Blanco) may be used.

The maximum level of contaminants, which allow in orange juices include: arsenic (As) 0.2 mg/kg, lead (Pb) 0.3 mg/kg, copper (Cu) 5 mg/kg, zinc (Zn) 5 mg/kg, iron (Fe) 15 mg/kg, tin (Sn) 250 mg/kg, the sum of copper, zinc and iron 20 mg/kg and sulphur dioxide 10 mg/kg (Codex, 2005).

When tested by appropriate methods of sampling and examination, the product shall (Codex, 2005):

(a) be free from microorganisms capable of development under normal conditions of storage; and

(b) not contain any substances originating from microorganisms in amounts which may represent a hazard to health.

2.5. Composition of orange juice

Australia and New Zealand Food Authority reported that the sweetness of fruit is mostly attributed to sugar (mainly sucrose). The amount of sucrose found in fresh oranges varies between 2 and 4%, depending upon seasonal and product variability. The other major sugars found include fructose and glucose, which together with sucrose accounts for more than 90% of the total sugars found in oranges. The total amount of these sugars is in the vicinity of 7 to 8%. The common available salts in orange juices include sodium (common table salt – sodium chloride), potassium and magnesium. Although there are no prescribed standards for sodium and potassium levels, they are the most common metallic ions in this food product. The following Table 2.3 demonstrated the mean and range of sodium, potassium and magnesium values for orange juice and orange fruit drink (Anonymous, 2003).

Table 2.3 The mean and range of sodium, potassium and magnesium levels for orange juice and orange fruit drink (Anonymous, 2003).

Category	No. Samples	Sodium	Potassium	Magnesium
OJ – sugar *	19	1.9 (0.4 – 4.1)	166 (140 – 220)	8.6 (7.2 – 10.8)
OFD + sugar	22	4.7 (1.4 – 7.3)	49 (35 – 66)	2.7 (1.6 – 4.0)

All results expressed in milligrams per deciliter (mg/dl).

*OJ – sugar : Orange juice – no added sugar

OFD + sugar : Orange fruit drink – added sugar

As indicated, the amount of sodium is slightly elevated in the fruit drink and is most probably due to the water addition because the water conversely affects the levels for potassium and magnesium. Sodium chloride could also be added to the product as it acts as a sweetness enhancer (Anonymous, 2003).

At present, consumers desire for high quality food products that are convenient, nutritious, with freshly prepared flavor, texture and color, with minimal or no chemical preservatives and above all safe. Orange juices are favorite for people because its high vitamin C and desirable flavor. However, a few studies about an effective quality control for natural unpasteurized orange juice shown that natural orange juices, even kept under refrigeration, have a short shelf life (de Souza *et al.*, 2004). Orange juice is susceptible to degradation by heat, microorganism, enzymes, oxygen and light during processing and storage. The shelf life of unpasteurized orange juice is only 12 days at 4.4^{0}C (Jia *et al.*, 1999).

During storage of fresh orange juices, their quality can changes such as loss of nutrition (vitamin C and carotenoids), off- flavor, changes in color, cloud loss and microbial spoilage (Lee and Coates, 1999). The stability of orange juice depends on the raw materials, processing conditions, packaging material and storage conditions (de Souza *et al.*, 2004). Product characteristics are also significantly dependent on the cold chain during storage and distribution, especially for unpasteurised orange juice (Zanoni *et al.*, 2004). These factors could cause microbiological, enzymatic, chemical and physical alterations that damage the sensorial and nutritional characteristics of orange juice. Therefore, several physical and chemical determinations (pH, total soluble solid content and total titratable acidity) are important for orange juice characterization and quality (de Souza *et al.*, 2004).

2.6. Characteristics of orange juice

2.6.1. Nutritional characteristics

2.6.1.1. Vitamin C is one of the principle nutritional components of orange juices and is known to degrade under less desirable storage conditions. Quantitative analysis of vitamin C content was considered as one of the simple approaches to predict the shelf-life of citrus products (Lee and Coates, 1999). Vitamin C is a water-soluble vitamin, which is present in fresh fruit, especially citrus fruit. It involves in wound healing, tyrosine metabolism, conversion of folic acid to folinic acid, carbohydrate metabolism, synthesis of lipids and protein, iron metabolism, resistance to infections and cellular respiration. In addition, vitamin C shows antioxidative effects and under certain conditions can protect against oxidatively induced DNA damage (Suntornsuk *et al.*, 2002). Therefore, the vitamin C content represents a stimulating factor for orange juices consumption (de Souza *et al.*, 2004).

In orange juice, the concentration of vitamin C is the most important indicator of nutritional quality. The Recommended Daily Intake (RDI) of vitamin C in Australia and in the United Kingdom is 40 mg and is 60 mg in the United States (Bull *et al.*, 2003). The Thai RDI of vitamin C is 35% for population over 6 years of age based on a 2,000 kcal diet. The amount of vitamin C in orange juice depends on many factors: the variety of orange, their ripeness, the climate and season in which they grew and how orange juice was handled, processed and stored (Anonymous, 2000).

Vitamin C is thermolabile and therefore in fruit and vegetables it provides an indication for the loss of other vitamins and acts as a valid criterion for other organoleptic or nutritional components, such as natural pigments and aromatic substances (Torregrosa *et al.*, 2005). In addition, cytochrome oxidase, ascorbic acid oxidase, and peroxidase are known to be present in citrus fruits and are responsible for the oxidation of vitamin C (Lee and coates, 1999).

Factors that affected the vitamin C levels include (Nagy, 1980) :

1. Production factors and climate conditions: High nitrogen fertilizer rates can lower vitamin C levels in citrus fruits. Proper potassium levels are also needed for good vitamin C levels. Additionally, climate, especially temperature, total available heat, affect vitamin C levels. Areas with cool nights produce citrus fruits with higher vitamin C levels. Hot tropical areas produce fruit with lower levels of vitamin C. Environmental conditions that increase the acidity of citrus fruits also increase vitamin C levels.

2. Maturity state and position on the tree: Vitamin C decreases during the ripening process. Immature fruit has the highest levels. The position on the tree also affects vitamin C levels. Since sunlight exposure enhances vitamin C levels, fruit positioned on the outside of the tree and on the south side have higher levels. Shaded inside fruit has the lowest.

3. Type of citrus fruit (species and variety): Early maturing varieties have higher levels than that late maturing types. Early Hamlin and Navel fruits have more vitamin C than the late maturing Valencia. Tangerines tend to have lower levels of vitamin C than oranges due to its lower acid levels. The peel had the highest levels of vitamin C followed by the pulp then the juice. Only 26% of vitamin C of a citrus fruit can be found in the juice. The peel had 53% and the pulp and rag had 21%.

4. Parameters used for processing into different products: Frozen concentrated orange juice (FCOJ) and reconstituted FCOJ almost always have higher levels of vitamin C and is above the 100% US RDA values. This is most likely due to blending of early-season fruit with late season fruit. Canned single strength orange juice will have lower vitamin C levels due to heating during the canning process. NFC, Not-From-Concentrate, will vary due to the varieties being processed.

5. Type of container: In cans, which are not used very much today, it was found that enamel-lined cans had higher losses of vitamin C than plain tin cans. This was due to residual oxygen and vitamin C reacting with the tin. Glass packed orange juice provides poor retention of vitamin C, losing 10% after 4 months of storage. Older cardboard cartons lost up to 20%. (Today, most cartons have specially designed multi-layered oxygen and light barriers to protect both loss of vitamin C, flavor and to enhance shelf-life). A study result also found that in orange juice containers, vitamin C loss was due to oxidation by a residual air layer trapped within the container during processing. The loss was faster in the first 2 weeks and was more evident at higher storage temperatures. Therefore, orange juice must be kept cool to prevent vitamin C degradation as it is accelerated at high storage temperatures.

6. Handling and storage: Oxygen is the most destructive ingredient in juice causing degradation of vitamin C. However, one of the major sugars found in orange juice, fructose, can also cause vitamin C breakdown. The higher the fructose content, the greater the loss of vitamin C. Conversely, higher acid levels of citric and malic acids stabilize vitamin C. Orange juice must be stored at proper cool temperatures with oxygen barriers for best retention of vitamin C levels. When fresh citrus is stored at 38°F for 12 weeks, there was no loss of vitamin C, but when stored at high temperatures, the loss was great.

Several analysis methods that can be used to determine vitamin C include (Suntornsuk *et al*., 2002) :

1. Ultra-violet (UV) spectrophotometry with zero-crossing technique can be employed to analyze a mixture of ascorbic acid, pyridoxine hydrochloride and tyrosine.

2. UV spectrophotometry and high performance thin layer chromatography (HPLC) for the simultaneous determination of vitamin C and dipyrone in pure form and in pharmaceutical dosage forms.

3. Microfluorometry and semi-automated fluorometry for quantitation of total vitamin C in vitamin preparations and food

4. Enzymatic method using ascorbate oxidase from starfruit (*Averrhoa carambola*) to determine vitamin C.

5. Titration methods can be carried out using different reagents, including (Denby, 1996):

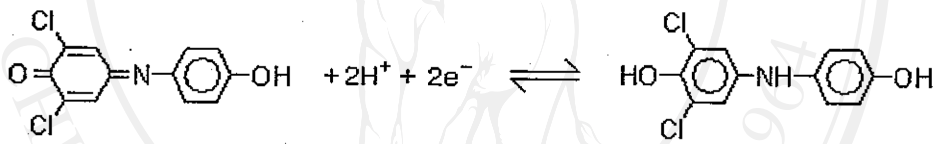
5.1. **Iodine and a starch indicator**. First, iodine reacts with ascorbic acid and when all the ascorbic acid has reacted, the excess iodine will form a blue/black complex with the starch indicator. This indicates the end point of the titration. A better method for this is reacting the ascorbic acid with iodine in excess, then back titration against sodium thiosulfate (using starch as an indicator).

5.2. Iodate and Iodine Ions. This is to generate the iodine in the presence of the ascorbic acid by the reaction of iodate and iodine ion in acid solution.

5.3. N-Bromosuccinimide. A much less common oxidising agent is N-bromosuccinimide (NBS). In this titration the NBS oxidises the ascorbic acid (in the presence of potassium iodide and starch). When the NBS is in excess (i.e. the reaction is complete) the NBS liberates the iodine from the potassium iodide which then forms the blue/black complex with starch, indicating the end point of the titration.

5.4. DCPIP. A commonly used oxidising agent is the dye 2,6-dichlorophenol-indophenol or DCPIP. The blue dye is run into the ascorbic acid solution until a faint pink colour persists for 15 seconds. The determination is thus based on colorimetric change caused by oxidation of ascorbic acid (Fig, 2.2).

DCPIP is an oxidising agent and in its natural state is a blue solid. However if a solution of the dye (also blue) is titrated with a reducing agent (such as ascorbic acid) it gains electrons and the newly formed compound is colourless (Denby, 1996).



DCPIP

Reduced DCPIP

Fig. 2.2 The reaction of DCPIP

2.6.1.2. Carotenoids

Carotenoids are a widely distributed group of naturally occurring pigments, usually red, orange or yellow in color. Carotenoids are used extensively as safe, natural colorants for food, feed and cosmetics. The compounds are known to be essential for plant growth and photosynthesis, and are a main dietary source of vitamin A in humans. They are thought to be associated with reduced risk of several chronic health disorders including some forms of cancer, heart disease and eye degeneration (Anonymous, 2006a). Carotenoids are also quality indicators for orange juice as they contribute both to the color and the nutritional value of the juice (Bull *et al.*, 2004). The most widely studied and well-understood nutritional role for carotenoids is their provitamin A activity. Deficiency of vitamin A is a major cause of premature death in developing nations, particularly among children. Vitamin A, which has many vital systemic functions in humans, can be produced within the body from certain carotenoids, notably β-carotene. Carotenoids also play an important potential role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen (di Mascio *et al.*, 1989).

Carotenoids absorb light in the 400-500 nm region of the visible light spectrum of the Ultra-Violet spectrum. This physical property is responsible for the characterristic pigmentation of the carotenoids (Anonymous, 2006). Bull *et. al.* (2003) found that during storage at 4 and 10^0C, the β-carotene content did not significantly decrease and storage temperature did not affect the concentration of this carotenoid in fresh orange juice samples.

2.6.2 Physical and chemical characteristics

2.6.2.1. Flavor of orange juices

The citrus flavor is among the most popular fruit flavors for beverages. The flavor of orange juice has been studied more than that of any other type of citrus fruit. This is partly because the orange juice is the most popular fruit beverage worldwide, and its great demand is a result of its nutritional and sensory properties. Its fresh and uniquely delicate flavor is due to complex combinations of several odour components that have interdependent quantitative relationships. The popular aroma is the result of a natural combination of volatile compounds in a well-balanced system of sugar, acids, and insoluble solids. More than 200 volatile compounds have been identified in the juice of sweet oranges (Selli *et al.*, 2004). The flavor compounds in orange juice are 0.02% of total weight. The 75 - 98% of flavor compounds are hydrocarbons, 0.6 - 1.7% aldehydes, 1% esters, 1% ketones and 1 - 5% alcohols. Limonene is the main flavor compound in quantity, but it is not the most important flavor compound in quality. Acetaldehyde, citral, ethyl butyrate, limonene, linalool, octanal, and α-pinene are the major contributors to orange juice flavor. Octanal and decanal are important flavor compounds in orange juice. Up to 40% of the limonene lost in aseptically packaged commercial orange juice during storage. The degradation of limonene to α-terpineol and other compounds produces off–flavor (Jia *et al.*, 1999). The concentration of these volatile compounds is generally low (1 g/l) and can be affected by a number of agronomic (variety, climatological conditions and ripening stage) and technological (harvest, post-harvest treatments, storage and processing conditions) factors (Riu-Aumatell *et al.*, 2004).

The fresh aroma of orange juice is adversely affected by industrial processing and/or storage conditions. α–terpineol can be used as an index of degradation of the aroma in the orange juice. It is also considered as an indicator of the age of orange juice. This component comes from the chemical degradation of limonene, and its presence becomes a problem at levels higher than 2 mg/g. Hexanal and ethyl butyrate, octanal and decanal are important contributors to the aroma in orange juice, and these components are considered as standards of identity in the essential oil of oranges. A decrease in their concentrations means an important loss in aroma quality. Esters and ketones are characterized as conferring aromatic quality to orange juice. Ethyl acetate and methyl and ethyl butyrate as indices of quality in the aromatic fraction of orange juice (Jordan *et al.*, 2003). The compound limonine can lead to the development of bitterness in the juice. Processing strategies to remove limonine from juice to reduce bitterness have been commercially implemented but these are relatively slow and moderately costly (Bull *et al.*, 2004). For volatile components in different orange juice products, they can be seen in Table 2.4.

2.6.2.2 The cloud loss in orange juices

Cloud stability has traditionally been considered as an important quality parameter for orange juices. The quality of orange juice during storage is usually related to cloud loss, a serious quality defect of orange juice due to the effect of enzyme pectin esterase (PE) activity leading to a clear upper layer and a bottom sediment (Tran and Farid, 2004). PE is a deesterification enzyme produced by higher plants, fungi, some bacteria and yeasts. It de-esterifies pectins starting from the reducing end of the molecule with release of methanol and mono- and polygalacturonic acids that react with calcium ions by precipitating under the form of pectinates, thus

Table 2.4 Volatile components in orange juices (mg/kg) (Jordan *et al.*, 2003)

	Fresh orange juice	Deaerated orange juice	Pasteurized orange juice
Alcohols			
3-Methyl-1-butanol	0.05±0.10a	0.08±0.06a	0.05±0.06a
1-Heptanol	0.01±0.02a	0.67±0.81b	0.75±0.55b
Octanol	3.95±1.69a	0.88±0.27b	1.02±0.50b
Linalool	7.87±3.39a	1.83±0.31b	1.81±1.03b
Terpinen-4-ol	2.82±1.17a	1.22±0.32b	1.15±0.11b
α-terpineol	3.69±2.15a	1.52±0.51b	1.03±0.27b
Nerol+citronellol	0.52±0.20a	0.20±0.04b	0.18±0.15b
Geraniol+linalyl acetate	0.41±0.18a	0.14±0.09b	0.17±0.06b
Aldehydes			
Valeraldehyde+2-pentanol	0.62±0.54a	0.01±0.01a	0.41±0.06b
Hexanal+ethyl butyrate	0.49±0.25a	0.08±0.07b	0.05±0.05b
Octanal	1.31±1.74a	0.25±0.18a	0.21±0.20a
Nonanal	0.31±0.22a	0.09±0.04ab	0.07±0.08b
Decanal	2.45±1.07a	0.81±0.21b	0.66±0.53b
Neral	0.18±0.13a	0.05±0.08a	0.08±0.06a
Geranial	0.85±0.36a	0.26±0.13b	0.16±0.11b
Decanal	2.45±1.07a	0.81±0.21b	0.66±0.53b

Table 2.4 (continued) Volatile components in orange juices (mg/kg)

	Fresh orange juice	Deaerated orange juice	Pasteurized orange juice
Neral	0.18±0.13a	0.05±0.08a	0.08±0.06a
Geranial	0.85±0.36a	0.26±0.13b	0.16±0.11b
Perillaldehyde	0.08±0.10a	0.01±0.02a	0.02±0.04b
Undecanal	0.07±0.06a	0.03±0.03a	0.03±0.03a
Dodecanal	0.27±0.13a	0.21±0.10ab	0.11±0.10b
Esters			
Methyl butyrate	0.02 ±0.01a	0.01±0.01a	0.06±0.05a
Octyl acetate	0.10±0.11a	0.04±0.09a	0.05±0.07a
Terpenyl acetate+citronellil acetate	0.06±0.10a	0.04±0.06a	0.07±0.08a
Neryl acetate	0.15±0.10a	0.07±0.05ab	0.04 ±0.04b
Geranyl acetate	0.11±0.07a	0.05 ±0.04a	0.07±0.06a
Ketones			
Ethyl-vinyl-ketone	0.71± 0.55a	0.46±0.93a	0.47±0.54a
Carvone	0.20±0.09a	0.07±0.05a	0.37±0.48a
Terpenic hydrocarbons			
α-Pinene	2.83±0.99a	0.54±0.55b	0.04±0.04b
Sabinene	1.55±0.94a	0.06±0.11b	0.12±0.09b
β-Pinene	0.34±0.14a	0.11±0.07a	1.01±1.79a
β-Myrcene	11.95±4.28a	4.12±1.57b	2.86±1.88b
α-Phellandrene	0.11±4.28a	0.05±0.09a	0.07±0.13a
Δ_3-Carene	0.56±0.32a	0.24±0.05ab	0.21±0.10b
α-Terpinene	0.32±0.16a	0.17±0.04b	0.12±0.08b
Limonene	772.36±355.96a	238.22±97.60b	165.36±119.12b
γ-Terpinene	0.65±0.26a	0.35±0.07b	0.45±0.26ab
β-Caryophyllene	0.11±0.05a	0.14±0.08a	0.09±0.06a
γ-Humulene	0.04±0.04a	0.03±0.02a	0.03±0.04a
Valencene	3.19±3.16a	2.38±1.10a	5.33±2.73a

Values within rows with common letters were not significantly different (*P<0.05): ± standard deviation causing spontaneous clarification (Spagna *et al.*, 2003). The enzyme is found in all tissues of the fruit, with juice sacs containing around 75% of the total activity (Nienaber *et al.*, 2006). Although the pH optimum for the enzyme was between 7.4 and 8.4, PE was active over a wide pH range (4-11) and low concentrations of metallic ions (Na^+ or Ca^{2+}) activated the enzyme (Ren *et al.*, 2000). Unprocessed orange juice presented PE activity of $6.5 \times 10^{-4} \pm 3.3 \times 10^{-4}$ PMEU/ml ^{0}Brix (Collet *et al.*, 2005).

PE is inhibited by high sugar concentrations. The juice of ripe fruits shows slightly high PE activity in blood than blonde cultivars. The studies published up until now that concern about PE determination in blonde varieties measure the PE enzyme based on several methods as followed (Ingallinera *et al.*, 2005):

1. Measurement of viscosity. A very simple method, although it has a disadvantage of being influenced by the activity of the other pectinolytic enzymes.

2. Spectrophotometry. This is an indirect method based on product coloration as a function of pH and the use of bromothymol blue. It has a disadvantage of not being particularly accurate.

3. Titrimetric method. This method is not very sensitive.

4 Measurement of released free methanol. Although the method uses longer and more complex reactions than the others, this method is probably the most sensitive since it measures the reaction product of the specific enzyme.

2.6.2.3. Color of orange juices

The intensity of the orange juice color depends on orange fruit cultivars, but also on the ripeness and climatic conditions, in particular a wide daily temperature range in the months of October–November that is essential for producing the distinctive red colour (Ingallinera *et al.*, 2004). Choi *et al.* (2002) studied the influence of ascorbic acid retention on color stability of blood orange juice investigated the color changes using CIE *L a* b**, hue, chroma, polymeric color and browning index during the storage period of the orange juice. They found that ascorbic acid content showed linear correlation with red color intensity (CIE a^* and chroma) in the juice and ascorbic acid appeared to have influence on lowering the intensity of redness in juice. In addition, the color of oranges is due to carotenoid pigments carried in the chromoplasts which form part of the suspended cloud. In fresh produced commercial citrus juices it has been demonstrated that nonenzymatic browning was mainly due to carbonyl compounds formed from L-ascorbic acid degradation. Increasing the L-ascorbic acid concentration extends the nutritional value of the product but also increases the severity of browning (Roig *et al.*, 1999).

Murata *et al.* (2002) reported about an orange juice model solution that gradually turned brown during storage. Adding sugar in orange juice made the juice turned brown after 2 weeks of storage.

2.7 Microbial spoilage of orange juices

Microorganisms causing off-flavors in foods are bacteria, molds and yeasts. They may spoil foods either by direct microbial action or indirectly by uptake of microbial metabolites from for example contaminated processing lines, packaging materials or storage facilities. Some of these contaminants may remain in the food even after extensive processing. Microorganisms from all three morphological groups (i.e. molds, yeasts and bacteria) can cause spoilage of juices. The most frequently found bacteria in juices are acetic acid and lactic acid bacteria (Zierler *et al.*, 2004).

Microbial spoilage of juice products may lead to off flavors, odors, turbidity and gas production. A limited range of yeasts, moulds and aciduric bacteria are capable of growth at the low pH of orange juice, typically pH 3.3–4.0 (Ingallinera *et al.*, 2005).

Fresh orange juice is spoiled with time due to the growth of microorganisms. Yeast and moulds, *Lactobacillus*, *Leuconostoc* and thermophilic *Bacillus* (*Bacillus subtilis* and/or *Bacillus pumilus* spore formers) are common microorganisms growing in orange juice. Recently, *Alicyclobacillus acidoterrestris*, which can be seen as a novel thermoacidophilic spore-forming bacterium and the target of juice industry due to their heat resistance, has been reported to cause spoilage in fruit juice (Tran and Farid, 2004). Microbial counts in the fresh/frozen juice were approximately 10^4 CFU/ml. This number was also typical for a fresh, nonpasteurized orange juice (Parish, 1998).

The sensorial aspect of fruit juice is directly related to consumer demand for the juice in the search for similarity to fresh juice sensory characteristics. The alteration in natural juices intensifies continuously after extraction, resulting in the development of undesirable flavor and color. Microbial growth in citrus juice is characterized by the production of unpleasant flavors and product deterioration who is commonly caused by yeasts (de Souza *et al.*, 2004).

de Souza *et al.* (2004) reported that a high initial yeast count may have occur due to contamination of the fruit during harvest or re-contamination during processing. If the initial contamination of the product was high for yeast, it was suggested that the raw material and/or its processing required better sanitary care.

Ingallinera *et al.* (2005) found that a high initial microbial populations of untreated Valencia juice limited the shelf-life of the untreated juice stored at 4^0C to 2 weeks and juice stored at 10^0C to 7 days. For an untreated Navel juice (pH 3.7) with a lower initial microbial population and a lower pH resulted in a longer shelf-life than that of the untreated Valencia juice (pH 4.3). The importance of storage temperature was also highlighted, as any microorganisms that may have survived were prevented from spoiling the product because of the acidic nature of the product and refrigerated storage.