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# FRUIT MORPHOLOGY, ANATOMY, AND PHYSIOLOGY



## I. FRUIT MORPHOLOGY

Citrus fruit is a modified berry or a specialized form of berry (hesperidium) resulting from a single ovary. In addition to citrus, this type of fruit is observed in five more genera: *Poncirus* (*Trifoliate* orange), *Fortunella* (kumquat which is eaten as is, with peel), *Microcitrus*, *Eremocitrus*, and *Clymenia* in the subfamily Aurentioidae of family Rutacae. The usually five-pointed or five-lobed calyx and button-like receptacle are attached to the peduncle – as seen in the orange fruit – at the stem end. The calyx remains attached to the branch if the fruit is naturally separated by abscission. Usually the small, green-colored button and calyx at stem-end is preferred in market. The size of citrus fruits range from nearly 2.25 cm for kumquats (*Forlunella* spp.) to more than 20 cm in diameter for pummelo (*C. grandis*). The shape is also variable: oblate in grapefruit, mandarins, and tangerines; globose to oval (spherical or nearly so) in sweet oranges; oblong in lemons (*C. limon*), (*C. medica*); and spherical in limes (*C. aurentifolia*). The rind, or peel, is leathery – more so when it loses some moisture. It is fragile and

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breaks on folding when turgid. Fruits generally have 8–16 segments. Grapefruits and pummelos have 17 or 18 segments. Seeds vary in number from zero in a few cultivars to many, leading to quite seedy fruit. Tahiti lime (*C. latifolia*) and navel oranges can be called truly seedless and have almost no seeds, while grapefruit and pummelo have 40–50 seeds. Seed size and shape also varies greatly among species.

## A. Fruit Characteristics

### 1. Mandarin

Most mandarins are easily peelable and deep orange to reddish-orange in color when fully mature. Fruit is small to large (5–8 cm in diameter at the equatorial axis); globose to oblate; base with a low to high collar, a deeply depressed apex and a medium-thick, loosely adherent rind; a relatively smooth surface sometimes pebbled with prominent, sunken oil glands; segments numbering about 10–17; a large and hollow axis; orange-colored flesh; tender, melting, and juicy; mild and strong flavor; seedless to seeded with nearly 3–7 or more seeds; small and plump; early to late season in maturity. Most mandarins lose quality and the rind 'puffs' if not picked when internally ripe.

Oblong and pyriform fruit has to be removed as it is not true-to-type and hence damages the impression of the whole lot of fruit. Fruit of small to medium size are preferred; very large fruits are associated with puffiness by the trade. Fruit with puffiness and over-maturity lack keeping quality. Some markets in the East prefer large mandarin fruits. Fruit with small neck or collar and near-flat base (at stem-end) are preferred for export as they suffer less damage. Most consumers like yellow-orange to deep-orange color, although fruit with a slight greenish tinge is acceptable. Fruit should be juicy. Flavor is another important attribute of fresh mandarins; this is the quality affected most in long-term storage and by various handling conditions. Most Indian consumers dislike excessive acidity, while a lack of acid renders fruit flat in taste. In European markets, seedless fruits with higher acids are preferred. Clementine and Satsuma mandarins are seedless. Seedless clones of the 'Nagpur' mandarin are being developed.

### 2. Sweet Orange

Unlike common sweet oranges, Navel oranges have navel-like structure at the stylar end, or apex. This difference is anatomical in nature and consists of a navel that is a rudimentary secondary fruit embedded in the primary fruit. Navel oranges, particularly the Washington Navel orange variety, are somewhat obovate or ellipsoid in shape. The fruit surface is usually smooth in all orange fruits of commercial importance; fruits with rough surfaces are removed while packing. In Navel oranges, the surface is moderately pitted and pebbled. Sweet oranges are mostly globose but oval to ellipsoid (Shamouti or Palestine Jaffa) are also common. Common Valencia is oblong to spherical. Fruits are flattened at the base in most varieties. Surface is finely pitted but smooth. Italian 'Moro' is

deep blood-orange variety on the inside, while 'Sangunello Moscata,' an Italian variety, and 'Doblefina' of Spain are both light blood oranges. Fruits of these varieties are deep orange to reddish in color from outside at maturity. Most sweet oranges in general have rind that is yellow to yellow-orange, thin to medium-thick, and firm and leathery. Flesh color is yellow to yellow-orange. The presence of a green or yellowish-green calyx and button at the stem end of an orange indicates the freshness of the fruit.

**3. Lemon**

Lemon fruit vary greatly in shape, size, color, rind texture, and juice content. Fruits of Assam lemon and Verna (Berna or Vernia of Spain) are long, obovate to oblong in shape, and medium to large in size. Fruits of Eureka are medium-small and elliptical or oval in shape. Lemons are seedless to seedy. The nipple is prominent and large in some varieties, while others have very small, inconspicuous nipples. Rind color is green to bright yellow at maturity. Fruits are with or without a collar at the neck. Fruits are slightly ribbed to without ribs. Rind is thick to thin and rough to smooth among varieties. Some varieties, such as Meyer lemons, are quite juicy while others have less juice.

**4. Acid Lime**

Acid limes are very small (3 cm diameter) to medium (5 cm in diameter) in size and round, obovate, or oblong in shape. They have very small necks, a flat base, and a small nipple at the apex. They have a thick to very thin and papery rind and are green to yellow in color. They are seedy to seedless. The rind surface is smooth and the flesh is tender, juicy, and yellowish-green. Fruits of *Citrus aurantifolia* Swingle are small (30–45 g) while fruits of *Citrus latifolia* are large (80–100 g).

**5. Grapefruit**

Fruits are medium to large in size (10–12 cm in diameter), oblate to spherical in shape, and slightly depressed from stylar end and flat on the stem end. The peel is medium-thick, yellow to pink-blushed, and smooth. Flesh is white and tender/melting with a central core that is usually open. Fruits are borne in clusters and are seeded to seedless. Pink- and red-fleshed cultivars are available. Flavor is strong.

**6. Pummelo**

Fruits are large to very large (15–20 cm in diameter or even larger), round to obovate in shape (some varieties are pyriform), and borne singly. The rind is thick to very thick (3–4 cm) with a smooth, green- to yellow-colored surface when mature. Flesh is firm and crisp, juice vesicles are separable, and core is open and hollow. Pink-fleshed cultivars are available. Fruit is seeded to seedless and flavor is mild to strong.

## II. FRUIT ANATOMY

Citrus fruit arises through the growth and development of an ovary and consists of 8–16 carpels clustered around and joined to the floral axis, which forms the core of the fruit. The carpels form locules, or segments, in which seeds and juice sacs (vesicles) grow. The pericarp (rind or peel) is divided into exocarp, or *flavedo*, and mesocarp, or *albedo*. The flavedo consists of the outermost tissue layers, which have cuticle-covered epidermis and parenchyma cells. The flavedo is the outer, colored part and the albedo is the inner, colorless (white) or sometimes tinted part (as in red grapefruit or blood oranges) (Fig 5.1).

The flavedo consists of the epicarp proper, the hypodermis, the outer mesocarp, and oil glands. Above the epicarp is a multilayered protective skin or

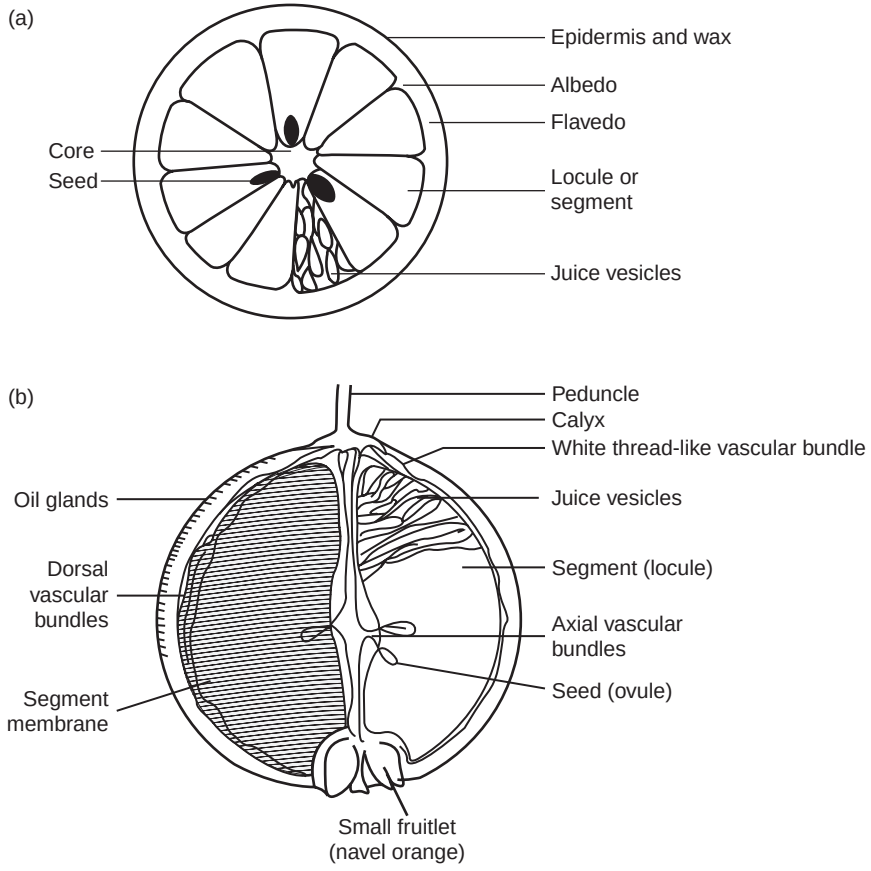


**FIGURE 5.1** (a) Transverse Section of Citrus Fruit Showing Various Anatomical Parts of Citrus Fruits and (b) Longitudinal Section Showing Vascular Bundles and Fruitlets of Navel Fruit.

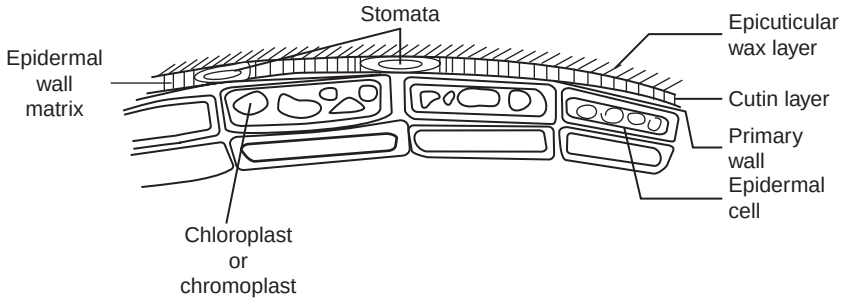


**FIGURE 5.2** Schematic Diagram of Epidermal Layer, Cutin, and Wax on Rind of Citrus Peel.

cuticle that is quite complex in origin, structure, and development. The cuticle consists of an inner layer of cutin, which is a heterogeneous polymer of fatty acids and cellulose, and an outer layer consisting of cutin (Baker et al., 1975). In all, the cutin matrix is formed with cutin, wax, and a cell-wall material. Wax deposition continues as fruit grows; the wax hardens and develops breaks naturally. Epidermal cells are known to synthesize lipids and waxes for depositing on the cutin layer (Fig 5.2).

Wax in the form of platelets, rods, and other shapes is embedded within and over the cuticular surface (Albrigo, 1972a; Freeman, 1978). Epicuticular waxes, which are mainly responsible for restricting water loss from the peel, are complex in nature and formed of alcohols, paraffin, aldehydes, ketones, etc. (Freeman, 1978). The wax layers of Pineapple and Navel oranges, Dancy tangerines, and Eureka lemons have been observed to be initially amorphous. Small protrusions and isolated regions of upright platelets develop thereafter (Freeman et al., 1979). All surfaces eventually crack and are uplifted to form large, flat, irregular plates.

This outer surface can be rubbed gently to give shining polish. By restricting water loss through evaporation, the cuticle plays an essential role in maintaining high water content within tissue that is necessary for normal metabolism. One estimate claims that cuticle reduces the rate of evaporation from living plant cells from about 3.6 to 0.14 $mg/cm^2/Pa/h$. Water loss varies with the type of fruit or commodity, its anatomy, and surrounding conditions. This coefficient of transpiration (the rate of water loss) is 42 for apple and 7400 for lettuce (Wills et al., 1998). Numerous *stomata* are scattered over the surface of the epidermal cell layer over the parenchymatous tissues between the oil glands (Turrell and Klotz, 1940). The number of stomata is greater in the stylar half of the fruit than at stem end, and there are few or no stomata around the stem and the calyx (Albrigo, 1972b). Stomata are plugged with wax as the fruit matures. These stomata remain functional even after harvest unless they are plugged by applied wax. Uptake of exogenously applied PGRs through stomata is less and mostly occurs through cracks in wax layers and cutin. Immature fruits do not have a

well-developed wax layer; the uptake of applied PGRs is relatively high in these fruits.

The epicarp also has cells with plastids containing chlorophyll (in other words, chloroplasts) which gradually change into chromoplasts as fruit color changes (Thompson, 1969). Fruits change color during the seasons from green to yellow and then to green again (in regreening). Photosynthetic activity is detected only in young green and regreened fruits. In yellow fruits a small amount of chlorophyll is detected. Electron microscopy showed that changing fruit color was the result of a typical transformation of chloroplasts into globular chromoplasts and vice versa (Ljubesic, 1984).

Colorless cells, called hypodermis and outer mesocarp, lie immediately below the epicarp and contain oil glands. The size of oil glands range from 10 to 100 μm or more. The terpenes (mainly *d*-limonene) and sesquiterpenes of oils in these glands give characteristic aroma and flavor to fruits of different citrus species. If the glands are ruptured by impact, oleocellosis develops – a characteristic lesion on the rind that can give fruit an unsightly appearance, as the oil is injurious to other cells of the rind (Wardowski et al., 1976). The rind of most citrus fruits is generally inedible, largely because of the oil. However, the rind of kumquats is sweet and can be eaten along with the pulp.

Albedo consists of inner mesocarp, which consists of parenchymatous cells with large air spaces (Scott and Baker, 1947). This is an extremely effective cushioning material against pressure and impact to fruits. The albedo is 1–2 mm thick in limes and tangerines, 2–5 mm thick in sweet oranges, and up to 20 mm thick in pummelos. Albedo is attached to flavedo on the outer side and connected with segment membrane from the inner side. The exocarp, or flavedo, and white spongy mesocarp, or albedo, are blended together. The endocarp is the inner side of the pericarp and a portion of the locular membrane. When the peel/rind is stripped, the entire exocarp and all but the inner portion of the mesocarp are removed.

The segments surrounding the central axis form the edible pulp of a mature citrus fruit. Each segment is surrounded by a continuous endocarp membrane. The juice in the fruit is contained in closely compacted, club-shaped multicellular sacs, also called juice vesicles, which completely fill the segments and are attached to a thin wall called the carpellary septum surrounding the segments. Each juice sac also has a very minute oil gland at the center. The seeds (ovules) are also attached to segment walls (toward the central column) by means of axial placentation.

The *central core* is composed of the same type of colorless or tinted, loose, spongy network of cells as the albedo. The core is connected with the albedo by membranes between each segment. The central axis of all citrus fruits is solid in the immature stages of development and also in mature fruit, particularly sweet oranges, grapefruits, lemons, and limes. In overmature stages, the central axis may open up in these fruits. The central axis of mandarins and their hybrids, and of pummelo, is normally open, with an air space in the center.

As the rind or peel is removed, the white, thread-like, vascular bundles forming the network in albedo and running parallel to the fruit axis along the

outside of the segments are also removed. These vascular bundles carry water and food to the juice vesicles during fruit growth and maturity.

The vascular system extends down the central axis of the fruit, reaching the blossom end (stylar or distal) first, then ramifies surrounding segments and back up the carpels to the stem end (calyx, proximal) of the fruit. Consequently, the distribution of photosynthates and sugars is higher in the blossom end than the stem end. The vascular bundles in citrus fruit provide nutrition to developing fruit and these form a highly ramified network of main and subsidiary traces whereby every cell in the various tissues is connected directly or is adjacent to a cell in contact with a particular section of the vascular system.

The vascular system of the peduncle (fruit stem) is similar to that of other young stems on the tree. It consists of concentric cylinders of phloem, lateral meristem or cambium, and xylem surrounding a central core of pith.

Studies (using labeled $CO_2$) on movement of photosynthates indicate that photosynthates accumulate in the peel. Most of them enter the fruit via dorsal vascular bundles and are partially hydrolyzed during slow transfer through non-vascular segment epidermis and juice stalks (Koch, 1984). Anatomical changes in the structures of the cells of the Persian lime have been studied during fruit maturation by Garcia and Rodriguez (1992). Chloroplast structure did not change during fruit development and ripening. Fruits are dark green when young and turn light green when mature and ripe. Gradual dissolution of the middle lamella on ripening occurred in the mesocarp. This is linked to polygalacturonase enzyme action and the ripening process. Juice vesicles had an epidermal cell layer and were covered by a cuticle. Vesicles had cell organelles initially; as the fruit developed, all organelles decreased. Juice vesicles showed senescent symptoms earlier than other tissues of the fruit.

## III. FRUIT PHYSIOLOGY

As citrus fruit develops, several physiological changes occur. There is a relationship between various functions of different organelles, tissues, organs, and the system as a whole. With growing age changes occur in functioning of the tissues and systems. Biochemical changes occurring with growth are discussed in Chapter 6. Some important physiological aspects with respect to postharvest life of the fruit are discussed here.

### A. Respiratory Activity

*Climacteric* is defined as a period in the ontogeny of certain fruits during which a series of biochemical changes is initiated by the autocatalytic production of ethylene marking the change from growth to senescence and involving an increase in respiration and leading to ripening (Biale, 1950; Rhodes, 1980). Citrus fruit are non-climacteric, hence their respiration rate and ethylene production do not exhibit remarkable increase along with changes related to maturity and ripening

as in mango or banana. These fruits do not ripen after harvest. Internal quality of citrus fruits is at its best when fruits are at optimum maturity on the tree.

Subramanyam et al. (1965) observed that respiratory rate of 450 mg $CO_2$/kg/h 30 days after fruit-set in acid lime fruit decreased to 200 mg after 60 days, 100 mg after 90 days and then increased up to 140 mg after 120 days. At 150 and 180 days, respiration rate was 50 and 40 mg $CO_2$/kg/h, respectively. Respiration was very high during rapid cell division. Spurt in respiration was observed at 120 days with a decline at later stages, indicating the possibility of climacteric peak between 90 and 120 days with maximum biochemical activity. Relationship among fruit age, epicuticular wax, weight loss, internal atmosphere composition, and respiration were investigated in maturing Washington Navel fruits by El-Otmani et al. (1986). Fruit epicuticular wax, internal $CO_2$ and internal ethylene increased, while with advancement of season, weight loss, and respiration decreased during storage. Concomitantly fruit conductance to $CO_2$ was reduced. Respiration rate of grapefruit peel was shown to be higher than pulp but it decreased after harvest while the rest of the fruit respiration remained constant (Vakis et al., 1970). Aharoni (1968) reported that respiratory climacteric can be detected if fruit are picked well prior to normal harvest time.

Response to exogenous ethylene of citrus fruit is reversible, hence they are not considered a climacteric fruit (Eaks, 1970). In Mosambi sweet oranges, respiration increased from initial rate of nearly 35 mg $CO_2$/kg/h to 80 mg $CO_2$/kg/h in ethylene-exposed fruit by the end of 48-h ethylene treatment. This reaction slowly declined (Fig. 5.3) after removal of the fruit from the ethylene atmosphere (Ladaniya, 2001).

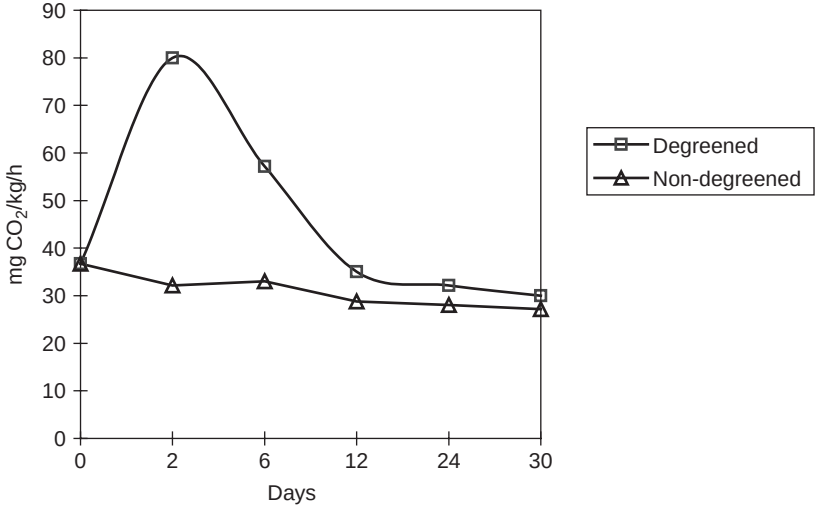


**FIGURE 5.3** Respiration Rate of Mosambi Orange Fruit as Affected by Ethylene Application During Degreening.

Respiration of citrus fruits is affected by temperature, humidity, air movement, atmospheric gases, and handling practices. Increasing the temperature increases respiration rate; lowering the temperature restores the original respiration rate with no evidence whatsoever of a climacteric (Vines et al., 1968). Eureka lemons produced 80.5 mg$CO_2$/kg/h at 37.7°C and 22.7 mg$CO_2$/kg/h at 21.1°C (Murata, 1997). The respiratory rate increases at higher storage temperatures. This significantly affects storage life because the heat of respiration, or vital heat, generated is also higher (Table 5.1). Heat evolution can be computed from the respiration rate.

**TABLE 5.1 Respiratory Activity ($CO_2$ Evolution in mg/kg/h) of Citrus Fruits as Influenced by Temperatures**

| Fruit | 0°C | 5°C | 10°C | 15°C | 20°C | 25°C | 30–32°C |
|---|---|---|---|---|---|---|---|
| Sweet oranges | 2–4 | 4–8 | 6–9 | 12–24 | 22–34 | 25–40 | 33–46 |
| 'Mosambi' orange | – | 7–8 | 11–12 | 18.5–19 | 27–28 | 38–40 | – |
| Kinnow mandarin | – | 5–6 | 10–11 | 12–14 | 17–18 | 28–30 | 48–50 |
| 'Nagpur' mandarin | – | 7–9 | 10–13 | 15–18 | 25–30 | 32–38 | 40–46 |
| Grapefruit | – | – | 7–10 | 10–18 | 13–26 | 19–34 | – |
| Lemons | – | – | 11 | 10–23 | 19–25 | 20–28 | – |
| Limes | – | – | 4–8 | 6–10 | 10–19 | 15–40 | 40–55 |

The heat evolution in Kcal/ton/24 h can be computed by multiplying above given respiration rates with a factor of 61.2.

Respiration of citrus fruit respond differently at temperatures above and below critical temperatures that correspond closely with the temperature at which chilling injury occurs. Citrus fruits exhibit cumulative time-temperature influence of chilling temperature on carbon dioxide evolution (Eaks, 1960). The $CO_2$ evolution is greater after exposure to 0°C than at 10°C. The chilling temperature also increases the production of ethylene and volatile components (ethanol and acetaldehyde) in fruit after a return to normal temperature (Eaks, 1980). Storage at 1°C for 14 days resulted in elevated respiration in Lisbon and Eureka lemons, with a peak occurring during the first 24 h. After 28 days at 1°C, peak respiration increased to 51 mg/kg/h for Lisbon lemons and 34 mg/kg/h for Eureka lemons. Respiration increased significantly, consistent with extensive chilling injury (Underhill et al., 1999).

The lowest safe temperature minimizes the respiration rate, which prolongs normal metabolism of the fruit and thereby its postharvest life. The 'Nagpur' mandarin has relatively low respiration rate: nearly 40–45 mg$CO_2$/kg/h at 25–30°C and 50–60 percent RH. Waxing reduced this rate by 34–35 percent. At low temperature (6–7°C), the respiration rate is 12–15 mg$CO_2$/kg/h. Waxing further reduces this rate by 10–11 percent. During storage, the respiratory activity of Shamouti and Valencia oranges declined and the internal $CO_2$ rose from a

range of 2–4 percent to 5–10 percent, while the $O_2$ declined from 17–19 percent to 10–12 percent (Ben-Yehoshua, 1969).

If fruits are stored at the lowest safe temperature (above freezing) that significantly suppresses respiration and fungal growth, the storage life of fruits can be increased significantly. While chilling temperature adversely affects fruit quality, the higher temperatures also reduce the storage life and quality. The severity of adverse effects depends on the time–temperature relationship under such conditions.

At lower humidity, the respiration rate of citrus fruits is lower than that at higher humidity (Murata and Yamawaki, 1989). Higher concentrations of oxygen (34.1–99.1 percent) increase the respiration rate of citrus fruits, while lower concentrations reduce the rate. Very low concentration of $O_2$ (0.5–5 percent) tend to increase the respiration rate. Citrus fruits produce higher quantities of ethanol and acetaldehyde at low $O_2$ levels and higher $N_2$ levels, indicating unaerobic respiration.

Particular care is needed during harvesting, storage, transportation, and marketing of citrus fruits because rough handling causes wounding, stimulation of respiration and ethylene production, and can induce physiological disorders and fungal rot. In 'Ponkan' (*C. reticulata*) fruits, respiration rate and superoxide dismutase (SOD) activity in both peel and flesh has been shown to increase during storage when fruits have compression bruising. (Increases were larger following compression at 6.0 kg, compared with compression at 4.5 kg.) Respiration rate and SOD activity decreased rapidly at 40 and 65 days after bruising respectively, to reach levels similar to those of the control fruits. Peel conductivity increased with the degree of bruising, indicating increased membrane permeability (Xi et al., 1995).

Higher respiration has also been recorded in 'Nagpur' mandarins during excessive shriveling, rough handling, and rotting. If the fruit is rotting from inside without any external symptom (as in *Alternaria* core rot), respiration rises, which can be interpreted as deteriorating quality and decay. Internal disorders such as granulation also affect respiratory activity. Climacteric peaks of 'Ougan' and 'Hongju' mandarins reached a maximum after storage for 120 and 45 days, respectively. The respiration rate of the peel of the 'Ougan' was higher than that of its pulp. On the other hand, the pulp of 'Hongju' had a higher respiration rate than its peel until full granulation only; thereafter the peel was higher than the pulp (Ye et al., 2000).

Gas-exchange properties and respiration seem to remain unaffected by insect damage to citrus peels. Regions damaged by rust mite, wind scar, and pitting from physical damage had similar gas-exchange properties as undamaged regions on the same fruit (Petracek, 1996).

## B. Biochemistry of Respiration

As the respiration rate is low and steadily declines after the harvest of citrus fruits, the available amount of sugar and organic acids is slowly converted into

$CO_2$, water, and heat. Since there is no starch, the sweetness of citrus fruits does not increase after harvest except for a slight increase in total soluble solids because of the activity of hydrolytic enzymes, or concentration effect, caused by rapid loss of water under dry storage conditions. At higher temperatures citric acid content also drops rapidly.

Detached fruits require energy for carrying out metabolic reactions to transport metabolites, to maintain cellular organization and membrane permeability, and to synthesize new molecules. This energy comes from aerobic respiration that is the oxidative breakdown of organic compounds such as sugars, organic acids (citric and malic acids present in vacuoles), lipids, and in extreme cases even proteins. The most common substrates in respiration of citrus fruit are glucose and fructose. One molecule of glucose produces energy equivalent to 686 kcal on complete oxidation. This chemical energy is stored in the form of adenosine 5'triphosphate (ATP), nicotinamide adenine dinucleotide (NADH), and $FADH_2$.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

In respiration, when sugars are consumed the ratio of oxygen utilized is equal to $CO_2$ produced: the respiratory quotient RQ = 1. (RQ = $CO_2$ produced ml/$O_2$ consumed ml.) In this case, respiration rate can be measured as either $O_2$ consumed or $CO_2$ evolved.

In cytoplasm, glycolysis takes place in which glucose is converted to pyruvate by the enzymes of the EMP (Embeden-Meyerhof-Parnas) pathway. The pyruvate is finally converted to $CO_2$ and energy in TCA (tricarboxylic acid cycle) by enzymes in mitochondria, the 'powerhouses' of the cells. During the initial fruit growth period, starch is broken down in to glucose by amylase. Phosphorylase enzyme converts glucose to glucose-1-phosphate. Sucrose is broken down to glucose and fructose by invertase. Sucrose synthase is also involved in formation of UDP-glucose (Uridine 5'-diphosphate) and then it is converted to glucose-1-phosphate and fructose-1-phosphate, which are then fed into the EMP pathway. Organic acids are directly utilized in the TCA cycle in mitochondria. Acids consume more $O_2$ for each $CO_2$ generated and hence RQ is 1.3. In case of fatty acids, the RQ is 0.7. If we measure both $O_2$ consumed and $CO_2$ evolved, we can know the substrate utilized in the respiration by the fruit. However, it is quite possible that several substrates are being utilized at a time and a correct picture may not be available simply on the basis of $O_2$ consumed and $CO_2$ produced.

### C. Transpiration

When water loss occurs from plant parts in the form of evaporation it is called transpiration. The stomata and cuticle on the epidermal layer of cells (outermost layer of cells) offer the least resistance to moisture loss. Stomata are mostly closed and covered with wax. The wax platelets on the fruit surface overlap; they are strongly hydrophobic. The spaces between these wax platelets

are often filled with air. The soft wax component made of alcohols, aldehydes, esters, and fatty acids determine the rate of transpiration. Citrus fruits contain 80–85 percent water. The loss of water has a greater consequence since it affects appearance and also weight. In citrus peels, water exchange was found to be approximately four to six times greater at the stem-end than in other regions (Petracek, 1996).

Usually, fruit peel loses water more rapidly than the flesh during storage under low-humidity conditions, and also becomes thinner. The fruit juice content (percentage) shows an increase (although erroneously) as it is recorded on a fresh-weight basis.

Softening of peel is due to flaccidity of the cell or hydrolysis of intercellular pectic compounds during long refrigerated storage. Under dry ambient conditions with low relative humidity, peel dries rapidly, thus becoming tough and leathery, and hinders normal gas exchange. This causes anaerobic conditions and an increase in alcohol levels inside the fruit. Citrus fruits have relatively long postharvest life if protected from water loss and decay causing microorganisms, mainly fungi. If the peel remains turgid and healthy, normal gas exchange can occur without accumulation of $CO_2$ or ethylene in and around fruit.

The loss of commercial value of Shamouti and Valencia orange fruit under various storage conditions is caused by transpiration, which lead to shriveling of the peel. During storage, respiratory activity declines and the internal $CO_2$ rises from a range of 2–4 percent to 5–10 percent while the $O_2$ declines from 17–19 percent to 10–12 percent. Drying of the peel causes a rise in resistance to gas diffusion, which in turn changes the internal atmosphere. The flavedo portion of the peel is the main site of resistance to gas diffusion (Ben-Yehoshua, 1969).

Stomata of harvested citrus fruits are essentially closed. However, ethylene, $O_2$, and $CO_2$ still diffuse through the residual stomatal opening (<1 percent), while water evaporates from epidermal cells. Ethylene, $O_2$, and $CO_2$ are constrained from using the water-transport pathway because their diffusivity in water is 104 times less than in air. Waxing fruits partially or completely plugs the stomata. This may increase the off-flavors if coating is not done properly by partially restricting $O_2$ and $CO_2$ diffusivity. Wax coating inadequately reduces transpiration because the new surface layer it forms has many pits and breaks. Sealing fruits individually in 10-micron-thick, high-density polyethylene film is more effective than waxing for increasing storage life. The film reduces water loss by 10 times without substantially inhibiting gas exchange (Ben-Yehoshua et al., 1983). The region of neck or stem-end loses water rapidly. Among citrus fruits, acid limes do not have cuticle and hence water loss is quite rapid in these fruits.

Loss of water not only affects appearance or esthetic value but also reduces saleable weight, thus causing direct economic loss. Citrus fruits have low surface-area-to-volume ratio and thus lose water more slowly than many leafy vegetables, but even 5–6 percent water loss can result in some change in appearance and firmness of the fruit that can be detrimental to its marketability.

### D. Role of Ethylene

Ethylene is also known as stress hormone. (Its level increases in plants and fruit with the application of stress, and it can create a stress-like condition in plants if applied exogenously.) Ethylene has a special role in fruit maturity, ripening, and senescence, and therefore has its own importance in postharvest management of citrus. Ethylene is known to soften the fruit by disintegrating cell membranes and making them leakier, eventually resulting in fruit softening. Chemical composition, flavor, and texture remain more or less unchanged with ethylene action in citrus. Acidity content decreases slightly with the exogenous application of ethylene. Treatment of fruits with ethylene or ethephon increases nootkatone levels in the rind of both harvested and unharvested Star Ruby grapefruit. The nootkatone level in the rind is therefore proposed as an indicator of ripening/senescence in grapefruits (Garcia et al., 1993).

Citrus fruits have a very low rate of ethylene evolution, in the amount of $<0.1\,\mu l/kg/h$. Even this rate can slowly build up ethylene concentration in closed chambers. The higher $CO_2$ concentration inside the fruit can counteract the ethylene action. If fruits are rotting in the box, the ethylene evolution is very high and can affect physiology of other fruits. Several other stresses such as freezing, excessive drying/shriveling, and even dropping of fruit can increase the ethylene buildup and respiration. Ethylene production per fruit basis is very low (2 nl/h/fruit), and even this low concentration can be effective endogenously to enhance maturation and senescence. Healthy Satsuma is reported to produce $0.16\,\mu l/kg/h$, while those infected with *Colletotricum gloesporioides* produced $11.80\,\mu l/kg/h$ (Hyodo, 1981).

Citrus fruits produce a very low quantity of ethylene after harvest and there is no associated rise in respiration. However, citrus fruits respond to exogenous ethylene by an increase in respiration, chlorophyll loss, calyx drying, and abscission, although they cannot synthesize large amounts of ethylene autocatalytically. Thus, when the supply of ethylene is terminated, the enhanced respiration decreases to the low level that existed before ethylene treatment.

Young, immature citrus fruits produce large amounts of ethylene, and their respiration increases parallel with a rise in ethylene production. This high ethylene production may be responsible for June drop. Wounding of harvested citrus fruit tissues causes a rise in ethylene production and accelerates coloring and related metabolic changes. This wounding could be due to fungal attacks (green and blue mold and other pathogens), insect damage, freezing injury, hailstorms, or postharvest stresses such as chemical injury, mechanical injury, gamma radiation, and chilling temperature. Preharvest injury and consequent microbial attacks also lead to fruit drop.

In citrus tissues, ethylene is produced from the amino acid methionine, as in most other plants, in an enzymatically controlled reaction as follows:

$$\text{Methionine} \xrightarrow{\text{SAM Synthetase (EC 2.5.1.6)}} \text{SAM(S-adenosylmethionine)}\ (\text{S-Ado-Met})$$

$$\text{SAM} \xrightarrow{\text{ACC Synthase enzyme}} \text{1-aminocyclopropane-1-carboxylic acid (ACC)}$$

The ACC (1-aminocyclopropane-1-carboxylic acid) is then converted to ethylene through the action of the ACC oxidase enzyme. $CO_2$ and cyanide are generated and cyanide is detoxified by another enzyme. The SAM is also a precursor for production of polyamines.

ACC is a fundamental intermediate in ethylene production. The ACC content of fresh fruit tissues is much less, but ACC content increases after wounding. Ethylene production in aged albedo tissue is markedly reduced by inhibitors of protein synthesis. This suggests that protein is required to maintain continuous evolution of ethylene (Hyodo, 1981). Conversion of SAM to ACC by ACC synthase enzyme is a rate-limiting step and its gene expression is tightly controlled (Wang et al., 2002).

Potential storage life of citrus fruits with fairly good appearance and eating quality can be obtained if fruits are stored under the most optimum conditions after harvest. Postharvest action that reduces the accumulation of ethylene around citrus and other non-climacteric produce during marketing can result in an increase in postharvest life (Wills et al., 1999). It is suggested that the threshold level of ethylene action on non-climacteric produce is well below 0.005 ppm than the commonly considered threshold level of 0.1 ppm. There is a 60 percent extension in postharvest life of the produce when stored at 0.005 ppm than at 0.1 ppm ethylene. Storage life can be linearly extended in oranges with a logarithmic reduction in $C_2H_4$ level.

Ethylene destroys chlorophyll and hastens color development by increasing carotenoid synthesis. Temperature and storage duration also affect color development. Carotenoid pigment synthesis takes place at 15–20°C without ethylene treatment. Treatment with ethylene increases chlorophyllase activity in the rind and reduces the number and the size of chloroplasts. Ethylene increases the appearance of chilling injury (CI) symptoms, stem-end rot, and the content of volatile off-flavors in the juice and fruit internal atmosphere. The protective effect of a small amount of ethylene during postharvest storage of Shamouti oranges reduced the amount of decay caused by molds (Porat et al., 1999). The small amount of endogenous ethylene produced by the fruit was considered to be required to maintain their natural resistance against various environmental and pathological stresses.

Plant hormones, such as auxins, GA, ABA, and cytokinin, play a role singly or in combination in the induction of ethylene production. At maturity, the fruit of early-maturing Hamlin and Pineapple oranges contained more ethylene and abscissic acid than late-maturing Valencia and Lamb Summer oranges. Both compounds increased most rapidly in Pineapple, resulting in increased cellulase activity and loosening of fruit separation zones at maturity (Rasmussen, 1974). Application of ABA to Shamouti orange peel plugs and to the excised segments or albedo discs of Satsuma mandarin fruit stimulated ethylene production (Hyodo, 1978). Ethylene and ABA play an interactive role in orange fruit maturation.

Ripening 'Pinalate' oranges (ABA deficient mutant) exhibited delayed degreening, developed a yellow color, contained lower concentrations of ABA, and contained lower concentrations of xanthophylls compared with wild-type fruits. Application of ABA to 'Pinalate' fruits accelerated degreening, and exogenous ethylene also influenced ripening. Both ethylene and ABA are involved in citrus fruit ripening, with ethylene possibly regulating the initiation of ripening and ABA the rate of ripening (Alfetrez et al., 1999).

Ethylene antagonists such as silver nitrate and 2.5-norbonadiene have been shown to inhibit chlorophyll breakdown induced by ethylene, thus inhibiting the degreening process (Goldschmidt et al., 1993; Goldschmidt and Galily, 1995: Goldschmidt, 1998). The compound 1-methylcyclopropene (1-MCP) inhibits the binding of ethylene to the ethylene receptor site, the ethylene binding protein (EBP) and thus blocks ethylene action and degreening (Porat et al., 1999). 1-MCP prevented infection-induced degreening, such that treated grapefruits retained their green, immature color compared to yellow, non-treated controls. However, 1-MCP treatment significantly increased whole fruit ethylene production. This suggested that in the presence of a pathogenic stress, blocking the EBPs prevented regulatory control of the ethylene biosynthetic pathway that resulted in an uninhibited expression of the ACS (ACC synthase) stress-associated genes, increased ACS activity, and elevated ACC accumulation and ethylene production. Blocking the EBPs with 1-MCP did not affect progression of the pathogen through the fruit (Mullins et al., 2000). Even in absence of pathogenic infection, 1-MCP increased ethylene synthesis.

### E. Color Development and Regreening

During maturation and development, citrus fruits change color from green to yellow or orange or orange-red as per the genetic character of the variety under favorable climatic and growing conditions. This is called natural degreening, or natural color development. In some citrus fruits, if held on the tree beyond maturity, the yellow-orange color again changes to green, which is called regreening. The regreening process has economic significance since regreened fruit, although internally mature, is not marketable. The regreening process occur on the tree and also after harvest. Huff (1984) observed that the regreening process results from a decrease in soluble sugars as observed in Valencia orange fruit. Ultrastructural studies have indicated that regreening takes place as a result of the reversion of chromoplast to chloroplast and not from the formation of new chloroplast (Wrischer et al., 1986). The chromo-chloroplasts are photosynthetically active and required photosynthetic proteins have been found in chloroplasts of regreened fruit. In most citrus fruits, regreening occurs when fruit is on the tree, but pummelo regreening has been observed in harvested fruit stored in natural light or fluorescent light (Saks et al., 1988). The process depends on light intensity and temperature. Electron-microscopy study indicates that globular chromoplasts in peel tissues of pummelo revert to chloroplasts during regreening

although only partially (20 percent chlorophyll level obtained after regreening). The proteins of photosysnthetic system have been detected in regreened peel (reconstructed chloroplasts). These reconstructed chloroplasts were not observed in yellow fruit.

### F. Fruit Abscission

In citrus fruit, leaves have abscission zones at the leaf lamina-petiole interface and another at the interface of petiole and stem. Similarly, fruit has two abscission zones: one at the fruit – calyx interface and another between fruit button and stem. When citrus fruit naturally abscises from the tree (as the calyx and button-like receptacle at the stem end has an abscission zone at its base) no button or calyx is attached to fruit. The calyx remains attached to the branch or peduncle. Natural abscission is dependent on several exogenous and endogenous factors. In fruit harvested with clippers with a 2–3 mm peduncle attached, the abscission of the calyx and button takes place naturally after some aging and drying of this part takes place during handling and storage.

In early-maturing oranges such as Hamlin and Pineapple, ethylene and abscissic acid levels have been shown to be higher than the late-maturing Valencia and Lamb Summer. Ethylene (up to 95 nl/l in the internal atmosphere) and abscissic acid (50 μg/kg dry weight of flavedo) increased most rapidly in Pineapple oranges, leading to increased cellulase activity and loosening of the fruit. Fruit of the late-maturing cvs contained less than 25 nl/l ethylene and 40 μg abscissic acid/kg dry weight of flavedo at peak maturity. Cellulase activity and loosening of the fruit of these late-maturing cvs was slight (Rasmussen, 1975). In Marsh grapefruit, high gibberellic acid and cellulase activities were noted in zone 'C' of the peduncle (nearest to the fruit) during June, when fruit drop was high and fruit growth was rapid (Pozo et al., 1989).

### G. Fruit Hormonal Balance

The physiological role of various endogenous plant growth substances is to regulate the growth, development, maturity, ripening, and senescence of fruit naturally (when there is no intervention by externally applied plant-growth substances) as a predetermined sequential process in normal growing conditions. Understanding the endogenous hormonal balance and physiology of fruit development is essential for exogenous application of PGRs in commercial citriculture.

Gibberellic acid can promote auxin action, while high doses of auxin can increase ethylene production (Burg and Burg, 1968). Auxins are mainly responsible for cell enlargement by increased water uptake and they increase the extensibility of cell walls. Greater activity of pectin methylesterase was observed in auxin-treated tissues (Osborne, 1958) with higher synthesis of pectic substances

(Albersheim and Bonner, 1959). Thus, there is an interaction between these growth regulators; time and concentration of exogenous application determines the result. Whatever may be the exact role and nature of interaction of these growth regulators, it is certain that all of them are important in fruit development, overall physiology, and shelf life. Growth regulators play a deciding role in growth and development of parthenocarpic fruit and their use is imperative in citrus fruit production.

Fruits are entities of plants: they are a portion that surrounds ovules (seeds). Development of fruit is linked to the development of the ovule. Mostly seedless fruits are small in size and this may be the reason that some sort of stimuli, perhaps hormonal, from the seeds (ovules) or pollens are regulating fruit growth. Larger fruit with more seeds is produced if more pollen is used in Valencia orange (Erickson, 1968). In fact, these stimuli start from the stage of flower formation and tissues of fruit are formed as primordial of flower ovary. Even before anthesis, the development of the ovary that is the fruit takes place. After pollination, ovules (seeds) play a leading role in fruit development and this regulation is mostly by hormonal means. In young ovaries of Washington Naval orange, indoleyl-3-acetic acid (IAA) has been reported (Nitsch, 1965). A higher cytokinin content was observed in ovaries of Washington Navel than in those of Navelate, suggesting that a higher flow of nutrients toward the vegetative organs occurs in Navelate, causing low fruit set and poor productivity. When Navelate trees were subjected to water stress, an increase was observed in the abscissic acid content of leaves and fruits (Furio et al., 1982). Cytokinins – Riboxylzeatin, zeatin, and isopentenyladenosine – have been detected in the developing fruits of Salustiana (seedless) and Blanca Comuna (seeded) (Hernandez Minana et al., 1988).

In general, the level of ethylene increases in fruit before maturation and ripening; this is more profound in climacteric fruit and less in non-climacteric citrus. Ripening is controlled by endogenous as well as exogenous level of ethylene in fruits. Ethylene promotes maturity/ripening, abscission, and senescence. Gibberellins and cytokinins have an antagonistic effect and they delay the ripening and senescence process as observed in oranges (Eilati et al., 1969). It can be said that growth substances direct the movement and utilization of nutrients and also stimulate cell metabolism as well as promoting cell division, enlargement, and maturation. Abscissic acid (ABA), which is known to promote abscission of flowers, leaves, small fruitlets, and fruits, was also reported from *Citrus medica* rind and pulp (Milborrow, 1967) in concentration of 0.097 mg/kg fruit weight. Abscissic acid concentration increases as the limes ripen, suggesting cessation of growth and changes leading to senescence and abscission of fruit (Murthi, 1988). The different citrus species varied in free ABA (3–8 μg/g dry weight) and conjugated ABA (10–39 μg/g dry weight). In general, the amount of conjugated ABA was four times that of free ABA. In the lemon flower, the combined style and stigma tissues contained the predominant (>65 percent) amount of both free and conjugated ABA. During fruit development, different tissues showed dynamic changes in ABA content. In the vesicles, free ABA showed progressive increases

with development and reached a high level at maturity, whereas the conjugated ABA showed a corresponding decrease. Free ABA increased in the seed with fruit development. The content of ABA in citrus fruit was correlated with the fruit weight (Aung et al., 1991). In Kinnow mandarins, auxin content in the seeds and pulp increased 34 weeks after fruit set, until just before harvest, while other substances, namely cytokinins, gibberellins, and abscissic acid, decreased during later stages of fruit development – although the decrease in the abscissic acid was less abrupt in the pulp (Dhillon, 1986).

Extracts of the seedless Clementine cv. Fino had higher contents of auxin-like compounds than those of the seeded cv. Monreal. These compounds were suggested to be involved in the parthenocarpic fruit set and development of Fino ovaries. Diffusable gibberellin-like compounds produced in the developing seeds were obtained from Monreal fruits after anthesis. Fruit ABA content was low in Monreal, whereas in Fino and in Monreal it increased markedly after anthesis with the seeds removed. Garcia and Garcia-Martinez (1984) suggested that the control of fruit set and development in Clementines is carried out through an equilibrium between auxin-like substances (in seedless Fino) or gibberellin-like substances (in seeded Monreal) and ABA. Applications of GA3 or paclobutrazol (PBZ) to fruitlets influences fruit retention (Turnbull, 1989a). In Valencia orange, 29 percent of GA-treated fruits were retained to maturity compared with 2 percent in untreated controls. Paclobutrazole, which inhibits GA biosynthesis, caused 100 percent fruit drop within 35 days of application. GA1, GA3, GA8, GA19, GA20, GA29, 3-epi-GA1, 2-epi-GA29, and iso-GA3 were identified in tissues of Valencia orange (Turnbull, 1989b). No major differences were found between tissues of immature seeded and seedless fruit, and developing seeds did not contain high levels of any GA. Seed-produced GAs are not considered essential for fruit retention and development in Valencia oranges.

During growth of citrus fruits the mass (mg/g dry matter) of ABA and GAs per fruit increases. Fruit size and retention were greater in leafy fruitlets (fruits near leaves or leafy inflorescence) than in leafless ones (Hofman, 1988), indicating the role of leaves in maintaining hormonal balance. Although the Blanca Comuna orange and its parthenocarpic mutant cv. Salustiana did not differ qualitatively in the GAs present during flower and fruit development, the Salustiana contained greater amounts as observed in their reproductive organ (Talon et al., 1990).

Satsuma is a male sterile cultivar that shows high degree of natural parthenocarpy, whereas Clementine varieties are self-incompatible and hence have a very low ability to set a commercial crop. $GA_3$ improves fruit set in Clementines and have little effect on Satsumas. Thus, it appears that self-incompatible Clementines do not have sufficient GA for fruit set. This suggests that endogenous GA content in developing ovaries is the limiting factor controlling parthenocarpic development of the fruit in seedless Clementine mandarins. Levels of 13-hydroxy $GA_3$ have been found to be lower in Clementines. At petal fall fruits of Satsuma and Clementine had 65 and 43 pg of $GA_3$ respectively (Talon et al.,

1992). In Spain, usually GA is applied at full bloom of Clementine to increase the set.

Changes in citrus fruit rind color on the tree are due to the weather; however, endogenous growth regulators play the role of internal control mechanism (Eilati et al., 1969; Rasmussen, 1973). Low temperature can provide sufficient stress to produce ethylene, which causes the destruction of chlorophyll and development of carotenoides (Grierson et al., 1982). Plant-water relation, stress conditions, and accompanying endogenous hormonal changes are also likely to change fruit physiology and result in the development of disorders. Concentrations of IAA, gibberellin-like substances, and zeatin have been found to be higher in the pulp than in the peel of cracked fruits, but lower in the pulp than in the peel of normal fruits of lemon (Citrus limon (L) Burm) cv. Baramasi. Abscissic acid concentration was higher in the peel than pulp of cracked fruits, and was higher in cracked fruits than in normal fruits (Josan et al., 1995).

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