CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Citrus species are small to medium-size shrubs or trees that are cultivated throughout the tropics and subtropics. Citrus is primarily valued for the fruit, which is either eaten alone (sweet orange, tangerine, grapefruit, etc.) as fresh fruit, processed into juice, or added to dishes and beverages (lemon, lime, etc.). All species have traditional medicinal value. Citrus has many other uses including animal fodder and craft and fuel wood (Manner *et al*., 2006). Brazil is the largest producer followed by the United States of America (USA), China and Mexico. Spain, USA and South Africa are the largest exporter countries followed by Turkey and Morocco (Citrus Commodity Notes, 2005). Citrus production for the 2008-2009 season were total 12.0 million tons, down 7% from the 2007-2008 season and 33% lower than the record high production of 17.8 million tons for the 1997-1998 season (Agricultural Statistics Board, 2009).

2.2 Citrus anatomy and biology

Citrus fruit are composed of an outer flavedo layer that contains the exterior fruit color and sesquiterpene oil sacs that protect the fruit from insects and microorganisms. Just under the flavedo is a white spongy albedo layer. Tangerines are characterized by a looser flavedo/albedo layer that makes them easier to peel. This loose rind makes juicing operations more difficult or messy. Tangerine fruit requires much gentler handling and usually cannot be stored for any length of time prior to processing. The looser rind facilitates easier peeling and sectioning (Kimball, 1999).

Under this layer are the fruit sections, divided by membrane material. Each section contains many vesicles called juice cells. These juice cells are also elongated and attached to the center of the fruit and consist primarily of enlarged vacuoles that contain the juice. The nucleus of these cells and the other organelles are located essentially in the membrane of the expanded juice vacuole. It has been shown that the juice in the vacuole is clear or devoid of cloud material (Bennett, 1987). As the fruit matures, carbohydrates and water from the sap flow of the tree accumulate in the juice vacuole. Mitochondria in the membrane of juice cell is active during maturation, producing, in the Krebs cycle, citric acid that also accumulates in the juice vacuole. This accumulation and subsequent dilution with water and carbohydrate accumulation result in the change in acidity of various juices with maturation. The general trend is for acid to reach high concentrations in early-season fruit and then to be diluted out by fruit growth or citric acid depletion or both through increased metabolic demand in warmer weather (Kimball, 1984). The combination of water, carbohydrates organic acids, pulpy textures, cloudy opaqueness, carotenoid and anthocyanin pigments, sesquiterpene oils, and trace flavor components produces the recognizable flavors, colors and textures were associate with citrus fruit and their products (Kimball, 1999).

2.3 Mandarins or tangerines

Mandarins or tangerines are known in Japan as *mikan*, in India as *suntura* or s*angtra*, in Italy and Spain as *madarino*, in Thailand as *som khieo wan*, and in French-speaking countries as *mandarine*. Mandarins clearly dominate the citrus industry in the Orient and are important in many other parts of the world. Since the characteristics of mandarins vary so widely, they are often referred to as exotics. Mandarins are characterized by their loose and easily peeled rind, an open core, and a deeper orange color than most other types of citrus. The flavor of mandarins is also unique and richer than that of most citrus species. Mandarins rank second behind oranges in global importance and are more cold resistance than other types of citrus, except that freeze damage to the fruit can be severed due to the loose nature of the rind. Satsuma tangerines sustain freeze damage at around -8ºC (18°F), whereas other mandarin fruit sustain damage at around -5.5 to -5ºC (22 to 23°F), which is a lower temperature than that for other citrus fruit. Increased heat during the latter part of the season results in lower acid levels and milder juice. Four basic types of mandarins have been assigned their own separate species classification: common, Satsuma, Mediterranean and King (Kimball, 1999).

2.4 Citrus fruit physiology and biochemistry

2.4.1 Respiratory activity

Climacteric is defined as a period in the ontogeny of certain fruit 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 (Rhodes, 1981). 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 climacteric fruit. Internal quality of citrus fruit is at its best when fruit are at optimum maturity on the tree.

Subramanyam *et al.* (1965) observed that respiratory rate of 450 mg CO_2/kg/h occurred 30 days after fruit-set in acid lime fruit decreased to 200 mg after 60 days, 100 mg after 90 days, but would increase up to 140 mg after 120 days. At 150 and 180 days, respiration rates were 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 between fruit age, epicuticular wax, weight loss, internal atmosphere composition, and respiration was investigated in maturing 'Washington Navel' fruit by El-Otmani *et al.* (1986). Fruit epicuticular wax, internal CO_2 and internal ethylene content 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 could be detected if fruit were 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 after removal of the fruit from the ethylene atmosphere (Ladaniya, 2001).

Respiration of citrus fruit 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. Heat evolution can be computed from the respiration rate.

Respiration of citrus fruit responds differently at temperatures above and below critical temperatures that correspond closely with the temperature at which chilling injury occurs. Citrus fruit exhibit cumulative time-temperature influence of chilling temperature on CO_2 evolution (Eaks, 1960). The CO_2 evolution after exposure to 0°C is greater 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 hours. After 28 days at 1°C, peak respiration increased to 51 mg CO_2/kg/h for 'Lisbon' lemons and 34 mg CO_2/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 a relatively low respiration rate nearly with 40-45 mg CO_2/kg/h at 25-30°C and 50-60% relative humidity. Waxing reduced this rate by 34-35%. At low temperature (6-7°C), the respiration rate was 12-15 mg CO_2/kg/h. Waxing further reduced this rate by 10-11%. During storage, the respiratory activity of 'Shamouti' and 'Valencia' oranges declined and the internal CO_2 content rose from a range of 2-4% to 5-10%, while the O_2 declined from 17-19% to 10-12% (Ben-Yehoshua, 1969).

If fruit are stored at the lowest safe temperature (above freezing) which significantly suppresses respiration and fungal growth, the storage life of fruit 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 fruit is lower than that at higher humidity (Murata and Yamawaki, 1989). Higher concentration of O_2 (34.1-99.1%) increased the respiration rate of citrus fruit, while lower concentrations reduce the rate. Very low concentration of O_2 (0.5-5%) tend to increase the respiration rate. Citrus fruit produce higher quantities of ethanol and acetaldehyde at low O_2 levels and higher CO_2 levels, indicating anaerobic respiration.

Particular care is needed during harvesting, storage, transportation, and marketing of citrus fruit because rough handling causes wound, stimulation of respiration and ethylene production, and can induce physiological disorders and fungal rot. In ‘Ponkan’ (*C. reticulata*) fruit, respiration rate and superoxide dismutase (SOD) activity in both peel and flesh have been shown to increase during storage when fruit has 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 fruit. Peel conductivity increased with the degree of bruising, indicating increased membrane permeability (Xi *et al*., 1995).

Higher respiration rate 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. This 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. However, 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).

2.4.2 Biochemistry of respiration

As the respiration rate is low and steadily declines after the harvest of citrus fruit, 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 fruit 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 (Ladaniya, 2008).

Detached fruit 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, 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 flavin adenine dinucleotide ($FADH_2$). In respiration, when sugars are consumed the ratio of oxygen utilized is equal to CO_2 produced: the respiratory quotient RQ = 1 (RQ = molecule of CO_2 produced /molecule of O_2 consumed). In this case, respiration rate can be measured as either O_2 consumed or CO_2 evolved (Ladaniya, 2008).

In cytoplasm, glycolysis takes place. Glucose is converted to pyruvate by the enzymes of the Embeden-Meyerhof-Parnas (EMP) pathway. The pyruvate is finally converted to CO_2 and energy in tricarboxylic acid cycle (TCA) 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 both O_2

consumed and CO_2 evolved are measured, the substrate utilized in the respiration by the fruit can be known. However, it is quite possible that several substrates are being utilized at a time and a correct picture may not be simply available on the basis of O_2 consumption and CO_2 production (Ladaniya, 2008).

2.4.3 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 determines the rate of transpiration. Citrus fruit contain 80-85% of 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, it becomes tough and leathery, and hinders normal gas exchange. This causes anaerobic conditions and an increase in alcohol levels inside the fruit. Citrus fruit 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 (Ladaniya, 2008).

The loss of commercial value of ‘Shamouti’ and ‘Valencia’ orange fruit under various storage conditions is caused by transpiration, which leads to shriveling of the peel. During storage, respiratory activity declines and the internal CO_2 rises from a range of 2-4% to 5-10% while the O_2 declines from 17-19% to 10-12%. 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 fruit are essentially closed. However, ethylene, O_2, and CO_2 still diffuse through the residual stomatal opening (<1%), 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 that in air. Waxing fruit 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 fruit 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 fruit, acid limes do not have cuticle, and hence, water loss is quite rapid in these fruit (Ladaniya, 2008).

Loss of water does not only affects appearance or esthetic value but also reduces saleable weight, thus causing direct economic loss. Citrus fruit have low surface area-to-volume ratio. Thus, they lose water more slowly than many leafy vegetables. However, only 5-6% water loss can result in some change in appearance and firmness of the fruit. There can be detrimental to its marketability (Ladaniya, 2008).

2.4.4 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 for softening 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 fruit 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 grapefruit (Garcia *et al*., 1993).

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

Citrus fruit produce a very low quantity of ethylene after harvest and there is no associated rise in respiration. However, citrus fruit 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 which existed before ethylene treatment (Ladaniya, 2008).

Young, immature citrus fruit 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. Wound of harvested citrus fruit tissues causes a rise in ethylene production and accelerates coloring and related metabolic changes. This wound could result from fungal attacks (green and blue mold and other pathogens), insect damage, freezing injury, hail storms, 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 (Ladaniya, 2008).

Potential storage life of citrus fruit with fairly good appearance and eating quality can be obtained if fruit 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 (0.005 mg/l) is less than the commonly considered threshold level (0.1 mg/l). There is a 60% extension in postharvest life of the produce when stored at 0.005 mg/l than at 0.1 mg/l ethylene. Storage life can be linearly extended in oranges with a logarithmic reduction in ethylene level (Ladaniya, 2008).

Treatment with ethylene increases chlorophyllase activity in the rind and reduces the number and the size of chloroplasts 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. 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. The small amount of endogenous ethylene produced by the fruit was required in order to maintain their natural resistance against various environmental and pathological stresses (Porat *et al*., 1999).

2.4.5 Color development and regreening

During maturation and development, citrus fruit 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 fruit, 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 occurs 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. Required photosynthetic proteins have been found in chloroplasts of regreened fruit. In most citrus fruit, regreening occurs when fruit are on the tree, but pummelo regreening has been observed in harvested fruit stored either 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% 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.

2.4.6 Carbohydrates

Carbohydrates are organic compounds composed of carbon, hydrogen, and oxygen. This group consists mainly of monosaccharides, disaccharides, and polysaccharides. Carbohydrates play a major role in citrus fruit physiology when the fruit is attached to the tree and also after its harvest. Citrus fruit have attractive color, texture, and flavor. In all these properties, carbohydrates, particularly sugars, either in a free state or derivatives, play a very important role. A fine balance of sugars and acids of fresh citrus fruit causes the appealing flavor. Specific flavors of different citrus often result from glycosides. Attractive colors of many citrus fruit are due to sugar derivatives of anthocyanidins. Texture is governed by complex structural polysaccharides. Ascorbic acid, which is commonly considered to be a sugar derivative, is found widely and abundantly in citrus fruit. Total sugar contents vary widely in different kinds of citrus fruit, and they are present in free form mostly as monosaccharides and disaccharides in the juices (Ladaniya, 2008).

Changes in sugars during fruit growth and storage

Nearly 75 to 85% of the total soluble solids of orange juice are sugars. The reducing, non-reducing, and total sugars increase as fruit continues to ripen on the tree. This trend is observed in almost all citrus fruit except in acid fruit. Small immature fruit also photosynthesize. The main source of nutrition is leaves. It is generally thought that the main sugar transported from leaf to fruit is sucrose. Sucrose is further utilized for synthesis of various polysaccharides including pectin. Starch is found in the outermost cells of the fruit. Immature oranges have fairly high sucrose content, but during maturation this slightly decreases (Sawyer, 1963). α- and β-glucose, fructose, sucrose, and a small amount of galactose have been reported in ‘Valencia’ orange juice (Alberala *et al.*, 1967). As the early and mid-season oranges and tangerines ripen on the tree, the total sugars in the juice increase rapidly due to an accumulation of sucrose. Sawamura and Osajima (1973) found that translocation from leaf to fruit occurs in the form of glucose and fructose which are converted to sucrose in the fruit.

2.4.7 Organic acids

Organic acids have acidic properties because they have a carboxyl group (COOH) in a free state. These acids are an important source of acidic taste in fruit and also form a source of energy in plant cells. Organic acids are dissolved in cell sap either free or in combined form with salts, esters, or glycosides. Most of the acid is probably present in the vacuole of the cell. Organic acids are water soluble particularly in short carbon chain, and weak acids with a dissociation constant of about 10-5 at 25°C. Citric is an important and abundant acid in citrus fruit (Ladaniya, 2008).

The free acidity or titratable acidity of the juice of most citrus fruit is due largely to citric acid. Citrus fruit also contain considerable amounts of cations, mainly potassium, calcium, and magnesium. The acids also combine with cations and form salts. The total acidity represents the sum of all acids (free and those combined with cations) (Ladaniya, 2008).

The pH of citrus juices also provides an idea about the acidity of fruit, and it could be one way of expressing acidity. The pH is an important parameter from a processing point of view. Generally pH varies from about two for lemons and limes to about 4-4.5 in over-mature tangerines. The pH of the juice of ‘Valencia’ and ‘Washington’ navel oranges varies between 2.9 and 3.9. In ‘Palestine’ sweet limes, citric acid content of 0.08% was recorded with a pH 5.7 (Clement, 1964).

Citric acid is the principal acid in the endocarp of all citrus fruit except the sweet lemon and the acidless orange. The peel has less acid than the pulp. The main acids of the citrus peel are oxalic, malic, malonic, and citric acids. Together they account for 30-50% of the anions present (Clements, 1964). L-quinic acid was found in the peel and pulp of various citrus fruit (Ting and Deszyck, 1959). Tartaric, benzoic, and succinic acids have also been reported to be presented (Braverman, 1933). In the juice of lemons, citric acid may account for 60-70% of the total soluble solids.

2.4.8 Pigments

Major pigments that give color to citrus fruit are chlorophylls (green), carotenoids (yellow, orange, and deep orange), anthocyanins (blood red), and lycopenes (pink or red). During growth and maturation, especially in the immature stage, chlorophylls predominate in the peels of all citrus fruit (Todd *et al*., 1961). There is a rapid synthesis of carotenoids in the chromoplast during ripening, which is accompanied by a simultaneous loss of chlorophyll. Chloroplasts change into chromoplasts. Carotenoids are long chain compounds (tetra terpenes) and include carotenes, (α, β, etc.) and xanthophylls (luteins, flavoxanthins, leuteoxanthin, zeaxanthin, violaxanthin, etc.). Total carotenoid, chlorophyll, and lycopene content varies greatly in the peel and pulp of various citrus fruit (Ladaniya, 2008).

2.4.9 Vitamins

The main contribution of citrus fruit in human nutrition is undoubtedly their supply of vitamins, especially ascorbic acid (vitamin C). Citrus fruit contain many other vitamins too. Vitamin C content of juice of different citrus fruit varies considerably. Oranges generally contain 40-70 mg vitamin C/100 ml juice, whereas grapefruit, tangerines, and lemons provide 20-50 mg/100 g. Ascorbic acid is usually high in immature oranges and grapefruit. As fruit ripen and increase in size, the concentration decreases (Ladaniya, 2008).

Compared with other foods, citrus juice may supply larger amounts of several vitamins on a per-calorie basis. Citrus juice, on a concentration basis, is higher in vitamin A, thiamine, and nicotinic acid (niacin) than milk but lower in riboflavin (Ting and Attaway, 1971). Rakienten *et al*. (1952) reported that inositol and tocopherol were present in fairly large amounts in the juice of citrus fruit. The other vitamins also occurred in amounts appreciable enough to be of dietetic importance.

2.5 Postharvest and preparation for market of tangerine fruit

Harvested tangerines should be brought to a packing area soon after harvest to begin the steps of preparing the fruit for market. These steps involve cleaning, grading, and packing. In addition, fruit destined for export may need to be treated with natural ripening agent ethylene in order to improve the external peel color. The ethylene treatment should be done prior to cleaning and grading (Ministry of Fisheries, Crops and Livestock New Guyana Marketing Corporation National Agricultural Research Institute, 2004).

2.5.1 Degreening

In order to improve external skin color and export market acceptance, tangerines can be treated with ethylene to degreen the peel. Ethylene breaks down the green chlorophyll pigment in the peel and allows the yellow or orange carotenoid pigments to be more prominent. The ethylene treatment does not change the flavor of the fruit.

The general degreening protocol involves exposing the green-skinned tangerine fruit to low concentrations of ethylene (usually between 1 to 10 mg/l) at 28°C (83°F), 90% to 95% relative humidity for several days. The best ethylene concentration and treatment duration varies by cultivar and growing conditions. Atmospheric ethylene concentrations above 10 mg/l can cause stem end rot and speed up decay.

In order to obtain adequate fruit degreening, ample internal air movement is needed in the treatment chamber so that the entire air volume is circulated every 2 to 3 minutes. The CO_2 levels inside the treatment chamber should not be allowed to rise above 2,000 mg/l as high levels of CO_2 will inhibit the effect of ethylene. The treatment chamber should be well insulated in order to maintain the required ethylene concentration.

A liquid ethylene-releasing compound, called ethephon [(2-chloroethyl) phosphonic acid], is also an effective degreening agent. It is applied by dipping the fruit in a tank of clean water at ambient temperature with 500 mg/l ethephon for 1 minute. It is important that the water be properly sanitized with sodium hypochlorite (i.e. 150 mg/l at a pH of 6.5) and a fungicide (i.e. 500 mg/l benomyl, 1,000 mg/l thiabendazole, or 1,000 mg/l imazalil) to prevent postharvest decay.

2.5.2 Cleaning

Generally, harvested tangerine fruit is not clean enough to pack directly from the field. Sooty mould, surface stains, dust, and other residues need to be removed from the peel to improve the external appearance of the fruit. Tangerines may be cleaned manually or semi-mechanically. Small scale operations typically submerge the fruit in a wash tank, followed by a gentle scrubbing of the fruit surface. It is important to use clean and properly sanitized water with a small amount of detergent. The water should be treated with hypochlorous acid (150 mg/l household bleach at a pH of 6.5). The concentration of hypochlorous acid and water pH should be frequently checked and maintained at the recommended levels during the entire cleaning operation.

Larger volume operations may choose to clean the fruit by passing them under high-pressure spray wash nozzles while moving along a series of roller brushes to gently scrub the fruit surface. Revolving brushes will remove most debris, after which soap or detergent is dribbled onto the fruit to enhance cleaning as the fruit continues across the brushes. Adequate cleaning usually requires about 30 seconds on the brushes, which should be horsehair grade and rotating at about 100 rpm. The tangerines should be thoroughly rinsed as they exit the brushes. The fruit surface should be dry prior to packing. A series of sponge rollers may be used to facilitate drying.

The spray wash water must be properly sanitized to reduce the spread of postharvest fruit rot. Chlorine and sodium *o*-phenylphenate (SOPP) are both effective sanitizing agents. However, chlorine is more readily available as it is sold as household bleach and typically comes in a 5.25% formulation. The effectiveness of chlorine is dependent upon water pH (ideal is 6.5), time of exposure, and the amount of hypochlorous acid present (ideal is 150 mg/l). A fungicide (same as recommended for degreening) should also be added to the wash water for maximum disease control. The fungicide can be added either to the wash tank or to the overhead spray water, depending on the packinghouse set-up. Heating the wash water to 40°C (104°F) for green-colored fruit has been found to provide enhanced decay control.

2.5.3 Grading

Grading and sorting of the fruit is done immediately after washing. Tangerines should be categorized according to size; color intensity and uniformity; shape; firmness; and the degree of surface blemishes. Only uniform appearing fruit should be packed into each container. Fruit which is damaged by insects, decay, or below market standards should not be packed for sale.

Grading can be done manually in small-scale operations or semi-automatically in larger volume operations. A series of perforated conveyor belts, roller bars, or drum rollers with different size holes openings are effective in mechanically sizing tangerine fruit.

2.5.4 Waxing

Tangerine fruit benefit from a postharvest wax application. The simplest ways to apply the wax are a manual rub or an overhead spray as the fruit are rotating on a bed of brushes made of horsehair. Various types of citrus wax formulations are available, but a water-emulsion wax is preferred. Waxing reduces moisture loss and shriveling of the fruit and extends the market life. Most of the fruit's natural wax is removed during washing, so it should be replaced. Waxing also imparts an attractive shine to the peel. Some commercial waxes have a postharvest fungicide incorporated, which provides additional protection against postharvest decay. Waxing can be detrimental to tangerines if it is applied too thick. This may reduce gas exchange through the peel and lower the internal O_2 concentration of the fruit resulting in off-flavor.

Degreening should always be done prior to waxing as the wax coating will partially restrict gas exchange through the peel and inhibit the action of ethylene.

2.5.5 Packing

Tangerines should be packed in strong, well-ventilated containers. Plastic crates are effective container for domestic marketing of tangerines. The ideal containers for export marketing of tangerines are carton boxes.

2.5.6 Temperature control

Tangerines do not maintain good quality when kept at ambient temperature. The fruit will have a high rate of decay after 2 weeks, and will be nearly all decayed or shriveled after 4 weeks at 24ºC (75ºF). Tangerine fruit is also susceptible to puffiness, in which the peel separates from the pulp at high storage temperatures. The ideal storage temperature for tangerines is 4°C (40ºF). At this temperature the fruit will have a storage life of 4 to 6 weeks.

2.5.7 Relative humidity

Although tangerines have a waxy peel, significant moisture loss can occur after harvest. Dehydration and shriveling of the fruit become apparent after the fruit has lost 5% of its original weight. In order to minimize postharvest water loss and preserve postharvest quality, tangerines should be stored at their optimum relative humidity of 90% to 95%. At a relative humidity of less than 70%, the peel will become thin, dry, and shriveled within 3 weeks. This will negatively impact the appearance and market potential of the fruit.

2.6 Coating application

Many coating methods are available. The type of method used depends on the nature of the coating constituents and the characteristics of the commodity to be coated.

2.6.1 Dip application

The dip application method involves submerging small quantities of produce into a vat of coating solution. Citrus fruit were the first types of fruit being coated by this method. Nowadays, other types of fruit, including tomatoes, rutabagas, and peppers have also been coated by dipping. Coating continuity is of paramount importance in the dip method, and can only be achieved by complete wetting of the entire fruit. After dipping, commodities are dried either at room temperature or with aid of a drier. Using the dipping method, several problems may occur including building up of trash, dirt, and microorganisms in the dipping tank. Other problems with dipping may include coating dilution due to addition of water from the fruit or vegetable surface. Lastly, coating applications from the dip method are usually thick, which may pose problems with respiration and storage characteristics (Grant and Burns, 1994).

2.6.2 Foam application

Foam coatings are made by adding a foaming agent to the coating or by blowing compressed air in to the coating tank. The foam is continually agitated and then dropped onto the commodity as it rolls by on rollers. Brushes and cloth flaps smooth the coating over the fruit while excess is removed and recycled. Unfortunately, the act of evenly distributing the coating may be difficult due to the constant movement of the fruit. The fruit is dried as in dip application. However, the process is not as extensive as dip coating due to the lower water content of foam coatings (Long and Leggo, 1959).

2.6.3 Spray application

Most coatings are applied by spray application. Such coatings are sprayed through nozzles onto produce which is traveling by on rotating brushes. The brush set up is important for coating application and distribution. Straight-cut brushes are more effective for coating distribution than round brushes, while spiral cut brushes may be used on irregularly shaped produce to facilitate tumbling and ensure even coverage. The number of brushes is also important. Too many brushes will remove too much coating, while too few will not generate even coverage. Typically, brush beds are made of 12-14 brushes, composed of equal portions of polyethylene and horsehair bristles. The distance between bristles is important. The recommended distance is no more than 0.95 cm between bristles. Brush wear should be often checked due to the tendency for natural bristles to fall away from the brush (Grant and Burns, 1994).

Coating flow from spray nozzles can be emitted as a full cone, tapered, even-edged flat or air atomizing in nature. Spray delivery allows for uniform coating of commodities. However, air currents between nozzles and fruit and vegetables can affect the coating application, and therefore, should be minimized. Adjustments to flow and bed fill should also be made when spray coating commodities of different surface areas.

2.6.4 Drip application

Drip application is the most economical coating application method. The drip method involves dropping various sized coating droplets onto a commodity or brush. Coatings are then dispersed by the rolling action of the brushes (Grant and Burns, 1994).

2.6.5 Controlled drop application

This method of application has been used only with certain types of produce. Controlled drop application involves delivering the coating to a rotating disk that disperses the coating into smaller sized droplets, which are then delivered through a spray nozzle to the food. The speed of the rotating disk will determine the coating droplet size; larger droplets are delivered by a slower rotation speed. Citrus commodities as well as apples have been coated using this method (Grant and Burns, 1994).

Regardless of which coating method is used, several factors are important for successful coating. Clean pumps, nozzles and brushes are essential for preventing clogs and product buildup. Buildup of coatings on rollers is particularly detrimental due to hardening of the coating, which may injure commodities passing over them. Prior to coating, all commodities should be free of surface water that will dilute the coating and may cause foaming of the coating materials. Brush wear and tear should also be monitored continually. Worn brushes will not give complete coating coverage. Making sure that commodities are thoroughly cleaned can also ensure complete coating. Sufficient drying time is also necessary. Insufficiently dried coatings which when handled can create incomplete coating coverage points may be sticky or tacky. Therefore, drying time coupled with the correct temperature, humidity and airflow are necessary for proper coating application.

2.7 Coating materials for fruit and vegetables

Most fruit and vegetables possess a natural waxy layer on the surface, called cuticle. This waxy layer generally has a low permeability to water vapor. Applying an external coating will enhance this natural barrier or replace it in cases where this layer has been partially removed or altered during postharvest handling or processing. Coatings provide a partial barrier to moisture and gas exchange, improve the mechanical handling property maintaining structural integrity, retain volatile flavor compounds, and carry other functional food ingredients.

Biopolymers such as proteins, polysaccharides, lipids, and resins are the common coating-forming materials that can be used alone or in combinations. The physical and chemical characteristics of the biopolymers greatly influence the functionality of resulting coatings (Sothornvit and Krochta, 2000). Selection of coating materials is generally based on their water solubility, hydrophilic and hydrophobic nature, easy formation of coatings, and sensory properties.

2.7.1 Lipid-based coatings

Lipid compounds include neutral lipids which are esters of glycerol and fatty acids, and the waxes which are esters of long-chain monohydric alcohols and fatty acids. On the other hand, resins are a group of acidic substances that are usually secreted by special plant cells into long resin ducts or canals in response to injury or infection in many trees and shrubs (Hernandez, 1994). Lipids including neutral lipids, fatty acids, waxes, and resins are the traditional coating materials for fresh produce, showing the effectiveness in providing moisture barrier and improving surface appearance (Kester and Fennema, 1986; Hernandez, 1994; Hagenmaier and Baker, 1994a, b; Hagenmaier and Baker, 1995).

Waxes (carnauba wax, beeswax, paraffin wax, polyethylene and others) have been commercially applied as protective coatings for whole fresh fruit and vegetables since the 1930s with the purpose of blocking moisture transport, reducing surface abrasion during fruit handling (Lawrence and Iyengar, 1983), and controlling soft scald formation (browning of the skin) in fruit such as apples by improving mechanical integrity and controlling internal gas composition of the fruit (Kester and Fennema, 1986). In general, wax coatings are substantially more resistant to moisture transport than other lipid or non-lipid coatings (Kaplan, 1986). Commercial applications of wax coatings are rather extensive on citrus, apples, mature green tomatoes, rutabagas, cucumbers, and other vegetables such as asparagus, beans, beets, carrots, celery, eggplant, kohlrabi, okra, parsnips, peppers, potatoes, radishes, squash, sweet potatoes, and turnips (Hardenburg, 1967), where high glossing and shine surface are desired. Waxes-based coatings are continuously evaluated for their applications in citrus fruit, melons, apples and pears (Mannheim and Soffer, 1996; Hagenmaier and Baker, 1997; Petracek *et al*., 1998; Hagenmaier, 2000; Alleyne and Hagenmaier, 2000; Bai *et al*., 2002; Da-Mota *et al*., 2003; Fallik *et al*., 2005; Porat *et al*., 2005).

Shellac and other resin-based coatings generally have lower permeability to O_2, CO_2, and ethylene gases. Shellac coatings also dry fast and produce a shiny surface on coated produce (Baldwin, 1994). Resin coatings are fairly effective at reducing water loss, but are the least permeable to gases among the available coating film-formers. This means that fruit can easily undergo anaerobic respiration and flavor changes that are usually undesirable. Some climatic fruit do not tolerate resin coatings at all due to impaired ripening from the MA created by these materials (Baldwin and Baker, 2002; Porat *et al*., 2005). The gas permeability of shellac and several experimental coating formulations, including candelilla wax and shellac carnauba, was measured by Bai *et al*. (2003b) on different varieties of apples. It was found that the shellac coating resulted in maximum fruit gloss, lowest internal O_2, highest CO_2, and least loss of flesh firmness for all of the apple varieties. However, the shellac coating gave an unusual accumulation of ethanol in freshly harvested and 5 months stored ‘Fuji’ apple. Candelilla and carnauba-shellac coatings maintained more optimal internal O_2 and CO_2, and yield better quality to ‘Fuji’, ‘Braeburn’, and ‘Granny Smith’ apples, although these coatings may present too much of a gas barrier for ‘Granny Smith’. It was recommended that the shellac and carnauba-shellac be the best coatings for ‘Delicious’ and ‘Braeburn’ or ‘Fuji’, respectively (Bai *et al*., 2003a).

The beneficial properties of lipid-based coating, including waxes-, resins-, neutral lipids-, and fatty acid-based coatings, include good compatibility with other coating-forming agents and high water vapor and gas-barrier properties in comparison with polysaccharides- and protein-based coatings (Greener and Fennema, 1992). However, lipid-based coatings present a greasy surface and undesirable organoleptic properties such as waxy taste and lipid rancidity (Guilbert and Biquet, 1986). Waxes and shellac tend to restrict the gas exchange of O_2 and CO_2 between atmosphere and fruit to the extent that the internal O_2 level becomes too low to support aerobic respiration, resulting in high levels of internal ethanol, acetaldehyde, and internal CO_2 (Petracek *et al*., 1998; Alleyne and Hagenmaier, 2000). This leads to accumulation of off-flavors fruit (Mannheim and Soffer, 1996; Baldwin *et al*., 1997; Hagenmaier, 2002). In addition, some lipid materials, such as shellac, are unstable when subjected to temperature changes, where a white waxy layer usually appears when moving fruit from cold storage to the grocery display shelves due to temperature fluctuation. Currently, lipid-based coating materials are usually studied in combination with polysaccharide- or protein-based coating materials for forming composite coatings, taking advantages of the desirable properties of different materials (Lin and Zhao, 2007).

2.7.2 Polysaccharide-based coatings

Polysaccharides that have been evaluated or used for forming films and coatings include starch and starch derivatives, cellulose derivatives, alginates, carrageenan, various plant and microbial gums, chitosan, and pectinates (Nisperos-Carriedo, 1994; Krochta and De Mulder-Johnston, 1997; Debeaufort *et al*., 1998). These coatings can be utilized to modify the internal atmosphere, thereby, reducing respiration of fruit and vegetables (Banks, 1984a, b; Drake *et al*., 1987; Motlagh and Quantick, 1988; Nisperos-Carriedo and Baldwin, 1990). Due to the hydrophilic nature of polysaccharides, the advantages of using these materials are more apparent as a gas barrier than retarding water loss. However, certain polysaccharides, applied in the form of high-moisture gelatinous coatings, can effectively retard moisture loss of food by functioning as sacrificing agents rather than moisture barriers (Kester and Fennema, 1986).

2.7.2.1 Starch and derivatives

Starch, the reserve polysaccharide of most plants, is one of the most abundant natural polysaccharides used as food hydrocolloid (Narayan, 1994) because of its wide range of functionality and relative low cost. Starch films are often transparent (Lourdin *et al*., 1997; Myllärinen *et al*., 2002) or translucent (Rindlav *et al*., 1997), odorless, tasteless, and colorless, and have low permeability to O_2 at low-to-intermediate relative humidity (Roth and Mehltretter, 1970). Starch films have low O_2 permeability comparable to ethylene vinyl alcohol copolymer, a commercial synthetic oxygen-barrier film, at ambient environment (such as 20°C, 50% to 60% relative humidity) (Forssell *et al*., 2002), but the O_2 permeability is greatly affected by the water content of the films (Gaudin *et al*., 2000; Forssell *et al*., 2002).

Dextrins, derived from starch with smaller molecular size, are often used as film-formers and edible adhesives (Smith, 1984). Coatings from dextrins provide a better water vapor resistance than starch coatings (Allen *et al*., 1963). Pullulan is an extracellular microbial polysaccharide from starch that is edible and biodegradable. Pullulan films cast from aqueous solution are clear, odorless, and tasteless, and have good oxygen-barrier properties (Conca and Yang, 1993). Pullulan-based coatings have shown potential for preserving fresh strawberries and kiwifruit because of their barriers to moisture, O_2, and CO_2 (Diab *et al*., 2001). In general, due to its good O_2 barrier, starch is a good candidate for coating fruit and vegetables having high respiration rates; thus, suppressing respiration and retarding oxidation of coated products.

2.7.2.2 Cellulose and derivatives

Cellulose is the structural material of plant cell walls. In general, cellulose derivatives possess excellent film forming property, but they are too expensive for large-scale commercial usage. The most common commercially produced cellulose derivatives are carboxymethyl cellulose (CMC), methyl cellulose (MC), hydroxyl-propyl cellulose (HPC), and hydroxypropylmethyl cellulose (HPMC).

Edible coatings made of CMC, MC, HPC, and HPMC have been applied to some fruit and vegetables for providing barriers to O_2, oil, or moisture transfer, and for improving batter adhesion (Morgan, 1971; Sacharow, 1972; Krumel and Lindsay, 1976; Maftoonazad and Ramaswamy, 2005). CMC coatings have shown the capabilities in retaining the original firmness and crispness of apples, berries, peaches, celery, lettuce, and carrots when used in a dry coating process (Mason, 1969), preserving important flavor components of some fresh fruit and vegetables (Nisperos-Carriedo and Baldwin, 1990), and reducing O_2 uptake without increasing CO_2 level in the internal environment of coated apples and pears by simulating a controlled atmosphere environment (Banks, 1985).

2.7.2.3 Seaweed extracts

Alginates are the major structural polysaccharides of brown seaweed known as Phaeophyceae (Sanderson, 1981). Alginate gel coating can act as a sacrificing agent, where moisture is lost from the coating before the food significantly dehydrates (Kester and Fennema, 1986). The coating can also improve the adhesion of batter to the surface of fruit and vegetables (Fisher and Wong, 1972). Alginate coatings are good O_2 barriers (Conca and Yang, 1993) that can retard lipid oxidation in various fruit and vegetables (Kester and Fennema, 1986). Calcium alginate coatings were found to improve the quality of fruit and vegetables, such as shrinkage reduction, oxidative rancidity, moisture migration, oil absorption, and sealing-in volatile flavors and appearance and color improvement in comparison with uncoated ones (Lin and Zhao, 2007).

Carrageenan, extracted from several red seaweeds, mainly *Chondrus crispus* (Whistler and Daniel, 1985) and a complex mixture of several polysaccharides, is another potential coating material for fruit and vegetables. Carrageenan-based coatings have been applied to fresh fruit and vegetables such as fresh apples for reducing moisture loss, oxidation, or disintegration of the apples (Lee *et al*., 2003). By acting as a sacrificial moisture layer, carrageenan coating was able to protect moisture loss of grapefruit (Bryan, 1972).

Other gums, including exudate gums (gum arabic or acacia gum and gum karaya) and microbial fermentation gums (xanthan gum), have also been studied as coating materials for fruit and vegetables. Xanthan gum provides uniform coatings with good cling and improves adhesion in wet batters (Arnold, 1968).

2.7.3 Other polysaccharide-based coatings

2.7.3.1 Chitosan

Chitosan, a linear polymer of 2-amino-2-deoxy-β-D-glucan, is a deacetylated form of chitin, a naturally occurring cationic biopolymer (Davis *et al*., 1988; Tharanathan and Kittur, 2003). It occurs as the shell component of crustaceans (crab and shrimp), as the skeletal substance of invertebrates, and as the cell wall constituent of fungi and insects (Anonymous, 1991). Applications of chitosan include flocculating agent, clarifier, thickener, gas-selective membrane, coating material, promoter of plant disease resistance, wound-healing factor agent, and antimicrobial agent (Brine *et al*., 1991; Goosen, 1997).

Chitosan is one of the most promising coating materials for fresh produce because of its excellent film-forming property, broad antimicrobial activity, and compatibility with other substances, such as vitamins, minerals, and antimicrobial agents (Park and Zhao, 2004; Chien *et al*., 2007a, b; Ribeiro *et al*., 2007). Chitosan-based coatings have shown effectiveness in delaying ripening and decreasing respiration rates of fruit and vegetables (Krochta and De Mulder-Johnston, 1997; Vargas *et al*., 2006), and reducing weight loss, color wilting, and fungal infection in bell peppers, cucumbers, and tomatoes (El-Ghaouth *et al*., 1991a, b; El-Ghaouth *et al*., 1992a, b). A commercial fruit coating, Nutri-Save (Nova Chem, Halifax, Canada), was developed to serve as both film former and natural preservative and to create a modified atmosphere for whole apples and pears to reduce respiration rate and desiccation of these commodities (Elson *et al*., 1985). Another very attractive function of chitosan is its broad antifungal property (Stossel and Leuba, 1984; Hirano and Nagao, 1989). This is done by inducing a plant-defense enzyme, chitinase, and in plant tissues which degrades fungal cell walls (Hirano and Nagao, 1989). The fungistatic property of chitosan coating, which inhibits spore germination, germ tube elongation, and growth of pathogens (*Botrytis cinerea* and *Rhizopus stolonifer*), have been reported by several researchers. Zhang and Quantick (1998) demonstrated the antifungal effects of chitosan coating on fresh strawberries and raspberries during cold storage. Iverson and Ager (2003) invented a chitosan-based antifungal coating mixed with an edible wax emulsion and/or a preservative such as sodium benzoate, and/or an adhesion additive such as zinc acetate, and/or a wetting agent to have a molecular weight sufficient to form a composition having a solid content of about 15% or higher. Han *et al*. (2004) reported that chitosan coatings extend shelf life of fresh strawberries and red raspberries by decreasing weight loss and delaying changes in color, titratable acidity, and pH during cold storage, and reducing the drip loss and improving the texture quality of frozen-thawed strawberries. Park (1999) demonstrated the antifungal function of chitosan coatings on fresh strawberries through a microbial challenge study and showed the excellent compatibility of chitosan with other antifungal agents. Vargas *et al*. (2006) evaluated high molecular weight chitosan combined with oleic acid for preserving the quality of strawberries and found that the addition of oleic acid does not only enhances chitosan antimicrobial activity but also improves water vapor resistance of coated samples. In addition, chitosan-based coatings can carry high concentrations of vitamins and minerals for increasing the content of these nutrients in the fresh and frozen fruit without altering its antifungal and moisture-barrier functionality (Han *et al*., 2004).

2.7.3.2 Aloe vera

A. vera is a tropical and subtropical plant that has been used for centuries for its medicinal and therapeutic properties (Eshun and He, 2004). Recently, there has been increase in the interest of using *A. vera* gel as a functional ingredient in drinks, beverages, and ice cream (Moore and MacAnalley, 1995), and as an edible coating material for fruit and vegetables driven by its antifungal activity (Martínez-Romero *et al*., 2003). *A. vera* gel-based edible coatings have shown to prevent loss of moisture and firmness, control respiratory rate and maturation development, delay oxidative browning, and reduce microorganism proliferation of sweet cherries (Martínez-Romero *et al*., 2003) and table grapes (Valverde *et al*., 2005).

2.7.4 Protein-based coatings

Coatings made of plant proteins (such as zein, soy protein, and wheat gluten) and animal proteins (such as milk protein) exhibit excellent O_2, CO_2, and lipid-barrier properties, particularly at low relative humidity (Gennadios *et al*., 1994; Baldwin and Baker, 2002). Protein-based coatings are brittle and susceptible to cracking due to the strong cohesive energy density of the polymers (Lim *et al*., 2002).

2.7.4.1 Plant origin

Zein and soy protein are the 2 major plant origin proteins studied as coating materials for fruit and vegetable applications. Zein is the key storage protein of corn and comprises approximately 45% to 50% of the proteins in corn. The ability of zein and its resins to form tough, glossy, and hydrophobic grease-proof coatings and their resistance to microbial attack have of commercial interest (Pomes, 1971). Zein-based coatings have water vapor permeabilities lower than or similar to those of other protein-based coatings (Krochta, 1992), but much higher than that of low-density polyethylene (LDPE) (Bakker, 1986). Its O_2 and CO_2 permeability is also lower than that of polysaccharides, polysaccharides/lipid composite coatings (Greener and Fennema, 1989), as well as common plastic films such as LDPE, propylene, polystyrene, and polyvinyl chloride, but higher than that of gluten coatings (Gennadios *et al*., 1993a, b).

Zein-based coatings have been applied to nuts and fresh and dried fruit, often as a substitute for shellac coatings. Zein coatings were able to retard ripening of tomatoes (Park and Chinnan, 1990; Park *et al*., 1994b), maintain the original firmness and color of broccoli florets, provide a continuous adhesive and stable coating with satisfactory sensory properties, and to reduce the growth of *Listeria monocytogenes* on cooked sweet corn. Compared to commercial shellac coatings, zein coatings are favorable for gloss and other quality characteristics on apples (Bai *et al*., 2002; Bai *et al*., 2003a).

Soy protein concentrate (SPC) or soy protein isolate (SPI) is extracted from defatted protein meal and contains 65% to 72% and 90% protein on a dry basis, respectively. Soy protein coatings generally exhibit poor moisture resistance and water vapor barrier properties due to the inherent hydrophilicity of the protein and the addition of hydrophilic plasticizers (Rhim *et al*., 2000). In contrast, soy protein coatings are potent oxygen barriers, especially in low relative humidity environments (Gennadios *et al*., 1993b; Ghorpade *et al*., 1995). The high oxygen-barrier capability of SPI coatings has led to their applications as microencapsulating agents of flavors and pharmaceuticals, or in coatings of fruit, vegetables, and cheese (Petersen *et al*., 1999).

2.7.4.2 Animal origin

Milk proteins such as whey protein and casein are important materials for coatings based on their numerous functional properties (Chen, 2002; Krochta, 2002). Caseinate-based and whey protein-based coatings have been applied on raisins, frozen peas, and peanuts to provide a barrier to oxygen and moisture transfer for extending shelf life of the products (Chen, 1995; Maté and Krochta, 1995). Caseinate and WPI coatings were reported to efficiently delay browning of apple and potato slices by acting as oxygen barriers (Tien *et al*., 2001). Such coatings, together with modified atmosphere packaging, protected carrots against dehydration and helped retain their firmness during storage (Lafortune *et al*., 2005). Milk proteins are important functional ingredients. Their solubility in aqueous solutions and unique surface characteristics (the balance of hydrophilic and hydrophobic forces) make them excellent emulsifiers. Hence, whey proteins are excellent candidates for developing composite or emulsion coatings with improved moisture barrier property (Lee *et al*., 2003; Certel *et al*., 2004).

2.7.5 Emulsion and bilayer coatings

Recent emphasis and interest in the development of edible coatings have been focused on composite or bilayer coatings, such as integrating proteins, polysaccharides, and/or lipids together in order to improve functionality of the coatings. This is based on the fact that each individual coating material has its own unique, but limited, functions, and thus, together their functionality can be enhanced (Krochta, 1997).

Composite coating can be categorized as a bilayer or a stable emulsion. For bilayer composite coatings, lipid generally forms an additional layer over the polysaccharide or protein layer, while the lipid in the emulsion composite coatings is dispersed and entrapped in the matrix of protein or polysaccharide. The amphiphilic character of proteins enables proteins to stabilize the protein-lipid emulsions through the balance between forces, primarily electrostatic and hydrophobic. Polysaccharides stabilize emulsions by strongly attaching to the surface of the lipid and significantly protruding into the continuous phase to form a polymeric layer or a network of appreciable thickness (Callegarin *et al*., 1997). In many cases, addition of emulsifier is required to improve emulsion stability.

The barrier and mechanical properties of the composite coatings are affected by the composition and distribution of the hydrophobic substances in the coating matrix (Debeaufort *et al*., 1993). In general, bilayer coatings are more effective water vapor barrier than emulsion coatings due to the existence of a continuous hydrophobic phase in the matrix. Their moisture-barrier property can also be improved by increasing the degree of lipid saturation and chain length of fatty acids (Kamper and Fennema, 1984a, b; Hagenmaier and Shaw, 1990). For emulsion composite coatings, the type of lipid, location, volume fraction, polymorphic phase, and drying conditions significantly impact moisture-barriers property (Gontard *et al*., 1994). Moisture barriers of whey protein-lipid emulsion coatings are improved when the hydrocarbon chain length of fatty acid alcohols and monoglycerides increase from 14 to 18 carbon atoms (McHugh and Krochta, 1994b, c). Beeswax and fatty acids are more effective in reducing water vapor permeability of WPI-based emulsion coatings than fatty acid alcohols due to the lipid polarity.

The improved moisture-barrier properties of composite coatings have made them promising candidates for coating fresh fruit and vegetables. Cole (1969) reported that a bilayer coating formed with amylose ester of fatty acids and protein prevents dehydration and oxidative degradation of fruit and vegetables. Wheat gluten with lipid (beeswax, stearic acid, and palmitic acid) based bilayer coatings significantly retained firmness and reduced weight loss of fresh strawberries (Tanada-Palmu and Grosso, 2005). Chitosan-lauric acid composite coatings prevented fresh-cut apple slices from browning and water loss (Pennisi, 1992). A casein-lipid emulsion coating formed a tight matrix that binds to the cut apple surfaces and protects apple slices from moisture loss and oxidative browning (Krochta *et al*., 1990). A sodium caseinate/stearic acid emulsion coating reduced white blush and respiration rate of peeled carrots. A calcium caseinate acetylated monoglyceride emulsion coating reduced water loss of apples, celery sticks, and zucchini as a result of increased water vapor resistance of the emulsion coatings (Avena-Bustillos *et al*., 1997). Caseinate-lipid emulsion coatings offer advantages over commercial wax coatings in that they can be applied to fresh produce at room temperature. The protein matrix also improves adhesion of the coatings to food surfaces. HPMC-lipid composite coatings consisting of beeswax or shellac significantly reduced texture loss and internal breakdown of plums (Pérez-Gago *et al*., 2003b). Composite coatings prepared from WPI or WPC as the hydrophilic phase and beeswax or carnauba wax as the lipid phase exerted an antibrowning effect on fresh-cut apples (Pérez-Gago *et al*., 2005; Perez-Gago *et al*., 2006). Locust bean gum, shellac and beeswax coatings prolonged the storability of the cherries by reducing moisture loss (Rojas-Argudo *et al*., 2005). An emulsion coating with CMC as the hydrophilic phase and paraffin wax, beeswax, or soybean oil as the hydrophobic phase also extended shelf life and reduced weight loss of apples, peaches, and pears (Toğrul and Arslan, 2005).

2.8 Mechanism of fruit coating

2.8.1 Permeability properties of coatings

Permeability of coatings to water vapor, gas, solute, or lipids is an important property to consider when selecting coating materials for specific commodities. Permeability properties of films are often unpredictable due to the absence of a homogeneous structure and the often hydrophilic nature of most formulations (McHugh and Krochta, 1994d). The chemical composition and structure of the film-forming polymer affect film permeability in general. Highly polar materials with a high degree of hydrogen bonding exhibit low gas permeability, especially under conditions of low humidity, and are poor barriers to moisture. Non-polar materials, such as lipids, provide good moisture barriers, but are permeable to gases such as oxygen. The type of functional group on a polymer can also have an effect, depending on the resulting chain interaction and motion and whether the functional group is hydrophilic or hydrophobic. Ionic functional groups create strong polymer chain interactions, which restrict chain motion. This usually results in good oxygen-barriers, but also hydrogen bonding with water and subsequent water absorption at high relative humidity, which in turn, results in high rates of water vapor permeation. In addition, absorption of water disrupts intermolecular chain interaction, which increases permeability in general. This is the reason that films are often more permeable at high relative humidity (Donhowe and Fennema, 1992). Non-polar groups result in a much less effective oxygen barrier film when present as the side chain but slightly improve water permeability.

Addition of low-molecular-weight components, or plasticizers, can affect film permeability and flexibility. They often increase both (especially water vapor permeability) by disruption of polymer chain hydrogen bonding. These components are generally added to decrease film brittleness by increasing elasticity/flexibility, resulting in less cracking and flaking of coatings (Park *et al*., 1993; McHugh and Krochta, 1994a, b).

The structure of the film-forming polymer is also important in terms of influencing permeability properties of a film. Polymer chain packing, whether it is tight or loose due to bulky side chains, results in increased or decreased permeability properties, respectively (McHugh and Krochta, 1994b). Molecular weight and crystalline structure of a polymer can have an effect (Park *et al*., 1993). Lipids can exist in different crystalline states, which result in different barrier properties with the higher degree of crystallinity resulting in lower permeability. Temperature affects polymer mobility, and thus, having permeability (Gennadios *et al*., 1993a). Higher temperatures result in polymers that are more mobile (plastic amorphous state) and have relatively increased permeability properties compared to lower temperatures (McHugh and Krochta, 1994d). Even without going through a structural transition, O_2 transmission through protein films was affected by temperature (Gennadios *et al*., 1993a). Orientation of polymers to the flow of permeate can affect permeability properties. For example, the packed arrangement of wax crystals perpendicular to the direction of gas flow presents a better barrier than the packed arrangement paralleling to the direction of flow (Hernandez, 1994).

Cross-linking of polymer chains with ions or enzymes can lower permeability values as well as change the pH (depending on the isoelectricpoint in the case of protein films) (Avena-Bustillos and Krochta, 1993; McHugh and Krochta, 1994d). The addition of hydrophobic materials (lipids) to a hydrophilic film-former making a composite coating can sometimes improve the moisture-barrier properties of the hydrophilic film former. This was demonstrated for a matrix of methylcellulose, and hydroxypropyl methylcellulose combined with saturated C_{16} and C_{18} fatty acids laminated with beeswax and with a chitosan film containing lauric acid (Wong *et al*., 1992). This can also be achieved by forming bilayer films from hydrophilic and hydrophobic materials. An example of this was reported for hydroxypropyl methylcellulose and a blend of stearic and palmitic acids (Kamper and Fennema, 1984a, b).

2.8.2 Effect on water loss

Water loss usually occurs in the vapor phase. Water vapor permeability describes the movement of water vapor through a coating per unit area and thickness, and determines the vapor pressure difference across the film at a specific temperature and humidity (Kamper and Fennema, 1984a, b). If pores, cracks, or pinholes form on the film surface, water vapor can flow through these areas directly. This is different from the dissolving and diffusion of water vapor through a film barrier (McHugh and Krochta, 1994c). Water vapor transfer through films is dependent on environmental conditions such as temperature and humidity, and thus should be tested under the conditions expected to be encountered by a specific product. Generally, the more hydrophilic the film-forming material, the more permeable the film will be to water vapor (Baldwin, 2007).

2.8.3 Effect on gas exchange of fresh fruit and vegetables

2.8.3.1 Creation of a modified atmosphere for coated fresh produce and effect on ripening

Cells of plant tissues, such as harvested fruit and vegetables, are physiologically active in that they consume O_2 and produce CO_2 as they respire. When fruit or vegetables are sealed in semipermeable plastic packaging or coating, the modified atmosphere is created within the packaging or in the internal atmosphere of the fruit, in the case of edible coatings, depending on the permeability of coating. During storage, fruit respiration continues to consume O_2 and release CO_2 (Baldwin, 1994). If O_2 levels fall too low (below 1-3%, depending on the produce and storage temperature), anaerobic reactions can occur. This results in off-flavors, abnormal ripening, and spoilage (Wills *et al.*, 1981; Kader, 1986). Climacteric-type fruit are often harvested immature and ripen off the mother plant with an accelerated respiration pattern and ethylene production (Wills *et al.*, 1981). The high rates of respiration and ethylene production, which turn on genes regulating ripening and senescence, can contribute a relatively short shelf life for this type of produce. Ethylene production, like respiration, is a process that requires O_2. Low O_2 (below 8%) and high CO_2 (above 5%) concentrations slow down respiration and retard ethylene production, and therefore, ripening (Kader, 1986). High storage temperatures increase fruit or vegetable respiration (Wills *et al*., 1981), and exacerbate the effect of a coating or other packaging on the internal atmosphere of the coated produce. Low temperature, on the other hand, slows down fruit ethylene production and respiration; thus, minimizing the effect of a film or coating in terms of modifying the atmosphere inside a fruit.

2.8.3.2 Retardation of weight loss and surface desiccation

Fruit and vegetables also lose water to the surrounding air in the form of water vapor through a process called transpiration. This entails the movement of water from fruit cells to the surrounding atmosphere following a gradient of high water concentration (~100% relative humidity in fruit intercellular spaces or internal atmosphere) to lower water concentration (% humidity of the storage environment). For this reason, fresh produce is often stored under conditions of high relative humidity (90-98%) to minimize water loss, subsequent weight loss, and shriveling (Woods, 1990). Edible coatings can help retard this movement of water vapor, but become more permeable to water vapor and gases under conditions of high relative humidity as explained above.

2.8.3.3 Structural integrity and appearance of coated products

Coatings on fruit and vegetables can act as lubricants to reduce surface injury, scarring, and chafing (Hardenburg, 1967). With less wounding of fruit, decay due to opportunist wound pathogens is lessened. In addition, the act of applying certain types of coatings reduces surface microbial populations (McGuire and Baldwin, 1994). For these reasons, waxed citrus fruit experience less decay compared to unwaxed fruit (Waks *et al*., 1985). For food consisting of multiple components, a film can be used to secure the components of the product during marketing (Donhowe and Fennema, 1994). Waxes are also used to encase cheese to prevent surface molding during the ripening and aging process. Resins, zein protein, and microemulsions of waxes can impart a high gloss to the coated product (Hagenmaier and Baker, 1994b; Hagenmaier and Baker, 1995). Shellac, polyethylene, and carnauba wax microemulsions are used on fruit (Baldwin, 1994), and carnauba, shellac, and zein have been applied to candies and confectioneries as well (Baker *et al*., 1994). Zein has been tested on tomato fruit, but so far, has not been used commercially on fruit (Park *et al*., 1994a, b). Candelilla wax microemulsions impart a glossy appearance, especially when combined with gelatin protein (Hagenmaier and Baker, 1996). Carbohydrate coatings, such as cellulose or pectin, result in an attractive non-sticky sheen when applied to products when dry, but often give an undesirable slippery texture when products become wet with condensation, as is often the case after removal from chilled storage. The polysaccharide film formers, however, do not result in the high gloss finish obtained with shellac, carnauba wax, or zein coatings.

2.8.4 Effect on respiration of fresh fruit and vegetables

The O_2 concentration in air around fruit is reduced to less than 10%, respiration rate is reduced in proportion to O_2 concentration. A concentration of 1-3% O_2, depending on the fruit, is required to induce anaerobic respiration (Kader, 1986). Under anaerobic conditions the glycolytic pathway replaces the tricarboxylic acid cycle (TCA) as the main source of energy provided by plant tissue, where oxidation of pyruvate in mitochondria is greatly reduced and accumulation of pyruvate and acetyl CoA activate the fermentation biosynthetic pathway. Pyruvate is converted to acetaldehyde and CO_2 by the enzyme pyruvate decarboxylase (PDC) and acetaldehyde is reduced to ethanol by nicotinamide adenine dinucleotide (NADH) and alcohol dehydrogenase (ADH) (Mathews and van Holde, 1996). Most fruit produce ethanol when exposed to anaerobic condition.

Anaerobic respiration may be regulated by two mechanisms: molecular control of PDC and ADH by increased concentration or production of new isozymes; and metabolic control by feedback mechanisms of products and co-factors inhibiting enzyme function (Perata and Alpi, 1993; Ke *et al*., 1995). Although induction of PDC, ADH, and their isozymes (Longhurst *et al*., 1990) occurs in anaerobic condition, enzyme concentration is not well correlated with enzyme activity except at very low concentration (Ke *at al.*, 1995). This implies that metabolic regulation of anaerobic enzymes takes place as a result of other factors such as changes in pH, substrate concentration, cofactors and/or inhibitors.

Induction of anaerobic respiration may involve reduction in cellular pH that selectively activates PDC and ADH. A decrease in cytosolic pH, associated with transient lactate fermentation, has been reported in avocado (Hess *et al.*, 1993), 'Bartlett' pears (Nanos and Kader, 1993), and in tomato root cultures (Rivoal and Hanson, 1994).

However, not all plants produce lactic acid before an ethanol increase occurs (Andreev and Vartapetian, 1992). A decrease in pH could come about following inhibition of proton pumping at low ATP concentration and proton release by ATP hydrolysis (Chervin *et al.*, 1996), by release of malic acid into the cytoplasm from the vacuole (Butler and Bangerth, 1982), or as a result of high CO_2 concentrations decreasing cytoplasm pH and thus inducing PDC activity (Blanke, 1991).

Anaerobic conditions consistently enhance acetaldehyde and ethanol concentrations in a wide range of fruit. Under anaerobic conditions acetaldehyde and ethanol can greatly exceed concentrations of several hundred $\mu l \cdot litre^{-1}$ with ethanol accumulation as high as 47 μl/l/kg/day at 0°C (Knee, 1991). When returned to air acetaldehyde and ethanol concentrations decrease to initial values over 1-2 weeks (Saltviet and Ballinger, 1983a, b). Fruit metabolism may be affected by acetaldehyde and ethanol that stimulate or inhibit various biochemical pathways involved in ripening (Dixon and Hewett, 2000).

2.9 Coating materials for citrus fruit

Formulations of coatings vary depending on the usage, commodity and qualities that they impart to the commodity. Citrus fruit are commonly waxed because washing removes much of the natural wax from the peel, thereby increasing shriveling and fruit drying (Wills *et al.*, 1998). Substances used for coating citrus include shellac- or resin-based, depending on the market (Grant and Burns, 1994). Shellac-based coatings are chosen to improve shine or gloss. There are numerous research reports studying gas exchanges of fruit waxes with formulations including shellac, wood rosin, candelilla wax, carnauba wax, beeswax, polyethylene and petroleum waxes (Hagenmaier and Shaw, 1992; Hagenmaier and Baker, 1994a, b; Hagenmaier and Baker, 1997; Hagenmaier, 1998a, b). Fungicides are often incorporated in the citrus coating for simplicity of application on the packing line.

Ben-Yehoshua *et al.* (1985) investigated resistance of either waxed or individually sealed citrus fruit to C_2H_4, O_2, CO_2, and H_2O mass transport. During the coating operation the liquid wax flows into stomatal openings and partially or completely plugs the stomata, effectively restricting the transport of O_2, CO_2, and C_2H_4. An intermittent discontinous layer is formed on the fruit surface after waxing. Contrary to the marked effects on resistance of O_2, CO_2, and C_2H_4, waxing results in inadequate reduction of water diffusion outside of the fruit. Individual sealing of fruit with high density polyethylene films reduced water transport by 90% without substantially inhibiting gas exchange, though the thickness of the plastic film was 15 μm whereas that of the discontinuous wax layer was less than 1 μm. This difference in effects of sealing and waxing explains the great risk that waxing may bring in restricting gas exchange by clogging the stomata.

Martínez-Romero *et al.* (2003) studied the coating treatments in postharvest behavior of oranges. They dipped oranges in imazalil solution (800 mg/l) and either coated with various waxes formations or seal packed with heated shrinkable polyolefin film. After 15 days of storage at 29°C and 70% relative humidity, they observed that weight loss was lower in coated fruit than in uncoated fruit and was negligible in seal packed fruit. Waxing and seal packing had no effect on the total soluble solids/acidity ratio. Weight loss was lower in coated fruit than in uncoated fruit and was the lowest in seal packaged fruit.

Rana *et al.* (1992) found that treated sweet orange fruit with sesame oil emulsion, after 40 days of storage reduced decay compared with untreated control. It was also found that the percentage of weight loss and total soluble solids of fruit increased with increasing storage time, while juice and ascorbic acid content decreased.

Grapefruit and oranges were coated with various fruit waxes. Compared to control, internal CO_2 concentration was markedly higher and weight loss markedly lower for coated fruit. Resistance of coated fruit to passage of CO_2 and water vapor was shown to be influenced by permeability of the coating but more so by the degree to which the coating seals openings in the fruit epidermis. For restriction of CO_2 exchange, the coating thickness and surface tension of liquid coating were of less importance than type of wax (Hagenmaier and Baker, 1993a).

Hargenmaier and Baker (1993a) found that oranges, grapefruit and tangerines that were coated with hydrocarbon-containing wax microemulsions had weight loss and internal CO_2 values less than half those coated commercially in Florida packinghouses. 'Valencia' oranges coated in layers with the two wax microemulsions had weight loss only 20-30% of washed control.

Late-season oranges and grapefruit were coated with polyethylene wax, carnauba wax, shellac and resin coatings. Amount of coating was varied from approximately 30 to 300 mg/fruit. The fruit coated with wax had gloss that was relatively stable during storage. All coating application rates this fruit had relatively high internal O_2 and low values of CO_2 and ethanol. Fruit coated with shellac or resin had gloss that was initially high, but decreased more during storage. This fruit had low O_2 and high values of CO_2 and ethanol. High ethanol content has been correlated with off-flavor, shellac and resin-based citrus coatings result in some sacrifice of flavor for initial appearance. Wax-based coatings might lead to better consumer satisfaction with fresh citrus fruit (Hagenmaier and Baker, 1994a, b).

Citrus fruit was coated with polyethylene wax, petroleum wax, synthetic petroleum wax, carnauba wax, and candelilla wax emulsified with fatty acids and other ingredients. Weight losses were low with coatings that contained hydrocarbon wax and for those waxes emulsified by stearic or palmitic acid rather than oleic acid. Oranges coated with wax had less weight loss, lower internal CO_2, higher internal O_2, and better water resistance than fruit coated with shellac or resin. Coatings formed on polymer films had proportionally higher resistance to water vapor when made with wax microemulsions rather than with mixtures of wax with shellac or wood resin (Hagenmaier and Baker, 1994a).

'Valencia' oranges and 'Marsh' grapefruit were treated with single or double layers of coating. Two coatings were applied, the first coating was a moisture-barrier wax; the second was either polyethylene wax or a mixture of shellac and resin ester. The inner coating reduced weight loss, and the outer coating imparted gloss. Fruit gloss decreased more rapidly during 1 week at 20°C with a single glossy coating than with the same coating applied as a second layer over a wax-based first coating. For citrus fruit, using resin ester or shellac as a high-gloss second coating tends to highly restrict the exchange of O_2 and CO_2 (Hagenmaier and Baker, 1995).

Hagenmaier and Baker (1997) studied the effect of edible coating on oranges stored at 23°C and 60% relative humidity. Waxes used were candelilla wax, beeswax, carnauba wax, polyethylene wax, and petroleum wax. All coatings were effective moisture barriers, limiting weight loss of oranges, with the best being those containing candelilla wax, beeswax and petroleum wax.

Chun *et al*. (1998) studied on the respiration rate of ‘Satsuma’ mandarin sealed in polyvinyl chloride (PVC) film 13.7 µm thick and wax-coated (6.8% solution of Prowax and 1.25% solution of Semperfresh) at different temperatures (4, 10 and 20°C). The results showed that sealing or coating treatments reduced apparent respiration rate, peel permeability and weight loss of mandarin, and maintained an internal atmosphere of lower O_2 and higher CO_2 compared with the unsealed and uncoated control. Respiration rate was more sensitive to temperature than peel permeability, which in some cases even decreased with temperature. Thus, high-temperature storage of some coated fruit induce high respiratory quotient (RQ), very low O_2 and high CO_2 internal concentrations, anaerobic respiration and accumulation of ethanol in juice.

Subedi *et al*. (1998) reported that treated mandarin (*Citrus reticulata* cv. ‘Suntala’) fruit with 6% paraffin wax significantly increased total soluble solids content and percentage of fruit weight loss significantly as storage time increased, while significant negative relationships between titratable acidity and length of storage period existed. Fruit rotting percentage reduced with paraffin wax.

‘Murcott’ tangerines were treated with coatings, and the coated fruit were stored 7 days at 21°C. The results showed that ethanol content of non-coated fruit increased slightly as the season progressed (about 20 mg/l/week) and the mean ethanol content of juice was 320 mg/l. The mean ethanol content was 800 mg/l for fruit with polyethylene coatings of high O_2 permeability. It was about 1,300 mg/l for carnauba wax coatings having medium permeability, and about 1,900 mg/l for high-gloss, shellac-resin coatings with low O_2 permeability (Hagenmaier, 2001).

Mandarin hybrids were treated with wax and resin coatings having differing oxygen permeabilities. After storage for 7 days at 21°C, fruit with coatings having O_2 permeability $\leq 1.1\times10^{-16}$ mol m s^{-1} m^{-2} Pa^{-1} were rated by a sensory panel as markedly less fresh than fruit with higher permeability coatings. Low-permeability coatings were those composed mainly of shellac and wood resin. The flavor changed most for fruit having mean internal $O_2 < 4\%$, internal $CO_2 > 14\%$ at 21°C and juice ethanol content >1,500 mg/l after 7 days storage at 21°C. All coatings seemed suitable for storage of citrus fruit for 7 days at 5°C. It is recommended that for mandarin hybrids, the most suitable are those composed mainly of waxes rather than shellac or wood resin (Hagenmaier, 2002).

Treated mandarin fruit with carboxymethyl cellulose (CMC) from sugar beet pulp cellulose and stored in storage chamber at 25°C and 75% relative humidity delayed the soluble solids, titratable acidity and ascorbic acid loses in comparison to the uncoated mandarins. Moreover, coating mandarin surface with emulsions containing CMC from sugar beet pulp was possible to extend the storage period with lower weight loss until 27 days by cellulose as a hydrophilic polymer (Toğrul and Arslan, 2004).

Oranges, bell peppers and apples were treated with different coatings. Measurements were made of gas permeance through the peel. Shellac and wood resin coatings reduced ethane permeance of orange and apple peels by approximately 95% from the values for non-coated peel, and carnauba wax coatings gave about 85% reduction. Application of coatings resulted in some fruit having markedly high values of internal CO_2 and low O_2. High-barrier coatings also resulted in much larger variation in internal gas concentrations in different individual fruit with the same coating, much larger than the variation between different individual non-coated fruit. Because fruit quality is much dependent on internal gas concentrations, high-barrier coatings result in fruit with higher variation in product quality (Hagenmaier, 2005).

Porat *et al*. (2005) found that all wax formulations (Tag with 5% polyethylene solids, Tag with 9% polyethylene solids, Tag with 13% polyethylene solids, Tag with 18% polyethylene solids, Tag with half shellac and Tag wax (Safepack Products Ltd., Israel) reduced water loss sufficiently, but it was necessary to include at least 13% of total solids and half the regular amount of shellac to impart the desired gloss and shine, comparable with that obtained with the commercial ‘Tag’ wax. However, serial dilutions of the total solids and of the shellac content in the wax formulation gradually reduced the decrease in O_2 and increase in CO_2 concentrations in the internal atmosphere of the fruit, and the accumulation of ethanol and off-flavor.

Ahmed *et al*. (2007) reported that coated with 20-30% of *trans* jojoba oil (TJO) concentrations proved to be the most capable treatments in maintaining ‘Valencia’ orange fruit quality up to 60 days storage at 5°C. TJO was found to be efficient enough for coating fruit equal to the exported wax (E. wax). TJO and E. wax were markedly reduced the weight loss and respiration rate than that of control fruit. Moreover, coated fruit stored for two months at 5°C withstand free from microbial pathogenic incidence. Although, soluble solid content showed insignificant differences due to TJO treatments throughout storage period, titratable acidity content showed lower decrease percent as TJO concentrations increased. Vitamin C content had been significantly decreased by expanding cold storage period with slight loss in fruit coated with highly concentrations of TJO wax.

Coating 'Clemenules' mandarins with 70 g/kg polyethylene wax and shellac and 100 g/kg polyethylene wax and shellac, fruit were stored at 5ºC and 90% relative humidity for 12, 22, 32, 42, 52 or 62 days, plus 7 days at 20ºC to simulate shelf life marketing conditions. Physicochemical quality was well preserved throughout storage, especially in fruit coated with 70 g/kg total solids water wax. Fruit from this treatment had the lowest weight loss and the greatest rind gloss. Mandarin-like flavor decreased throughout the storage period, which was highly related with ethanol build-up (Marcilla *et al*., 2009).