CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Citrus fruit are immensely popular worldwide for their flavour and nutrition (Baldwin, 1993). Total production and consumption of citrus fruit has grown strongly since the 1980s. Current annual worldwide citrus production is estimated at over 105 million ton, with more than half of this being oranges (Wikipedia, 2008a). It belongs to the Order Geraniales, family Rutaceae and subfamily Aurantioideae which contain an orange or lemon-like fruit classified as a hesperidium or berry of special structure. These fruit are characterized by a juicy pulp made of vesicles within segments. Only three genera in this subfamily (*Citrus, Fortunella* and *Poncirus*) produce edible juice vesicles (Baldwin, 1993). The commercially important species are orange (*Citrus sinensis* (L.) Osbeck), mandarin and tangerine (*Citrus unshiu* Marc. and *Citrus reticulata* Blanco), lemon and lime (*Citrus limon* (L.) Burm. f. and *Citrus aurantifolia* Swingle), grapefruit (*Citrus paradisi* Macf.), pomelo (*Citrus grandis* Osb.) and their hybrids (Murata, 1997).

The origin of almost all citrus species was probably at the southern slopes of the Himalayas in northeastern India and adjacent Burma from at least 2000 BC (Radha and Mathew, 2007). Currently, citrus is cultivated in the subtropical and tropical regions of the world between 40°north and south latitude in over 137 countries on six continents. The fruit have differentiated into many varieties adapted to new surroundings, and many cultivars have been bred in various countries (Ismail and Zhang, 2004). Brazil is the largest producer followed by the United States of America (USA), China, Mexico, Spain, USA and South Africa are the largest exporter countries followed by Turkey and Argentina (FAO, 2008b).

In South-East Asia, sweet oranges, particularly mandarin and tangerine are popular as everyday fruit, particularly among the Chinese population. The names tangerine and mandarin have often been used synonymously. The term tangerine

refers to most mandarin types in most of the USA and to the more deeply pigmented mandarins in Australia and China. They grow best in regions with sunny conditions and adequate irrigation. The four citrus groups recognized in Thailand are orange, tangerine, lime and pomelo (Yaacob and Subhadrabandhu, 1995; Thompson, 2003). Tangerine fruit is grown commercially in the north of Thailand. This is grown mainly on large plantations in Chiang Mai province. Important cultivar is 'Sai Num Pung'. In general, these fruit have short on-tree life, resulting in a short season and the trees being subject to alternate bearing. The tangerine flowering period in Chiang Mai province is in December through January. After full bloom, tangerine fruit reach maturity within 9-10 months and are harvested in November and continue to be available until February. The fruit is also spherical, 7-11 centimeters in diameter with greenish gold to bright orange skin, surface almost smooth, base necked or depresses, rind thin, easily peeled; pulp orange, texture tender and melting with 8-12 segment, juice abundant, sweet and slightly acidic, aromatic, of very fine quality and with few seeds. Growers can programme the production to coincide with periods of heavy demand, particularly during local festivals, such as the Chinese New Year (around early February), when the price is usually high.

At present, the production of commercial tangerine fruit is enough for local consumption in Thailand. The Thai government has tried to promote tangerine fruit exports to foreign markets. The Ministry of Commerce and the Ministry of Agriculture and Co-operatives have tried to increase the tangerine yield and tangerine exports as much as possible. If tangerine fruit are properly processed and their quality during transportation and storage is maintained, they have the potential to compete with any other citrus fruit in the world market (Yaacob and Subhadrabnadhu, 1995).

2.2 Chemical composition, nutritive and medicinal value

Tangerine fruit have high nutritional qualities and merits. They are refreshing and delicious to eat, possess minerals and vitamins especially a high content of vitamin C, which is an antioxidant. Antioxidants help prevent chemical damage of cells and can promote vision health, keep the immune system healthy, support cardiovascular health and help to prevent cancer. They contain β-carotene,

xanthophylls (diol), cryptoxanthin (monol) and violaxanthin (5, 6-epoxide) as carotenoids, which cause the orange colour of the rind and juice. β-carotene is a precursor of vitamin A and can be converted in the human body to retinol, an active vitamin A compound. Besides, tangerine fruit contain flavonoids (or bioflavonoids) in the rind and juice segments, known for their antioxidant properties and ability to increase levels of Vitamin C within the body's cells. Moreover, tangerine fruit are also a source of folic acid, a B-complex vitamin that can help prevent birth defects and a great fiber source that is high in the soluble fiber pectin. Fruit juice contains sugars (glucose and sucrose) and acids (primary citric and a little of malic acid). Soluble solids content in tangerine fruit varies from 6 to 12% and acidity from 0.5 to 1.5%. Rind is rich in pectin, essential oils and glucosides (Murata, 1997; Radha and Mathew, 2007). The chemical compositions and nutritional values of fresh tangerine fruit are shown in Table 2.1.

2.3 Anatomy of citrus fruit

Anatomically, citrus fruit are superior ovaries composed of 6 to 20 united carpels which form locules. The pericarp exterior to the locules is subdivided into the exocarp (flavedo or exterior peel), mesocarp (albedo or interior peel) and endocarp (locule or segment membrane). The juice vesicles, which are the edible portion of citrus fruit and therefore of economic value, arise from epidermal or subepidermal primordial on the surface of the endocarp and grow to fill the locular cavity (Albrigo and Carter, 1977; Roth, 1997) (Figure 2.1).

Flavedo

The exocarp or flavedo, which is the coloured portion of the peel, contains pigments in chloroplasts or chromoplasts and oil glands formed by special cell that produce terpenes and oils. The oil gland cavity forms schizogenously and/or lysogenously by the separation and/or lysis of cell walls of the central cells. Flavedo epidermal cells produce cutin sand waxes and contain actinocytic type stomates (Roth, 1977; Baldwin, 1993).

Table 2.1 The chemical compositions and nutritional values of fresh tangerine fruit per 100 gram of the edible part

Ingredients	Quantity
Moisture	83.40 g
Fat	0.31 g
Fiber	1.80 g
Protein	0.81 g
Ash	0.41 g
Carbohydrates	13.34 g
Energy	50.00 kcal
Calcium	37.00 mg
Phosphorus	20.00 mg
Potassium	166.00 mg
Magnesium	12.00 mg
Manganese	0.07 mg
Iron	0.15 mg
Sodium	2.00 mg
Zinc	0.07 mg
Carotene	0.09 mg
Vitamin A	57.50 IU
Ascorbic acid, Vitamin C	26.70 mg
Thiamine, Vitamin B_1	0.058 mg
Riboflavin, Vitamin B_2	0.036 mg
Niacin, Vitamin B_3	0.376 mg
Pantothenic acid, Vitamin B_5	0.216 mg
Vitamin B_6	0.078 mg
Folate, Vitamin B_9	16.00 μg

(Source: Wikipedia, 2008b)

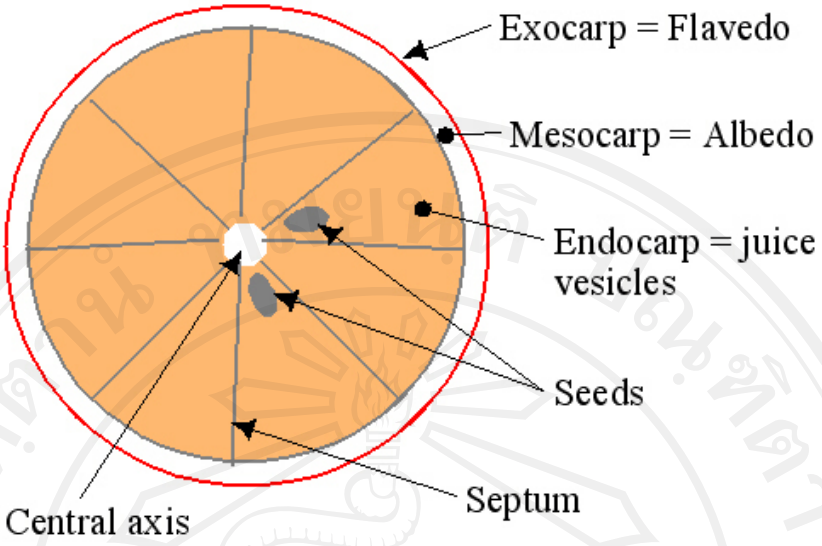

Figure 2.1 Cross-section of a citrus fruit
(Source: Mark's Fruit Crops, 2008)

Albedo

The mesocarp or white albedo portion of the peel consists of colourless cells which are typically eight-armed, parenchymous, highly vacuolated and tube-like (Albrigo and Carter, 1977). The tissue contains large air spaces, imparting a spongy nature where two arms of adjacent cells meet, the transverse wall remain thin and develops pits to enhance translocation of assimilates. A network of vascular tissue branches through the albedo and extends from the main bundles that run parallel to the fruit axis to the outside of the segments at three locations per segment from where juice vesicles are attached (Koch *et al*., 1986). The albedo serves as a storage parenchyma and contains higher flavanone content than juice vesicles, section membranes or flavedo and is also an important source of limonin. The albedo, although much more vulnerable to pathogen invasion than flavedo if exposed, was found to contain a polygalacturonase inhibitor and believed to be a form of defence against pathogens such as *Diplodia natalensis*, which uses polygalacturonase to gain entrance onto fruit tissue. The core of the fruit resembles albedo and contains vascular bundles and parenchymous tissue (Albrigo and Carter, 1977; Baldwin, 1993).

Peel

Albedo and flavedo together make up what is called the peel or rind, and contain more bitter principles and pectin than other parts of the fruit. The higher concentration of glycosides (flavonones), limonin and water-soluble pectins are inversely correlated to processed juice flavour and these components are found in higher quantities in juice that contains more peel extract from hard squeezed fruit (Baldwin, 1993). The presence of the leathery rind protects the fruit from damage during handling and desiccation during storage (Albrigo and Carter, 1977).

Juice vesicles

The endocarp portion of citrus fruit is the most complex, giving rise to juice sacs or vesicles. They are classified as both emergences or multicellular hairs and form elliptical-shaped single or branched body structures on a non-vescular stalk (Roth, 1977). Juice Sac cells are highly vacuolated and the narrow cytoplasm contains lipid droplets in plastids, leucoplasts and chromoplasts. Juice within the vacuole of these cells contains essentially all the titratable acids and other soluble materials such as amino acids and salts. Calcium oxalate, hesperidin and naringin crystals can occur in citrus fruit in the peripheral layers of the rind and juice sacs. Limonin and naringin contribute bitterness to juice flavour (Roth, 1977; Baldwin, 1993).

2.4 Postharvest technology

2.4.1 Harvest maturity indices

Tangerine fruit cannot be picked immature for after-ripening since the fruit contain little starch and are non-climacteric. Unfortunately, colour is not a good indicator of maturity, so percentage soluble solids (°Brix, of which 70-85% is composed of sugars) and titratable acid of the juice are used as a maturity index. A soluble solids content (SSC): acid ratio ranging from 8 to 10 is generally accepted as a measure of minimum maturity, while a ratio of 10 to 16 is considered to be acceptable quality. If the fruit remain on the tree, the SSC continues to increase and the acid diminishes, until eventually overripe and unpleasantly sweet fruit reach a SSC: acid ratio of 20 or more (Baldwin, 1993).

2.4.2 Harvesting methods

It is best to harvest tangerine fruit on a clear, sunny day with low humidity. The fruit should be harvested as soon as the dew has evaporated. On a cloudy day, the fruit should be harvested in the afternoon. Fruit should not be harvested at all on a rainy day. Fruit are either pulled from the limb or clipped in some cases to avoid plugging (rind tearing). Plugging predisposes the fruit to fungal infection and desiccation during transit and packing. In most areas the picker places the fruit into a canvas or plastic sack which he then empties into a plastic container. The container is then moved from the orchard to a truck for transport to the packinghouse (Devies and Albrigo, 1994).

2.4.3 Packinghouse procedures

2.4.3.1 Delivery and cleaning

After harvesting, the fruit can be delivered to the central packinghouse in plastic container or bins. Small scale operations usually dip 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. (Devies and Albrigo, 1994; Arras, 2001).

2.4.3.2 Washing, waxing, antifungal treatment and sorting

The fruit are washed in brushing-washing machine with detergent. Waxing consists of applying synthetic waxes which reduce water loss besides conferring a shine to the fruit, improving their appearance. Waxing is often done together with antifungal treatment using imazalil and thiabendazole, the latter proving more efficacious. Fruit are dried after waxing. The produce is sorted by hand on conveyor belts since it identifies rotten or damaged products (Arras, 2001).

2.4.3.3 Sizing and packing

Grading can be done manually in small-scale operations or semi-automatically in larger volume operations. The latest technology consists of electronic systems which sort the fruit by diameter, weight and colour. The mechanical sizers used in central packinghouses (cup belts, expanding sorting belts) have proved to be sufficiently accurate and reliable in relation to their technical features, if regularly serviced. However, electronic sizers by weight and diameter are more efficient than

mechanical sizers and can also separate the fruit by species. More use could therefore be made of machines, with a consequent increase in productivity. Fruit are packed into several types of containers but the most common are corrugated cardboard cartons, netted bags or plastic container and the fruit are mostly similar in size. These materials can be used to package fruit for distribution to wholesalers, while plastic container is more practical for retailers (Devies and Albrigo, 1994; Arras, 2001).

2.5 Postharvest physiology

Tangerine fruit are non-climacteric, not exhibiting any respiratory climacteric coupled with steep rise in ethylene production after harvest. Internal quality of these fruit is the best at the time of their optimum maturity on the tree. Fruit show a rise in the respiration rate with ethylene application during degreening, but it is a temporary change in peel color only. Its chemical composition, flavor and texture remain more or less unchanged, while the acidity content decreases slightly with an exogenous application of ethylene (Devies and Albrigo, 1994; Murata, 1997).

The respiratory rate of the rind is nearly ten times as high as that of vesicles. Therefore, the rind had an important physiological role in the qualitative changes that take place during storage of the fruit. Respiratory rates of tangerine fruit are affected by temperature, humidity, air movement, atmospheric compositions, handling practices and microbial infection (Murata, 1997). The respiratory rate increases with storage temperatures that significantly affect storage life because the heat of respiration or vital heat generated is also higher. Heat evolution can be computed from the respiration rate of the commodity. The respiration rate of tangerine fruit under different temperatures at 5°C (4 to 8 miligrams (mg) $CO_2kg^{-1}hour^{-1}$), 10°C (6 to 10 mg $CO_2kg^{-1}hour^{-1}$), 15°C (12 to 20 mg $CO_2kg^{-1}hour^{-1}$) and 20°C (20 to 30 mg $CO_2kg^{-1}hour^{-1}$). Respiration rates at optimum storage temperatures are generally <10 mg $CO_2kg^{-1}hour^{-1}$ (Arpaia and Kader, 2005).

Tangerine fruit respire differently at temperatures above and below critical temperatures, which closely correspond with the temperature at which chilling injury occurs. Such fruit exhibit cumulative time-temperature influence of chilling temperature on carbon dioxide evolution, which is greatening after exposure to 0°C

than at 10°C. The chilling temperature also increases the production of ethylene and volatile components (ethanol and acetaldehyde) in fruit after a return to normal temperature (Ladaniya, 2002).

Temperature and storage duration also affect the color development. Carotenoids are the major pigments in the rind giving tangerine fruit their characteristic color. Synthesis of these pigments takes place at 15-20°C without ethylene treatment, for treating with ethylene increases the chlorophyllase activity in the rind and reduces the number and the size of the chloroplastes (Ladaniya, 2002).

If fruit are stored at the lowest safe temperature (above freezing) that significantly suppresses respiration and fungal growth, the storage life of fruit can be markedly increased. While chilling temperature adversely affects fruit quality, higher temperatures also reduce the storage life. The severity of adverse effects depends on the time-temperature relationship under such conditions. Higher concentrations of oxygen (34.1-99.1%) increase the respiration rate of tangerine fruit, while lower concentrations reduce the same. Very low concentrations of O_2 (0.5-5.0%) tend to increase the respiration rate. Tangerine fruit produce a higher quantity of ethanol and acetaldehyde at low O_2 levels and higher N_2 levels indicating anaerobic respiration (Ladaniya, 2002).

Tangerine fruit with fairly good appearance and eating quality can be obtained provided they are stored under most optimum postharvest conditions. Ethylene at very low (<0.1 microliters (μl) $kg^{-1}hour^{-1}$ at 20°C) concentrations can be physiologically active in tangerine fruit (Arpaia and Kader, 2005). Postharvest action that reduces the accumulation of ethylene around non-climacteric produce during marketing can result in an increase in their postharvest life. It is suggested that the threshold level of ethylene action on non-climacteric produce is below 0.005 ppm than the commonly considered level of 0.1 ppm. There is 60% extended in oranges with logarithmic reduction in ethylene level (Wills *et al*., 1999).

Particular care is needed during harvesting, storage, transportation and marketing of tangerine fruit because rough handling causes wounding, stimulation of respiration and ethylene production and can induce physiological disorders and fungal rot (Ladaniya, 2002).

2.6 Refrigerated storage of tangerine fruit

Tangerine fruit 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 24°C. Tangerine fruit is also at risk to puffiness, in which the peel separates from the pulp at high storage temperature. The object of the cool storage is to slow deterioration without predisposing the fruit to abnormal aging or the other undesirable changes, thereby maintaining the fruit in a condition acceptable to the consumer for as long as possible. Cool stores for fresh tangerine fruit are generally required to operate within relatively close temperature limits (i.e. ±1°C), both in space (i.e. throughout the room) and time (i.e. constantly, in order to maximize storage life, minimize desiccation, avoid chilling and injury due to high CO_2 and low O_2 (Ladaniya, 2002).

Storage of mandarins is limited to 2 to 3 weeks or at the most 4-6 weeks. These fruit can be stored best between 4-6°C in general with a slight variation in temperature range, depending on tolerance of the specific cultivar. Recommended storage conditions and storage life of some cultivars are shown in Table 2.2.

Citrus fruit of the mandarin group are considered to be specialty fruit and include Temples (natural tangor) and tangelos besides tangerines and mandarins. These fruit have relatively shorter storage life, except Sutsuma and are not stored for more than 4-6 weeks. These fruit should be marketed promptly after storage. Temples, Orlando tangelos and tangerines have shown susceptibility to chilling injury at 0-1°C and, hence, are stored at 4°C. At low-temperature, mandarins develop surface pitting while Orlando tangelos and Tample oranges are more susceptible to chilling injury (Arras, 2001). Ladaniya (2002) recommended 2°C with 93% RH of Dancy tangerines grown in Hawaii. Careful handling of the tangerines intended for storage, along with pre-storage application of an approved decay-control treatment is necessary.

Table 2.2 Storage temperature, relative humidity and storage life of fresh mandarin and tangerine fruit

Commodity	Temperature (°C)	Relative humidity (%)	Approximate storage life (weeks)
Avana mandarin	5-6	85-90	4-8
Clementine mandarin	3-4	85-90	4-6
Dancy tangerine	5-6	90-95	2-5
Fortune mandarin	6-7	85-90	6-7
Kinnow and Coorg mandarin	4-5	85-90	8-12
Nagpur mandarin	5-6	90-95	6-8
Ortanique mandarin	4-5	85-90	8-11
Sutsuma mandarin	1-3	80-85	12-18
Tardico Ciaculli mandarin	4-5	85-90	7-8

Note: Various supplementary procedures such as wax coatings, application of fungicides and growth regulators and polyethylene lining in the cartons are used during storage at low-temperatures given above

(Source: Arras, 2001; Ladaniya, 2002)

Satsuma mandarin and Ponkan are loose-skinned fruit and easily puff up and suffer from quality loss during storage at a high humidity. Sustsuma mandarin fruit subjected to long-term storage are usually cured and reduced by 3-5% of their weight before storage. Prestorage curing of the fruit also reduces the incidence of puffing, decay and chilling injury during storage. The storage life of mid-season and late Satsuma mandarins is relatively long. A ventilation facility is essential in mandarin storage as air movement reduces the level of volatiles from the fruit and excess vapor in the storage room. Satsuma mandarins grown in tropical areas can be stored up to 45

days at 4°C and >85% RH with minimum decay losses and loss of green color (Guerra *et al*., 1988; Murata, 1997).

Fruit water content also decreases during storage and shipment. Use of shrinkwrap plastic films on individual fruit is quite effective in reducing water loss, although gas exchange may also be impeded causing anaerobiosis, ethanol and aldehyde accumulation and off-flavours to develop if fruit are held at high temperatures (>30°C) for extended periods. Natural waxes and the postharvest application of wax influence the rate of loss. Waxed fruit also develop off-flavours if stored at high temperatures for too long because of poor gas exchange (Hagenmaier, 2002). Li *et al*. (1998) reported that Fortune mandarin coating with rhamnolipid 0.5% is reduced percentage of decay, water loss and respiration rate. Moreover, vitamin C content and firmness are greater in coated rather than in non-coated fruit when stored at temperatures 0-1°C.

2.7 Green mold rot of tangerine

2.7.1 Disease cycle and epidemiology

Green mold rot of tangerine is caused by the fungus *P. digitatum* Sacc. which is ubiquitous to all citrus growing regions. Spores of this organism are airborne and large numbers are produced by the fungus on the surface of infected fruit. These spores will contaminate the packinghouse and its equipment, storage room, transit containers and even the retail marketplace. Spores will also accumulate in water used in drenchers and soak tanks (Barkai-Golan, 2001; Timmer *et al*., 2003). The fungus survives in the field on soil debris and produces spores that infect split and bruised fruit on the tree and on the ground. At cooler fall and winter temperatures that favor fungal development, large numbers of spores are produced and carried by wind currents to surfaces of fruit in the tree canopy (Brown, 2005).

The spores germinate and infect fruit when nutrients and moisture are released by injuries that are formed during harvesting and handling. Even injuries that involve only a few oil glands are sufficient enough to induce infection. The fungus can also invade fruit through certain physiologically induced injuries, such as injuries associated with chilling injury and stem-end peel breakdown (Barkai-Golan, 2001;

Timmer *et al*., 2003; Brown, 2005). Fruit decaying with green mold produce relatively large amounts of ethylene gas which is a natural plant hormone that promotes respiration, senescence and premature color development. This fungus does not usually spread from infected to healthy fruit in packed cartons, but it can cause a condition known as soilage when masses of spores produced on infected fruit are disseminated to surfaces of healthy fruit. Such soiled fruit must be cleaned before retail sale. The infection and sporulation cycle can be repeated many times in a packinghouse and in storage rooms during extended storage (Murata, 1997; Timmer *et al*., 2000; Barkai-Golan, 2001; Timmer *et al*., 2003; Brown, 2005). The dusting of sound fruit with *Penicillium* spores from decayed fruit is often of greater economic importance in retail cartons, than it to the decayed fruit itself (Stange and Eckert, 1994).

2.7.2 Symptoms

In the early, pinhole stage, the decay appears as a soft, watery, slightly discoloured spot 6-12 mm. The spot enlarges to 2-4 cm within 24-36 hours at 24°C and the rot soon involves the juice vesicles. White mycelium appears on the peel surface and after it reaches a diameter of approximately 2.5 cm, olive green spores are produced. The sporulating area is surrounded by a broad zone of white mycelium and an outer zone of softened peel. The entire fruit is soon encompassed by a mass of olive green spores, which are easily dispersed if the fruit is handled, shaken, or exposed to air currents. If the relative humidity is low, the whole fruit shrinks to a wrinkled, dry mummy. If the relative humidity is high, other molds and bacteria become involved, and the fruit collapses into a soft, decomposing mass (Timmer *et al*., 2000; 2003) (Figure 2.2).

2.7.3 Causal agent

Colonies of *P. digitatum* Sacc. on Czapek Yeast Extract Agar (CYA) 35-55 mm, plane, surface texture velutinous to deeply floccose; mycelium white; conidial production moderate to heavy, greyish green to olive; reverse pale or brownish. Colonies on Malt Extract Agar (MEA) of variable diameter, from 35 mm to greater than 70 mm, plane, relatively sparse, strictly velutinous; conidial production moderate, dull yellow green; reverse pale or brownish (Pitt, 1979; Pitt and Hocking,

1998) (Figure 2.3).

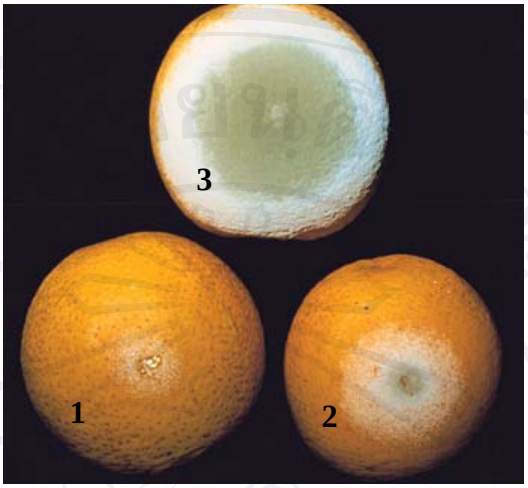

Figure 2.2 Three stages of green mold rot on sweet oranges

(Source: Brown, 2008)

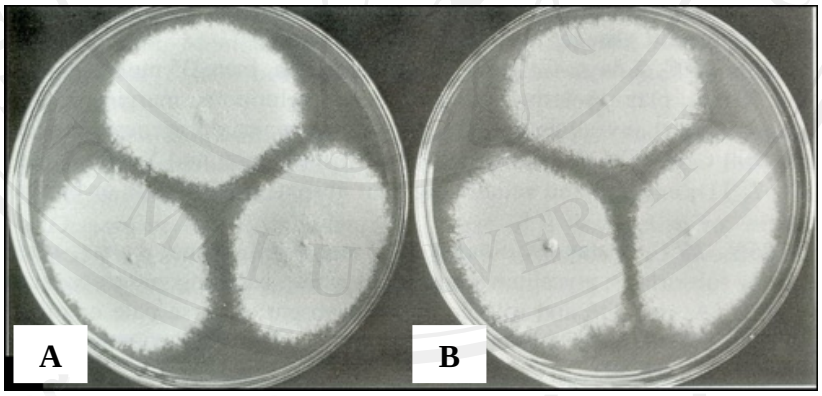

Figure 2.3 *Penicillium digitatum* colonies on CYA (A) and MEA (B), 7 days at 25°C (Source: Pitt, 2008)

Conidiophores borne from surface or aerial hyphae, stipes 70-150x5-7 µm, with thin smooth walls, bearing terminal penicilli, when best developed terverticillate but frequently biverticillate or irregular; rami 20-30x5-6 µm, smooth walled; metulae in verticils of 2-3, commonly 15-25x5-6 µm; phialides in verticils of 3-5, ampulliform to cylindroidal, 10-15(-20)x4-5 µm, narrowing abruptly to large cylindroidal collula, 2.0-5.0x2.5-3.0 µm; conidia ellipsoidal to cylindroidal, 6-8(-15)x2.5-5(-6) µm,

smooth walled, borne in disordered chains (Pitt, 1979; Pitt and Hocking, 1998; Timmer *et al*., 2000) (Figure 2.4).

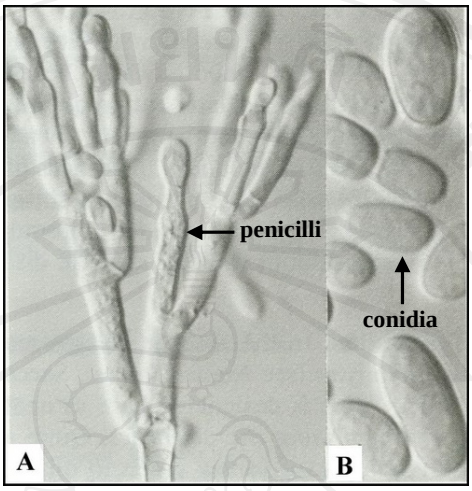

Figure 2.4 *Penicillium digitatum* (A) penicilli x750 (B) conidia x1875
(Source: Pitt, 2008)

Penicillium digitatum can grow at temperatures between 6-7°C and 37°C, at a minimum a_w for growth of 0.90 at 25°C. The minimum inhibitory concentration of sorbic acid preventing growth of *P. digitatum* is 0.02-0.025% at pH 4.7 and 0.06-0.08% at pH 5.5. The lethal temperature has been reported between 50-60°C. This variation in lethal temperature can be traced in part to exposure duration (Pitt and Hocking, 1998). Spores of *P. digitatum* germinate to a minor extent in pure water, whereas the addition of fruit juice greatly accelerates germination. *Penicillium* spore germination is also stimulated by the addition of oil derived from the peel of orange, lemon, grapefruit or other citrus fruit. Inserting the fungus into the oil glands of the citrus fruit peel leads to higher infection rates than its insertion into the peel between the glands (Barkai-Golan, 2001). Searching for the reason for this phenomenon, Eckert and Ratnayake (1994) found that a mixture of volatiles evaporating from the abrasions of wounded oranges were capable of accelerating or inducing germination of *P. digitatum* spores on water agar as well as within the injury of the peel. The major components of this mixture were the terpenes, limonene, α-pinene, β-nyrcene and sabinene accompanied by acetaldehyde, ethanol, ethylene and CO_2 as identified

by gas chromatography.

Fruit are particularly susceptible to infection during wet or humid weather, and the postharvest temperature is another factor determining the green mold fungi development. The optimal temperature range for fungi is 22-27°C. Although fungal growth is reduced at lower temperatures, a very slow rate has still been recorded at 4.5-10°C, allowing the fungi to progress under these conditions when storage is extended or in overseas shipments. At 0-1°C, the growth of the *P. digitatum* is arrested, but these temperatures result in chilling injury, or are expressed in pitting and internal physiological injuries (Timmer *et al*., 2000; Barkai-Golan, 2001).

Currently, green mold is primarily controlled by the application of synthetic fungicides, but resistance to benzimidazoles, biphenyl, imazalil, sodium *ortho*-phenyl phenate and thiabendazole has developed in most countries to a greater or lesser degree (Pitt and Hocking, 1998; Palou *et al*., 2002; Brown, 2005). The use of synthetic chemicals on harvested fresh produce is becoming more difficult to justify (Schirra *et al*., 2000). A number of fungicides are no longer registered for use on fresh citrus, including those that were used to effectively control postharvest disease. There are only three fungicides (imazalil, sodium *ortho*-phenyl phenate and thiabendazole) currently registered for postharvest use on citrus (Wilson *et al*., 1994; Ben-Yehoshua *et al*., 1997). Recently, there has been an increased demand for fresh horticultural commodities with less or no chemical residues. Chemical-free technologies to extend the storage and shelf life of fresh produce are needed. The environmental contamination and human health risks are associated with fungicide residues. The widespread use of these chemicals in commercial packing houses has led to the proliferation of resistant strains of the pathogens (Palou *et al*., 2002).

2.8 Chilling injury

2.8.1 Chilling temperature of tangerine fruit

Temperature plays a key role in metabolism of fruit and vegetables. Low-temperature decrease metabolism and prolong the shelf life. Refrigeration is a common method used in posthavest technology to maintain the quality of fruit and vegetables. Unfortunately, the tropical and subtropical commodities face a serious

problem during low-temperature storage, because of the physical and or physiological disorders induced by low-temperature such as chilling injury (Kays, 1991). Chilling injury in citrus fruit occurs at below a critical threshold temperature, but above their freezing point (Kader, 2002). Tangerine fruit are subjected to chilling injury when stored at temperatures below 5-6°C and 90-95% RH. The highest freezing temperature reported was -0.80°C (Ladaniya, 2002). Precise freezing temperatures very greatly among species and varieties of citrus fruit (Table 2.3). The other tropical and subtropical fruit were injured by temperatures in a range of 7-13°C such as banana (12-13°C), lime, muskmelon and pineapple (7-10°C) and cucumber, eggplant and papaya (7°C) (Kays, 1991).

2.8.2 Symptoms of chilling injury

The chilling symptoms usually develop during cold storage, but in some cases, they may appear after transferring the fruit to higher shelf-life temperatures. The development of chilling disorders depends on the genetic characteristics of each variety, as well as on its sensitivity to low-temperatures, cultivars, maturity and harvest seasons (Murata, 1997; Porat, 2004). Chilling injury symptoms of tangerine fruit were rind pitting, which appears visually as dark sunken lesions of collapsed epidermal tissue all over the peel surface (Figure 2.5). Other forms of chilling injury disorders of tangerine fruit include scald, brown staining is characterized by large, poorly-defined areas, at first the tissues are of normal consistency then they soften and collapse. The colour is at first pale yellow then light brown. The injury is sometimes associated with a watery breakdown (Arras, 2001; Arpaia and Kader, 2005). Rind pitting and sunken areas, which coalesce to form larger lesions are the common symptoms of chilling in Nagpur mandarin, Mosambi sweet orange and Kagzi acid lime when these fruit are stored at 3.5-5.0°C, 3.0-5.0°C and 5.5-7.0°C, respectively (Ladaniya, 2002).

Table 2.3 Approximate lowest safe temperatures, freezing points and characteristic of low-temperature injury in citrus fruit

Commodity	Approximate lowest safe temperature (°C)	Freezing point (°C)	Characteristic low-temperature injury symptoms
Grapefruit (*C. paradise* Macf.)	10.0-15.5	-1.0	Scald, pitting, watery breakdown
Lemons (*C. limon* (L.) Burm. f.)	10.0-13.0	-1.2	Pitting, membranous staining
Limes (*C. aurantifolia* Swingle)	7.5-9.0	-1.5	Pitting
Oranges (*C. sinensis* (L.) Osbeck)	4.0-7.0	-0.5 to-1.0	Pitting, brown sunken areas
Tangerines and Mandarins (*C. reticulata* Blanco)	4.0-7.0	-0.5 to-0.8	Pitting, watery breakdown

(Source: Ladaniya, 2002)

2.8.3 Mechanism of chilling injury

The current knowledge strongly suggests that membranes of chilling sensitive fruit undergo alterations in biophysical properties related to their composition that can alter functionality. Modern theories focus on the plasma membrane as the site for chill-induced membrane damage. The primary event in chilling injury is the induction of a phase change in the membrane lipids. This would be reversible until secondary events had caused a modification in the normal properties of the membrane lipids. Lateral phase separations may be reversible up to the point in time where lipid degradation and accumulation of lipid degradation products induce irreversible membrane damage. Permanent and extensive chilling injury symptoms may be due to

the irreversible phase of the reaction (Marangoni *et al*., 1996).

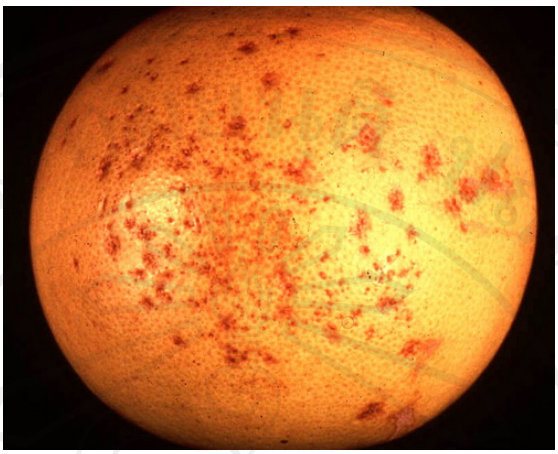

Figure 2.5 Postharvest chilling injury of citrus fruit
(Source: Amador, 2008)

Further support for the membrane damage hypothesis comes from the observation that sensitive fruit can be acclimated. If fruit are exposed to non-chilling low-temperature prior to refrigerated storage, their tolerance is increased. This "preconditioning" caused a compositional change in membrane lipids such as decreased rate of phospholipid degradation, increased unsaturation of phosphatidylcholine, decreases in free sterol : phospholipid ratio, increases in linoleic acid and free sterols. All these changes demonstrated the relationships between membranes and chilling injury (Marangoni *et al*., 1996).

2.8.3.1 Lipid composition and chilling injury

In plant tissue, most of the lipids are membrane components. The fatty acids present varying totals in the number of *cis*-double bonds (Ohlrogge and Browse, 1995). Changes in membrane lipids have been associated with increased leakage which varied with the fruit, but included an increase in sterol content, a decrease in linoleic acid (18:2) content and the double bond index, and an increase in palmitic acid (16:0) and the saturation index. Lyons *et al*. (1964) reported that the degree of unsaturation of fatty acid in membrane lipids was higher in chilling-resistant tissues than in chilling-sensitive tissues. Wang and Gemma (1994) also found the ratio of unsaturated/saturated fatty acids in the peel tissues of banana decreased during the

chilling peroid. Wang and Baker (1979) observed that the higher degree of unsaturation of fatty acids coincided with the higher chilling resistance in cucumbers and sweet peppers. Polyunsaturated fatty acids, very abundant in galactolipids molecules, are the preferential substrate of peroxidation and hydrolytic enzymes (Sahsah *et al*., 1998). Malondialdehyde (MDA) is one of the final products of stress induced lipid peroxidation of polyunsaturated fatty acids and has been considered a marker for cold sensitivity (Queiroz *et al*., 1998).

2.8.3.2 Membrane permeability and leakage

There are many symptoms of chilling injury of horticultural crops associated with the changes in membrane permeability. Membrane permeability is an expression of the freedom that water and solutes can pass through the membrane. Increased permeability of membranes may cause the promotion of an enzyme-substrate interaction, resulting in the occurrence of browning. Ion leakage has been used as an indicator of damage to the plasma membrane and chilling injury. If damage to biological membranes is the cause of chilling injury, one would expect to detect earlier increases in ion leakage during the chilling treatment. A possible problem with ion leakage measurements is that phase transitions and formation of lateral phase separations are reversible (Marangoni *et al*., 1996). Ion leakage measurements are usually performed at room temperature that may reverse the effect. Perhaps ion leakage measurements should be performed on chilled fruit tissue at chilling temperatures, without allowing the tissue to warm up at all (Palta, 1990). Many scientists presented data indicating that increased membrane permeability and an increased rate of ion leakage are associated with the chilling injury of sensitive tissue (Paull, 1981; Wang, 1990; Saltveit, 2000). Membrane permeability as a result of enzymatic lipid peroxidation in plants is mainly catalyzed by lipoxygenases (LOXs) (Berger *et al*., 2001).

Lipoxygenase (LOX); lineolate:oxygen oxidoreductase; EC 1.13.11.12 are nonheme iron-containing dioxygenases which are widely distributed in plants and animals. LOX catalyzes the addition of molecular oxygen to polyunsaturated fatty acid containing a (Z,Z)-1,4- pentadiene system to produce an unsaturated fatty acid hydroperoxide. In plants, linolenic and linoleic acids are the most common substrates for LOX (Siedow, 1991). LOX gene expression is regulated by different effectors

such as the source/sink status (Fischer *et al*., 1991), methyl jasmonate (Creelman and Mullet, 1997), abscisic acid (Melan *et al*., 1993) and also by different froms of stress, such as wounding, and/or pathogen attack (Melan *et al*., 1993). In addition, LOX genes isolated from different plant species show differential organ specific expression (Kolomiets *et al*., 2001).

2.9 Heat treatment

Postharvest decay is the major factor limiting the extension of storage life of many fresh harvested commodities. All fresh fruit and vegetables for domestic or export markets should be free of dirt, dust, pathogens and chemicals before they are packaged. The susceptibility of freshly harvested produce to postharvest disease increases during prolonged storage as a result of physiological changes that enable pathogens to develop in the fruit (Fallik, 2004). The concept of killing pathogenic fungal spores by heat treatment is not new. In early 1930s, fruit were passed through hot dips for a few minutes at 49°C to kill mold spores on citrus fruit. What is new is the initiative to use non-chemical means of mold control (Irtwange, 2006).

In the first decades of the 20th century, postharvest heat treatment was used on a commercial scale to control fungal diseases and insect infestation of horticultural crops. However, with the development of synthetic fungicides, the use of heat treatment was abandoned because of the greater advantages of fungicide treatments in terms of effectiveness, lower cost and ease of application. Many factors, however, have recently contributed to the implementation of strategies for reducing the dependence on agrochemicals. These include the enhanced proliferation of resistant strains of fungus due to prolonged use of agrochemicals, the prohibitive costs of selecting, synthesizing and testing new active ingredients and the difficulties of registering them (Ben-Yehoshua and Porat, 2005).

Increased consumers awareness has, in recent years, brought about a resurgence of interest in the use of non-chemical treatment for the preservation of fresh produce. The use of heat is a method which has been studied for a number of fruit species (Barkai-Golan and Phillips, 1991). There are three methods used to heat commodities, includes hot water, vapor heat and forced hot air. Hot water was

originally used for fungal control, but its use has been extended to disinfestations of insects. Vapor heat treatment was developed specifically for insect control, while forced hot air has been used for both fungal and insect control. The thermodynamic and operational characteristics of the three methods are summarized in Table 2.4. Since water is a more efficient heat transfer medium than air, it is preferred as medium for most applications. Treatment with hot water becomes increasingly accepted commercially and significant improvement has been made with the addition of brushing (Lurie, 2005; Irtwange, 2006).

Table 2.4 The thermodynamic and operational characteristics of heat treatments

Method	Heat Transfer Coefficient ($Wm^{-2} \times K^{-1}$)	Heat Capacity ($kJ\ s^{-1}$)	Advantages/Disadvantages
Water	50-10,000	7,300	High heat transfer coefficient High heat capacity Used as immersion
Vapor	5,000-100,000	640	Developed as an improvement over air
Air	10-200	120	Low specific heat capacity Low surface heat transfer coefficient

Note: Values based on the following assumptions: air and vapor flows 3 meter/second, water flow 50 liter/second/square meter. Temperature difference of 35°C. Heat capacity includes latent heat of evaporation for vapor treatment $Wm^{-2} \times K^{-1}$ = watt/square meter/kelvin; $kJ\ s^{-1}$ = kilo joule/second

(Source: Bollen and Dela Rue, 1999)

Changes in fruit ripening during and following heat treatments can be divided broadly into two types. The first type of response is the normal stress cellular responses that lead to modification of chilling sensitivity, delayed or slowed ripening

and some slight modification of quality. Preharvest conditions, species, cultivar, the stage of ripening, temperature and its duration and possibly diurnal variations are modifying factors. The second type of response occurs when the stress exceeds a threshold and the cells ability to recover is lost. This response is associated with the disruption of various aspects of ripening. The site of the lesion may be associated with either or both transcription and translation and a cellular threshold of metabolic disruption (McDonald *et al*., 1991; Rodov *et al*., 1995; Schirra and D'hallewin, 1997; Paull and Chen, 2000).

Heat treatments exert their effects either by slowing pathogen growth or by killing its germinated spores. Also, heat treatment enhances the host pathogen-defence responses and thus renders the commodity more resistant. Moreover, heat treatments may also partially melt the epicuticular surface of fruit of vegetables and thus occlude and seal micro-cracks and wounds which could serve as possible pathogen invasion sites. Finally, postharvest heat treatment also can reduce chilling injury in many wounds of fruit during subsequent low-temperature storage as well as reduce pathogens level and disease development (Schirra *et al*., 2000; Irtwange, 2006).

2.10 Hot water treatment (HWT)

Hot water dips (HWD) have generally been utilized for fungal pathogen control, since fungal spores and latent infections are either on the surface or in the first few cell layers under the peel of the fruit or vegetable. Postharvest dips to control decay are often applied for only a few minutes and temperatures used are higher than those for hot air or vapor heat, because only the surface of the commodity is heated (Barkai-Golan and Phillips, 1991). These treatments are effective within narrow ranges of temperature and duration, because heat has two effects: Inhibition of the pathogen, and preventing damage to the fruit, which is not. The principle is to use a temperature that is high enough to inactivate the pathogen without damaging the fruit (Ben-Yehoshua and Porat, 2005). The time and temperature applied depend on the cultivar, fruit maturity, fruit size and conditions during the growing season (Fallik, 2004). Many fresh horticultural products can tolerate hot water temperatures of 50°C to 60°C for up to 10 minutes, but shorter exposure at this temperature can control

many postharvest plant pathogens (Barkai-Golan and Phillips, 1991). These treatment temperatures and times can also be effective for enhancing the resistance of the commodity to chilling injury (Lurie, 1998).

Treatments for citrus fruit can range from 36°C for 3 days to 62°C for 2 seconds (Watkins and Ekman, 2005). Schirra and D'hallewin (1997) found that pre-storage dipping of Forture mandarins in water at 50, 52 or 54 °C for 3 minutes reduced decay both during cold storage at 6°C and stimulated shelf-life at 20°C without causing adverse effect to the rind surface. However, temperatures of 56-58°C induced heat damage in the form of rind browning, dull-coloration and resulted in enhanced decay development and water loss. Physiological behavior and the internal quality attributes between the untreated and those treated at 50-54°C were minimal. Those dipped at 58°C, however, developed off-flavor, which was probably due to the increased ethanol level. The benefits of HWT for control of decay caused by *Penicillium* spp. on citrus have been reported (Schirra and D'hallewin, 1997; Porat *et al*., 2000; Palou *et al*., 2001; Ben-Yehoshua, 2003). For citrus, HWD at 50-53°C for 2-3 minutes were shown to be as effective as curing at 36°C for 72 hours in controlling postharvest decay and chilling injury in various citrus fruit and are much less expensive, mainly because of shorter treatment duration (Rodov *et al*., 1995). Table 2.5 summarizes the optimal temperature and time of exposure for control of postharvest decay, quarantine treatment or for preserving quality of citrus fruit.

2.11 Heat treatment and host pathogen interactions

2.11.1 Pathogen response to heat treatment

The efficacy of heat treatment on pathogens is usually measured by reducing viability of the heated propagules. However, heat effects may be lethal or sublethal (Schirra *et al*., 2000). The response of decay-causing agents to heat can be influenced by several factors such as the moisture content of spores, their metabolic activity, age of the inoculum, inoculum concentration and location of the pathogen upon the host(Barkai-Golan and Phillips, 1991), as well as factors inherent within the host (i.e., physiological, maturity and stress) (Klein and Lurie, 1991).

Table 2.5 Hot water treatments for citrus fruit, optimal temperature and aim of heat treatments

Commodity	Treatment	Optimal temperature °C (time)	Aim
Clementine	HWD	45 (2.5 min)	Decay control
Grapefruit (cv. Hass)[1]	HWRB	59-62 (20s)	Decay control, chilling and decay resistance, better quality
Kumquat	HWRB	58 (20s)	Decay control, better quality
Lemon	HWD	52-53 (2 min)	Decay control, decay resistance
	HWRB	62.8 (15s)	Decay control, quality maintenance
Mandarin (cv. Fortune)	HWD	50-54 (3 min)	Decay control
Orange (cv. Shamouti)[1]	HWRB	56 (20s)	Decay control, better quality
Orange (cv. Tarocco)	HWD	53 (3 min)[2]	Decay control, chilling resistance
	HWRB	62.8 (15s)	Decay control
Tangerine (cv. Minneola)	HWRB	56 (20s)	Decay control

Note: [1] = Commercial treatment; [2] = Season-dependent

HWD = Hot water dips; HWRB = Hot water rinsing and brushing

(Source: Fallik, 2004)

Although reports have indicated a linear relationship between the logarithm of the reduction time and the temperature of heat treatment, pathogen kill is not always proportional to the temperature-time product of the treatment. The sensitivity of fungal spores to heat treatments does not necessarily depend upon the spore size, shape or inoculum age. Fungi express considerable species variation in sensitivity to high temperatures (Barkai-Golan and Phillips, 1991). *In vitro* experiments showed that *Botrytis cinerea* was found to be more sensitive to heat than *Alternaria alternata*, while *A. alternata* was more sensitive than *Fusarium solani* (Fallik *et al*., 2000).

In general, germinated or moist conidia are more sensitive to heat than dry spores or mycelial growth and phathogens located on the outer surface of the commodity are much more easily eradicated than those located deep in side it (Fallik *et al*., 1993; Ben-Yehoshua and Porat, 2005). *Penicillium expansum*, the main decay-causing agent on stored apples in Israel, was found to be relatively resistant to high temperature. The time to reduce spore germination of *P. expansum* by 50% (ET_{50}) was found to be 12, 23 and 45% shorter than mycelial growth at 38, 42 and 46°C, respectively (Fallik *et al*., 1995). Barkai-Golan (2001) reported that germinated spores of *A. alternata* were more sensitive to heat than non-germinated spores. Hot water treatments were found to be ineffective in killing dormant spores (Schirra *et al*., 2000; Barkai-Golan, 2001).

The above results obtained by *in vitro* experiment could explain the direct effect of heat treatment *in vivo*. A certain threshold of inoculum level is needed to initiate decay development (Trapero-Casas and Kaiser, 1992). As a result of heat treatments, which reduce fungal viability, the effective inoculum concentration which causes decay development is reduced, thus reducing rot development. Hot water rinsing and brushing was reported to significantly reduce decay development on several fresh harvested commodities (Fallik *et al*., 1999; Prusky *et al*., 1999; Fallik *et al*., 2000; Porat *et al*., 2000). Employing hot water rinsing and brushing resulted in a 3-4 log reduction of the total microbial counts (colony-forming unit-CFUs) of the epiphytic microorganism population, compared to untreated fruit as was revealed by scanning electron microscopy analysis (Fallik *et al*., 2000; Porat *et al*., 2000). Another explanation for the reduction in rot development could be a reduction in spore survival of various decay causing agents such as with *Penicillia* in 'Valencia' oranges. Heat may also cause changes in nuclei and cell walls, may denature proteins, destroy mitochondria and outer membranes, and disrupt vacuolar membranes and formation of gaps in the spore cytoplasm (Barkai-Golan and Phillips, 1991).

2.11.2 Fruit responses affecting pathogen defence

The mechanisms of fruit defence against pathogens involve complex interactions with many lines of response such as mechanical barriers to microorganisms, antimicrobial chemical compounds and pathogenesis-related (PR) proteins (Porat *et al*., 2002). In citrus fruit several defence mechanisms against

pathogens have been progressively identified and analyzed. The first line of defence comprises constitutive antifungal materials and the second comprises induced mechanisms that are elicited by infection with the pathogen. The latter include increased deposition of lignin-like material in the cell wall, the production of the phytoalexins scoparone and scopoletin and the production of PR proteins (Ben-Yehoshua *et al*., 2000; Ben-Yehohua and Porat, 2005).

Studies on various citrus fruit cultivars have shown that the curing of *Penicillium spp.* inoculated fruit prevented the development of the pathogen and promoted biosynthesis of the phytoalexin scoparone by cells adjacent to the wound. Hot water dipping induces the occurrence of scoparone and scopoletin in wounds of grapefruit approximately 12-14 hours after treatment (Schirra *et al*., 2000). However, their appearance proved not to depend only on pathogenic infections. Ultraviolet illumination, gamma-irradiation, and biological antagonists also induce the production of phytoalexins (Ben-Yehoshua *et al*., 1992; Rodov *et al*., 1992; Wilson *et al*., 1994). When heat treatment was carried out before wounding, neither scoparone nor scopoletin was detected in post-treated inoculated wounds. Hot water dipping applied to green lemons 2 days after inoculation with *P. digitatum* induced scoparone production as soon as 24 hours after treatment, and scoparone and scopoletin rose to an effective dose to inhibit the pathogen within 2 days of the treatment. Wounding, either followed by a hot water dip or not, induced scoparone production to a much smaller extent than the combined inoculation and heat treatment (Schirra *et al*., 2000).

Recently, Porat *et al*. (2000) reported that hot water drenching of 62°C for 2 seconds with additional brushing applied to Star Ruby grapefruit before the inoculation with *P. digitatum*, induced resistance against decay. This resistance was most effective than when the inoculation was carried out 1 day after the brushing and was less effective when the fruit were inoculated at the same day or 7 days after the brushing. The heat treatment was essential to decay control as brushing with cold water did not enhance any resistance.

It has been demonstrated that PR proteins such as chitinase and β-1,3-glucanase are constitutively present in orange peels and that their levels increased following pathogen inoculation and subsequent heat treatment (Porat *et al*., 2000; Porat *et al*., 2001). Schirra *et al*. (2000) reported that both chitinase and β-1,3-

glucanase were induced by a hot water drenching which included additional brushing of the Star Ruby grapefruit which was applied before the inoculation with *P. digitatum*. They suggested also that various heat shock proteins (HSPs) may be involved in the hot water brushing-induced resistance responses.

Two major groups of proteins may be activated by the HWT that induce fruit resistance: HSPs and PR proteins. HSPs comprise a diverse group of protein, ranging in molecular weight from 15 to 115 kDa, that are expressed in all organisms in response to elevated temperatures and are believed to play a major role in thermotolerance (Sabehat *et al*., 1998). In plants, HSPs function as molecular chaperones and assist in protein folding, assembly and transport and act to prevent protein aggregation at high temperatures (Boston *et al*., 1996).

PR proteins are acid-soluble, low molecular weight, PR proteins that accumulate in the intercellular spaces as well as the vacuoles of various plant cells during incompatible interactions with viruses, bacteria or fungi. Many studies have suggested a direct relationship between the accumulation of PR proteins and the expression of disease resistance in infected plants (Vidhyasekaran, 1997; Huang, 2001; Mohammadi and Karr, 2001). Induction of PR proteins following elicitor treatment, wounding or tissue infection is accompanied by a massive alteration in the pattern of gene expression and activation of other defence responses such as phytoalexin accumulation, lignin deposition and the synthesis of cell wall hydroxyproline-rich glycoproteins (Huang, 2001; Mohammadi and Karr, 2001). Induction of constitutive plant enzymes or PR proteins which are capable of inhibiting pathogen development would contribute to greater resistance. Induction can be triggered by elicitors that are biological, chemical, molecular or physical. Elicitors can be used for the exploitation of defence mechanisms in plants by either directly acting as signal molecules or generating signals to activate gene(s) that code for enzymes (Fajardo *et al*., 1998).

Among PR proteins, chitinase (the PR-3 family) and β-1,3-glucanase (the PR-2 family) are the most studied and well characterized defence-related proteins in plants. Chitinase (poly-β-1,4-(2-acetamido-2-deoxy-D-glucoside) glycanohydrolase, EC 3.2.1.14) catalyzes the hydrolysis of β-1,4 linkages of *N*-acetyl-D-glucosamine polymers of chitin, a major component of fungal cell walls. Since higher plants lack

of chitin, it has been suggested that plant chitinase act as a defence response against chitin-containing pathogens (Punja and Zhang, 1993). Up to six classes of chitinases have been identified in higher plants including several families of exo- and endochitinases in microorganisms (Vidhyasekaran, 1997; Huang, 2001). Specific classes of chitinases have been shown to possess antifungal activities *in vitro* (Ponstein *et al*., 1994). In combination with vacuolar isozymes of tobacco β-1,3-glucanase I, class I chitinase caused structural distortions and eventual lysis of hyphal tips of pathogenic fungi (Sela-Buurlage *et al*., 1993).

Glucanase (endo-β-1,3-glucan 3-glucanohydrolase, EC 3.2.1.6) or laminarinase catalyzes the hydrolytic cleavage of the β-1,3-D glucosidic linkages in β-1,3-glucan, which is a component of mycelial cell walls. Alternatively, β-1,3-glucanase can release elicitor-active glucan oligomers from the mycelial walls of pathgenic fungi, thereby inducing its own synthesis as well as that of other defence-related enzymes involved in both the production of phytoalexins and cell wall deposition (Porat *et al*., 2002; Wang *et al*., 2003). These two enzymes are of particular interest because many pathogenic fungi contain β-1,3-glucans and chitin as major structural cell wall components. It had been demonstrated *in vitro* that the two enzymes β-1,3-glucanase and chitinase are able to degrade fungal wall components, resulting in the growth inhibition of fungi (Van Loon and Van Strien, 1999). Furthermore, breakdown products of fungal wall components, released by the activity of the two enzymes, have been shown to act as elicitors of plant defence responses (Wang *et al*., 2003). Studies on the subcellular localization of β-1,3-glucanase and chitinase *in vivo* showed that these hydrolases accumulated at higher concentrations in infected host plant tissues and on fungal cell walls, especially, at sites where host cells were in close contact with fungal hyphae. In incompatible plant-pathogenic fungal interactions higher activities of both hydrolytic enzymes were detected compared with the compatible interactions. This indicates that β-1,3-glucanase and chitinase could play an important role in the active defence of plants against fungal pathogens (Kang and Buchenauer, 2002). Indeed, in some cases, transgenic plants over expressing β-1,3-glucanase and chitinase genes appeared to be more resistant towards pathogen attacks (Wang *et al*., 2003).

Peroxidase are part of this muti-component defence system (Vidhyasekaran, 1997; Huang, 2001). Plant peroxidase (EC 1.11.1.7) are heme-proteins that use H_2O_2 to oxidise a large variety of hydrogen donors such as phenolic substances, amines, ascorbic acid, indole and certain inorganic ions (Caruso *et al*., 2001). These proteins are widespread in the plant kingdom and peroxidase isoenzymes are known to occur in a variety of plant tissues. The pattern of expression of each isoform varies in the different tissue of healthy plants and is developmentally regulated and influenced by environmental factors (Huang, 2001). These enzymes are involed in catalysing the polymerization of cinnamyl groups into lignin (Lagrimini *et al*., 1993), suberization of cell walls (Brownleader *et al*., 1995), accumulation of phenolic polymers (Graham and Graham, 1992) and polymerisation of hydroxyproline-rich glycoproteins (Caruso *et al*., 2001), but also in the regulation of cell wall elongation (Caruso *et al*., 2001), wound healing and resistance against infection by pathogens (Graham and Graham, 1992; Reimer *et al*., 1992). Wall toughening in the defence reaction can include linification and suberization, as well as insolubilization of the cell wall structural proteins. The first two processes involve the synthesis of aromatic polymers that are covalently attached to carbohydrates of the cell wall and/or are attached to cell wall proteins. The deposition of these phenolic polymers as barriers on the walls to wound-healing or infected tissues may act to prevent entry by the pathogen. The polymerization of the phenols is catalysed by a cell wall associated peroxidase (Lurie *et al*., 1997). Mature green tomatoes are strongly resistant to infection. The increased susceptibility of tomatoes to decay during ripening has been associated with the decrease and disappearance of mRNA encoding an anionic peroxidase (Schirra *et al*., 2000). Heat treatment of mature green tomatoes was proven to delay the degradation rate of such mRNA and to maintain antifungal resistance in the fruit tissue to decay (Lurie *et al*., 1997).

2.12 Heat treatment and chilling injury

Since chilling injury such a crucial problem in the postharvest handling of citrus fruit, many studies have been conducted to elucidate the effects of various postharvest parameters on its development and various postharvest technologies have been developed to enhance citrus fruit cold tolerance and to reduce chilling injury

damage. Postharvest heat treatments induce cold tolerance in citrus fruit and reduce the development of chilling injury symptoms following cold storage (Porat, 2004).

Table 2.5 tabulates some of the citrus fruit that have been tested for high-temperature induction of resistance to low-temperature injury. What is apparent from the table is that a time temperature regime can be found for some commodity, which will reduce chilling injury. The successful treatment is arrived at empirically, by trial and error, and what works on one cultivar may not be as successful on another. For example, Israeli citrus fruit respond well to a HWD at 53°C for 3 mintues (Rodov *et al*., 1995). A number of citrus varieties including grapefruit, lemon, oroblanco and kumquat all had reduced chilling injury after this treatment and low-temperature storage. However, Tarocco blood oranges grown in Italy will be heat damaged by a HWD at 53°C for 3 mintues, but respond favorably at 50°C for 3 mintues by reduced chilling injury (Schirra *et al*., 1997). HWT control chilling injury on a number of fruit.

During high-temperature treatment the synthesis of many proteins ceases and HSPs are synthesized instead. HSPs are produced from evolutionarily conserved gene families that are found in all of the major subcellular compartments of plant cells (Vierling, 1991). The correlation between HSPs and thermotolerance has been established in many organisms and it appears that they can also protect against low-temperature injury. Recently it was found that rice seedlings and tomato discs could have chilling injury reduced if they were heat-treated following a period of low-temperature, suggesting that the HSPs might be involved in recovery from stress and not protection during the stress (Saltveit, 2001).

The reduction of sensitivity to chilling injury of heated commodities may not be due solely to the presence of HSPs. Chilling injury has long been thought to begin with membrane damage (Marangoni *et al*., 1996) and a heat treatment of above 35°C may cause membrane alterations. High temperature (35 to 40°C) increases membrane leakage, but after removal from heat stress the tissue recovers and leakage returns to levels found in tissue held at 20°C (Lurie and Klein, 1991). A kinetic analysis of ion leakage showed two compartments, a fast extracellular reservoir of ions and a slow cellular level. The change in leakage following chilling was due to an increase in the extracellular reservoir, indicating that ions had leaked out of the cellular reservoir

during chilling to increase the content of the extracellular pool (Saltveit, 2002).

An examination of the lipid composition of apple plasma membrane showed that after heating in air at 38°C for 4 days and storage at 0°C for 4 months there were more phospholipids and greater fatty acid unsaturation in heated than in nonheated fruit (Lurie *et al*., 1995). Whitaker *et al*. (1997) also found greater fatty acid unsaturation though not greater phospholipids content in apple fruit heated by the same method. This would indicate more fluid membranes in fruit after exposure to conditioning temperatures and correspond with lower indiscriminate leakage from conditioned fruit and vegetable tissue.