Chapter 2
Literature Review

2.1 Mango

Mango (*Mangifera indica* L.) is a major tropical fruit in the domestic and export markets of Thailand. It belongs to the family Anacardiaceae which has 75 genera and 700 species (Lizada, 1993). They are considered as one of the choicest fruit of the world because of their attractive color, delicious taste and excellent nutritional properties (Table 2.1). Thailand is one of the major mangoes exporting countries in the world (Mitra and Baldwin, 1997). In 2004 Thailand exported around 4,661,475 metric tons, earning 171,397,810 bath (Customs, 2005). Mango plantations are found in many provinces but the large-scale plantations are located mainly on the east and the west coasts of the gulf of Thailand. In 2003, Thailand having an average production of 1,802,431 tons per year (DOAE, 2004).

'Nam Dok Mai' mango is the most popular variety and its total production ranks the first among commercial mango varieties. The fruit are usually harvested mature-green, about 91-105 days from bloom and with a starch content of about 18-20% (Tungtirmthong, 1998). After harvest, the mango remains alive and normal life process continue, the fruit can fully ripen in 4-5 days at ambient temperature (Yantarasri *et al.*, 1994).

Nam Dork Mai Sri Thong mangoes are one of the mango's cultivars that exported because their shape, color and taste are match to the consumer' s demand. Moreover, they have physical properties that are suitable for transportation. Difference between Nam Dok Mai Si Thong and Nam Dok Mai No.4 are shown in table 2.1.

Table 2.1 Difference between Nam Dok Mai Si Thong and Nam Dok Mai No.4.

Characteristic	Nam Dok Mai Si Thong		Nam Dok Mai No.4	
	Mature	Riped	Mature	Ripe
Peel color	YG14B	YO15A	YG11B	YO15B
Flesh color	YO17A	N/A.	YO21A	N/A.
Peel thickness	0.11cm.	0.08	0.14 cm	N/A.
Flesh thickness	2.24 cm.	N/A.	2.19 cm.	N/A.
%Fiber	little	little	little	little
%TSS in flesh	10.36	18	10.40	22
%TA in flesh	2.03	0.52	2.07	0.53

source: DOAE, 2004 and Chidtragool, 1996.

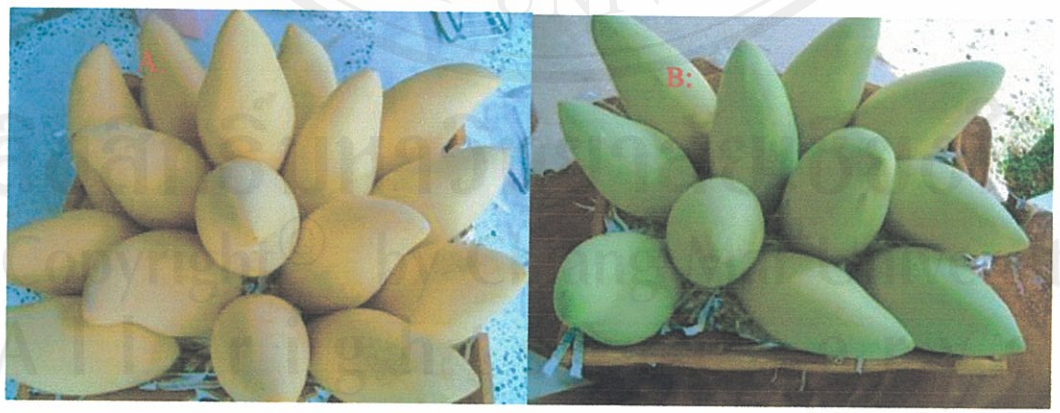

Figure 2.1 Mango cv. Nam Dok Mai Si Thong at A: maturiy stage and B: ripening stage.

2. 2 Chemical composition, nutritive and medicine value

The mango fruit is one of the highly prized fruits of the tropics. It has rich aromatic flavour and is decent in taste having well blended mixture of acidity & sweetness. Unripe fruits are usually acidic and used for pickles, chutney, amchur and culinary preparations. Ripe fruits are preserved by canning or used in the manufacture of juice, squash, jams and jellies, preserves (murabba) and Ampapar (Amavat).

Table 2.2 Analysis of the flesh of green & ripe mangoes gave the following Composition (per 100 gms):

Content	Green Mango	Ripe Mango
Moisture	90.0%	86.1%
Protein	0.7%	0.6%
Vitamins C	3mg/100g	13mg/100g
Carbohydrate	8.8%	11.8%
Phosphorus	0.02%	0.02%
Vitamin B2	30ug	50ug
Calcium	0.01%	0.01%

source: Vitamins-minerals-supplements.org (2006)

The mango fruit also contains fluorine, iodine, copper, potassium, sulphur and magnesium. The sugar and acid contents may vary as per condition and variety of mango. Sucrose, glucose, fructose-are the principal carbohydrates present in ripe mango; maltose is also present. It has total sugars 11.20-16.80% with reducing sugars 1.40-4.83% with and non-reducing sugar 8.19-13.81%. Small amounts of cellulose, hemicelluloses and pectins are also present. The green tender fruit is rich in starch; during ripening the starch is hydrolysed into reducing sugars and a part of the latter is synthesised into sucrose. In the post-ripening stage, sucrose decomposes into reducing sugars.

Unripe fully developed mangoes of pickle variety contain citric, malic, oxalic, succinic acids besides two di & tri-basic acids; citric acid is the dominant constituent. As the fruit ripens, acid content decreases to more than half. Among amino acids, mango has asparatic acid, glutamic acid, alanine, glycine, methionine, leucine and cystein.

2. 3. Poshtarvest Storage

The basic concept of storage is to extend the shelf life of products by storing them in appropirate conditions to maintain their availability to consumers and processing industries in their useable form. They can either be stored naturally in the field, or in built storages (Raghavan and Gariépy, 1985 and Pantastico *et al.*, 1975). In natural storage the product islest in the field and harvesting is delayed, while in artificial storage favorable conditions are provided which help to maintain product freshness and nutritional quality for a longer period. During storage, the mango physiology and its ripening involves many physiochemical activities, such as cummulative physiological loss in weight and volume, pulp and shin color change, acidity, loss in firmness, increase in total solids and sugar concentration.

According to FAO Year Book, 2000 the world import of fresh mango are projected to increase by 53% to 459,000 tonnes by year 2005 and it can further be increased by improving storage conditions and controlling disease and insect contamination. There are various techniques, which have been developed to improve the storage life and maintain the quality of fresh horticultural commodities.

2.3.1 Storage at low temperature

International trade of fresh mango has been limited because of its highly perishable nature and susceptibility to postharvest disease and injury and hence mangoes are still consider as luxurious and expensive item in the markets of many industrialized countries. While storing the commodities there are many factors which influence product quality; temperature is one of them. For successful storage it is necessary to efficiently control the temperature throughout the storage period.

The principal behind cold storage is to delay the period of ripening of a product by slowing down its physiological activities. While storing fruit priority is to maintain the quality of the product. Many commodities when stored at low temperature are subjected to damage caused by chilling which will promote fungus and diseases. Mangoes are tropical fruits and are therefore sensitive to chilling when stored below a critical temperature (Chaplin et al., 1991; Lizada, 1991). If stored at low temperatures for prolonged time, storage could have an effect on ripening. It has been reported that cv. Nam Dok Mai can be safty be stored at 13-2-13°C for 2-3 weeks, while Philippine mango such as 'Carabao' can be stored at 7-10°C for 15 days (Mendoza and Wills, 1984). However, there are variation in reports about the optimal temperature, which may be attributed to the characteristic of cultivar and/or the stage of maturity and ripeness when kept in low temperature storage.

2.3.2 Controlled and modified atmosphere storage

Respiration is the major physiological activity of concern in postharvest storage. It is a metabolic process that occurs continuously in all living cells. Respiration is the oxidative breakdown of complex material such as starch, sugar and other organic compound into simple molecules such as a carbon dioxide, water and energy as represented by the following equation (Whiting *et al.*, 2006).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + Energy \qquad (2.1)$$

Respiration rate (RR) is usually expressed in terms of O_2 consumed and CO_2 produced or heat released and it is expressed as $mg.kg^{-1}.h^{-1}$. RR can be lowered by lowering the temperature. During the postharvest life of the product, the respiration provides defence mechanism against spoilage. However higher RR, leads to faster deterioration of fruits and shortening of storage life.

By elevating carbon dioxide and lowering oxygen amount surrounding the product, there will be a decrease in respiration and a decrease in the rate of ethylene production. In closed storage, the respiration will simultaneously lead to build-up of carbon dioxide and depletion of oxygen. If O_2 level goes too low it will create anaerobic conditions, which results in fermentation and unwanted by-products that can cause damage to certain products. If mango fruits are stored at 1% O_2 and 15% CO_2 level condition. Off-flavor and skin discoloration of fruit will occur (Hatton and Reeder, 1966).

The basic idea of controlled atmosphere storage (CA) is to maintain the best product quality. This can be accomplished by keeping CO_2, O_2 and ethylene gases at predetermined levels (gas levels differ depending on the type of fruit being stored). Usually decrease O_2 and increase CO_2 level at low temperature with high RH are suitable for stored commodities. Ca can provide an effective storage environment for different fruits and vegetables (Raghavan *et al.*, 2003, 1984; Bender *et al.*, 2000; Gariépy and Raghavan, 1991, 1986, 1984; Kader, 1986).

Modified atmosphere (MA) is referred to as a relationship between product respiration and gas exchange within any form of structural enclosed. MA storage technique can be used to maintain the postharvest quality of differ fruits (Ding *et al.*, 2002; Rodov *et al.*, 2002; Meir et al., 1998; Illeperuma and Jayasuriya, 2002; Prabhanjan et al., 1992). MA packaging inhibited the mango ripening process (Sornsrivichai *et al.*, 1989, 1992). In 1994, Yantarasri et al., found that film perforation delay softening and can reduce the weight loss of mango cv. Nam Dok Mai.

2.3.3 Storage at low pressure

Low pressure storage, also called hypobaric storage, deals with the control of air pressure, temperature and humidity. The main principle of low pressure is that the pressure of chamber is directly proportional to the O_2 level. With a pressure decrease, the amount of O_2 decreased reducing the respiration rate of the product. This system easily maintains O_2 and relative humidity. The advantage of this system is that it can easily remove the metabolites. But this system cannot be used commercially as it has certain construction disadvantages with airtight chambers being costly. Furthermore, this storage provides unsatisfactory ripening and poor aroma and flavor to the fruits (Ramachandra, 1995)

Spalding and Reeder (1977) noted that when cvs. Tommy Atkins and Kent were stored at 13°C at a pressure of 76-152 mm Hg with a relative humidity of 98-100% for up to 3 weeks, a greater percentage of market acceptable fruits were obtained; and when they were placed under normal pressure they took a longer time for ripening as compared with those stored at 760 mm Hg. The fruits were more greenish in color compared to those stored at normal pressure.

2.3.4 Storage by use of coating

Waxes are commercially used to reduce the moisture loss from the fruits. Aqueous wax emulsion consisting of mineral petroleum like paraffin and vegetable waxes with or without emulsifier used to increase the storage life of mangoes (Dalal *et al.*, 1971). However, coating of mango with refined mineral oil resulted in fruit injury (Mathur and Srivastava, 1956). Oil coating decreased the respiration more than wax coating and results in severe anaerobic condition that injured the fruit.

Fungicidal wax, wax emulsion containing hydrazide, maleic and polysaccharide-based coating also delays ripening process. Selective films like Polyvinyl chloride (PVC) film also prolong shelflife of mango fruit (Ketsa and Raksritong, 1992). The wax can be applied by roller brushes in a specially designed wax applicator or by hand. Dipping in wax is to be avoided, and a uniform application of wax is necessary otherwise some fruits receive too much wax and other

too little. Before application of wax fruits must be dried, otherwise foaming of water-emulsion waxes may occur.

2.3.5 Storage by using ionizing radiation

For safe storage of fresh fruits and vegetables, the US Food and Drug Administration approved the use of irradiation at a dose of 100 Krad (United States Food and Drug Administration, 1986). Irradiation includes use of ionizing energy such as gamma rays, electrons, X-rays and microwaves. Spalding and Von Windeguth (1988) reported that the percentage of decayed mango is minimized when they are exposed to 750 Gy or higher, but fruit peel shows scald-like symptoms with irradiation doses over 500 Gy.

Radiation also affected ripening, because of the specific biochemical processes. According to Spalding and Reeder (1986), combination of irradiation and HW (53°C), or 0.2% hot imazail (53°C) was more effective for storage purpose. Gamma irradiation (30 Krad) caused ripening delay of seven days in comparison to mango stored at room temperature.

2.3.6 Storage by using chemicals

Treatment efficiency varies with infection level and storage regime. The length of shelf life depends on cultivar, injury, maturity at harvest, calciun spray, and exposure to ethylene (Anonymous, 1988, Coates *et al.*, 1995). A dip in 46% calcium chloride can increase the shelf life of some cultivars (Singh et al., 1993). Ethylene is used to reduce time for ripening initiation and it can also enhance skin color of the fruit (S. P. Burg and Burg, 1962). In South Africa, a benomyl dip for 5 min at 55°C is recommended just after picking of fruits, which can control soft brown rot (Sepiah, 1986), same as in Thailand, benomyl 500 ppm dip for 5 mins at 52°C after picking can control anthracnose (DOAE, 2003). Prcchloraz also provides good protection from anthracnose and Alternaria rot in mango (Johnson and Coates, 1993). Prior to harvest Gibberellic acid (GA3) spray can retarded mango ripening at ambient temperature for up to six days of storage (khader, 1991). Calcium chloride treatment

resulted in low ethylene production, low respiration, and helped to reduce the occurrence of storage decay (Eeden, 1992).

2.4 Ripening of mangoes

Mangoes are harvested at mature green stage and subsequently allowed to ripen. The recommened temperature for ripening of mango fruits ranges from 20-25°C but it varies according to the variety and origin. Ripening at higher temperatures results in off flavor and spotting of the fruits. During ripening of the mango fruit, different occurrence have been observed:

2.4.1 Respiration

Generally, fruit can be classified as climacteric and non-climacteric. Climacteric fruit displays a characteristic peak of respiration activity during ripening, termed the respiratory climacteric (Tuker, 1993). Mango is a climacteric fruit. The respiration decreases as the fruit matures and the respiratory rise then commence with ripening (Mitra and Baldwin, 1997). The patterns of respiration and ripening behavior vary among the varieties, the climacteric conditions and the local places.

2.4.2 Ethylene production

In climacteric fruit, there have been accepted that ethylene plays an important role in ripening. A massive production of ethylene commence at on set the respiratory climacteric peroid. Exogenously and endogenously ethylene induces ripening (Yang and Hoffman, 1984). Ethylene is biosynthesized from methionine. The two key enzymes in the pathway are those catalyzing the conversion of s-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC to ethylene, called ACC synthase and ethylene forming enzyme (EFE), respectively (Gomez-Lim, 1993) (Figure 2.2).

2.4.3 Carbohydrates

Mature greenstage of mango is characterized by accumulation of starch, which is mobilized during ripening. This phenomenon is evident in the chloroplast where the starch granules become progressively smaller as ripening proceeds. The primary consequence of starch hydrolysis is an increase in total sugars. Main monosaccharides include glucose, fructose, and sucrose. Sucrose being the predominant sugar, it contributes 57% of total sugar in ripe mangoes cv. Keitt while fructose and glucose are present at 28 and 15% respectively (Medicott and Thompson, 1985).

2.4.4 Color changes

The external color of the fruit is an important factor in consumer's preference. Peel color of the fruit changes during ripening as chloroplast in the peel is converted into chromoplast, which has red or yellow pigments, while some cultivars showed reddish blush because of anthocyanins, while some remain green (Lizada, 1993). During ripening the carotenoid pigment level also varies among cultivars. It has been reported that the level of carotenoid increases with gradual decrease of anthocyanin in mangoes cv. Tommy Atkins (Medicott *et al.*, 1986). Mango fruit cv Nam Dok Mai, 6 days after storage at 35°C, peel color changed more rapidly. Chlorophyll a and total chlorophyll contents of peel decreased while β-carotent content increase rapidly (Yindee, 1994).

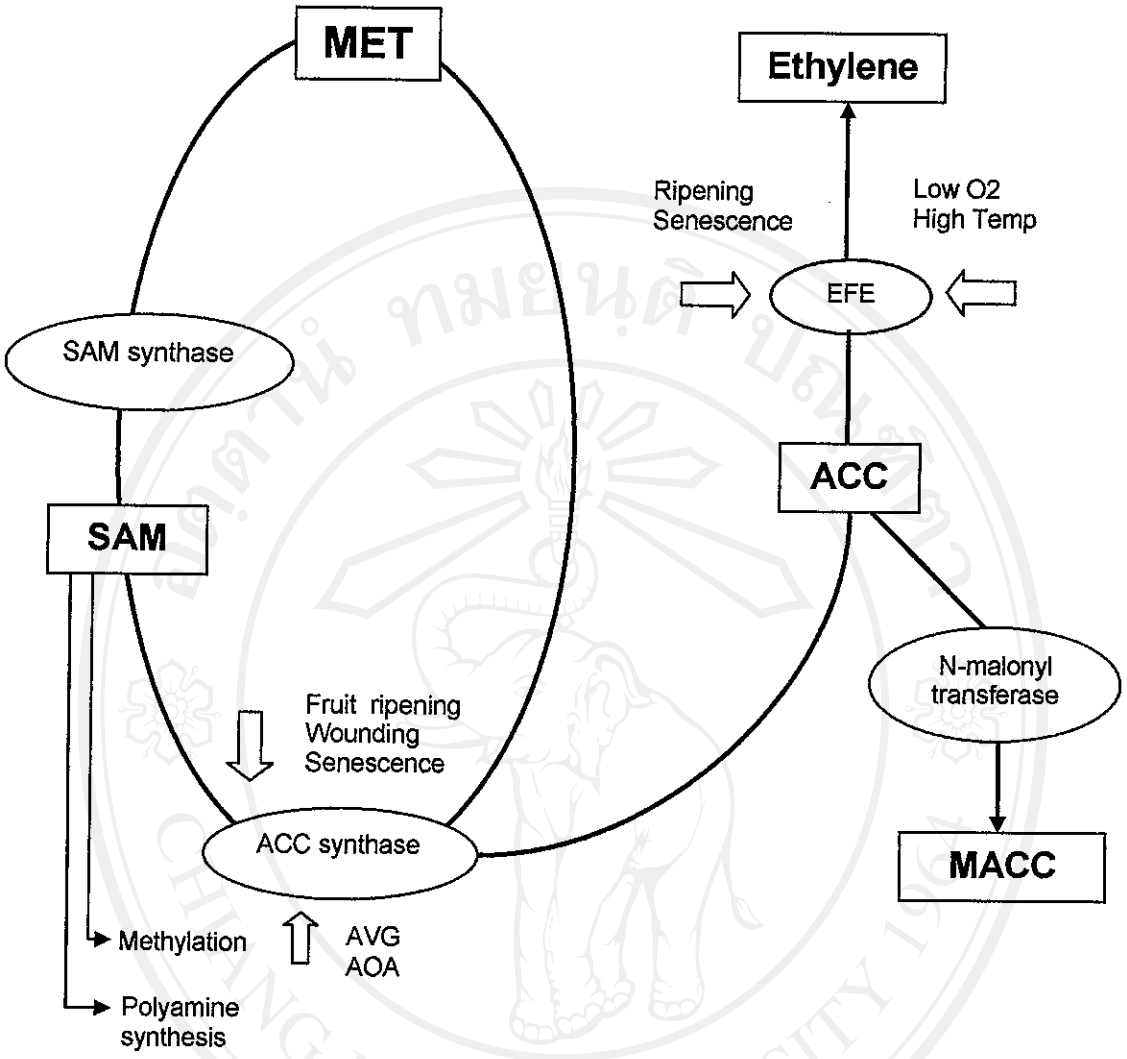

Figure 2.2 Pathway of ethylene biosynthesis (Yang and Hoffman, 1984)

2.4.5 Texture changes

Many fruits show soften character during ripening and this is the quality that dictates shelf life. Fruit softening may arise from loss turgor or breakdown of the fruit cell walls. Loss turgor is largely a non-physiology process associated with the postharvest dehydration of fruit. Loss of water equivalent about 5-10% of a fruit's fresh weight will cause commercially unacceptable of the fruit (Gomez-Lim, 1997).

In generral, texture damage during the ripening of fruit is thought to be largely the result of cell wall degradation. The structural component of cell wall is 90-95% of

carbohydrate polymers, which can be grouped together as cellulose, hemicellulose or pectin. The major constituent of the cell wall is pectin, a mixture of polymer from sugar acids, such as D-galacturonic acid, which are connected by (α1-4) glycosidic link (Figure 2.3) (Heldt, 1997).

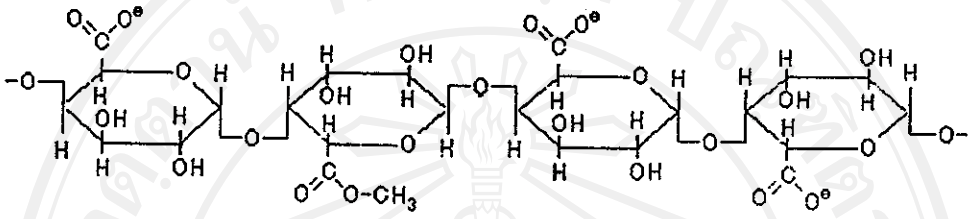

Figure 2.3 Constituent of pectin (Heldt, 1997)

Pectinmethylesterase (PME) removes the methyl group from the C-6 position of a galacturonic acid. Polygalacturonase (PG) hydrolase the α(1-4) adjacent to demethylated and galacturonic acid residue. These two enzymes can act synergistically with PME, generating site for PG action. However, the extent of this interaction in situ during ripening is unclear (Tuker, 1993).

Ripening of the mango fruit is characterized by softening of flesh. Softening is thought to be the result of by hydrolysis of various cell wall components. Fruit cell wall contains relatively more pectin material and less hemicellulose than other plant cell walls (Mitra and Baldwin, 1997).

Mango ripening causes by solubilization of pectin (Roe and Bruemmer, 1981; Lazan *et al.*, 1986 and Brinson *et al.*, 1988). The enzyme, polygalacturonase (PG) is responsible for pectin solubilization. There is a correlation between the increasing of PG's activity and solubilization of pectin. There is a report on the correlation between the loss of firmness and the increase of PG activity in mango (Tuker, 1993). PG has more active in degrading demethylated pectin. Pectinmethylesterase is a group of enzymes that catalyze deesterification of galactosyl uronate methyl esters of pectin to their free carboxyl groups. They are important factors during fruit ripening (Gomez-Lim, 1993).

2.5 Chilling injury

Temperature plays a key role in the metabolism of fruits and vegetables, as a low temperature decreases metabolism and prolongs the shelf life, refrigeration is therefore a common method used in postharvest technology to maintain the qualities of fruits and vegetables. Unfortunately, the tropical commodities face a serious problem during low temperature storage, because of the physical disorders induced by low temperature known as chilling injury (CI) (Parkin *et al.*, 1989, Wang, 1994).

There is difficulty to measured physiological or visual changes that are unique to chilling injured plant tissue (Morris, 1982). Chilling injury is not translocate (Eaks and Morris, 1957). Localized regions of injury, indicate distinct regions of tissue that are more susceptible than the surrounding tissue to chilling temperatures (Wang, 1990). The critical threshold temperature below which chilling stress occurs varies widely between fruit species. For example, banana fruit are injured by temperatures in the 12-13°C range; mango, 10-13°C; lime, muskmelon and pineapple, 7-10°C; cucumber, eggplant, and papaya, 7°C (Kays, 1991); lemon, below 1°C for more than 14-21 days (Hale *et al.*, 1976).

2.5.1 Mechanism of chilling injury

The event leading to chilling injury can be separated into primary event and secondary event. The primary cause of chilling injury is the membrane damage, the secondary cause may include ethylene production, increasing of respiration, interference with energy production, accumulation of toxic compounds (ethanol and acetaldehyde) and alteration of cellular structure. If the product is stored below the critical temperature for the short period, the plant can repair the damage, if the exposure is prolonged, the irreversible damage occurs and visible symptoms often result (Mitra *et al.*,1997; Wang, 1982; Will *et al.*, 1989; Neilson and Orcutt, 1996).

For many years, CI had been considered to be a consequence of the transition of a bulk lipid-phase of membrane occurring at a critical temperature that leaded to a complete loss of permeability control (Lyon, 1973). Evidence for the existence of a lateral phase separations came from the work of Platt-Aloia and Thompson (1987),

these authors reported the chilling induced lateral phase separations in the plasma membrane of avocado fruit, however, lateral phase separations might be reversible if the produce was removed from the critical surrounding before the occurrences of the lipid degradation and accumulation of lipid degradation products which induced irreversible membrane damage (Figure 2.4).

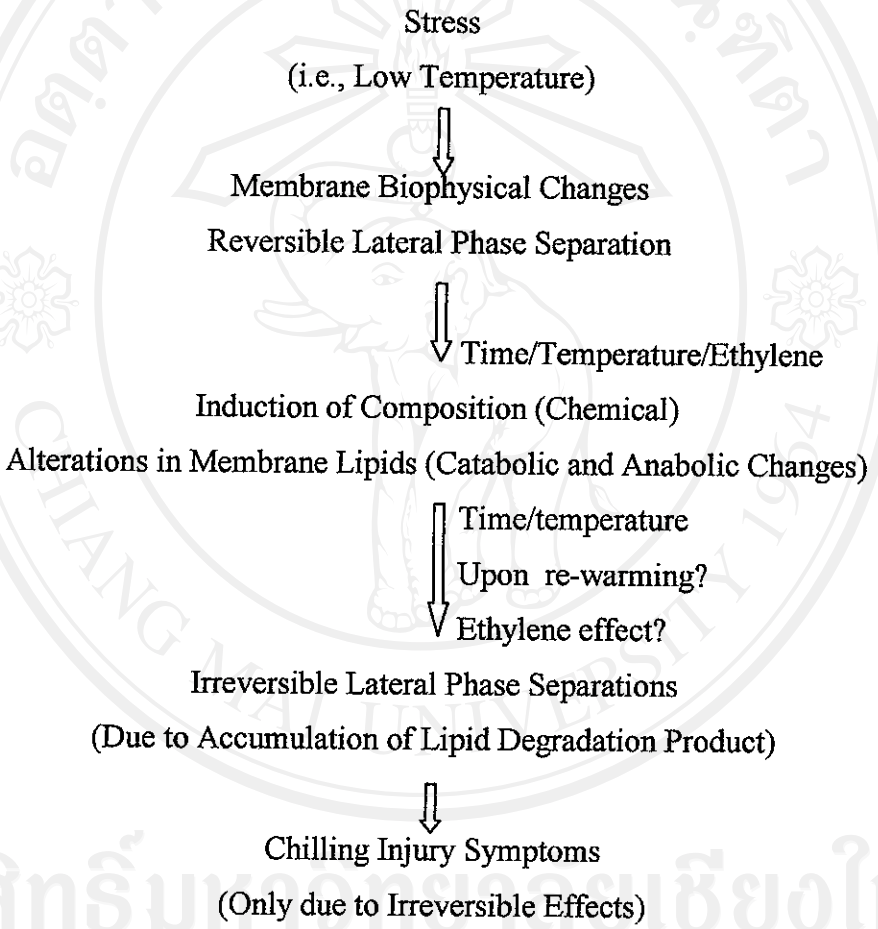

Figure 2.4 A sequence of metabolic events leading from a stress-induced alteration in the properties of membrane to observable macroscopic tissue damage (Marangoni *et al.*, 1996)

Marisela and Yahia (1995) used Differential Scanning Calorimeter to characterize thermograms of avocado and mango, and to relate with their sensitivity/tolerance to insecticidal atmospheres (<0.5%O_2 and/or > 50% CO_2), fresh and lyophilized tissues of fruits stored in air or in an insecticidal atmospheres were scanned at a temperature range of 10 to 145 °C at rate of 10°C /min, there were qualitative and quantitative differences between thermograms, there were fewer endotherms in thermograms of fresh tissue than in lyophilized tissues. Avocado thermograms showed a major endotherm at 15°C and two minor ones at 105-110°C. In addition, the mango thermograms showed another major endotherm at 80°C, which was not present in the thermograms of avocado, and might represent a contributing factor in the tolerance of mango to insecticidal atmosphere. The phase behavior of thylakoid polar lipids from oleander was studied by Differential Scanning Colorimetry (Raison and Orr,1986), it was shown that the calorimetric exotherm provided direct evidence of a thermally induced transition in leaf polar lipids of the plant at chilling temperature, the initiation of the transition was similar to the temperature below which injury developed in tomato plant.

Membrane peroxidation would appear to occur during chilling and might be responsible for the formation of irreversible lateral phase separations, in tomato fruit (Sharom *et al.*, 1994). Phospholipid hydrolysis, fatty acid peroxidation and break down to hydrocarbons would induce the formation of gel phase lipid that in turn lead to gel-phase formation (Paliyath and Droillard, 1992). Additional support for oxidation hypothesis came from the fact that antioxidation enzymes, superoxide dismutase, catalase (Spychalla and Desborough, 1990; Bruggemann *et al.*, 1999), superoxide dismutase (Sen-Gupta *et al.*, 1993) and antioxidants (α-tocopherol) (Spychalla and Desborough, 1990) are involved in preventing chill-induced oxidation of lipids, it was found out that after one week of chilling, there was an increase in the saturation index of tomato fruit (Palma *et al.*, 1995), this suggested an initial loss of unsaturation, followed by acclimation during chilling and induction of accelerated senescence after removal from the chilling stress (Marangoni *et al.*,1996).

One more support for the membrane damage hypothesis came from the observation that sensitive fruit could be acclimated, if they were exposed to non-

chilling low temperature prior to refrigerated storage, for example, acclimation of tomato (Maragoni *et al.*, 1990; Heureux *et al.*, 1994), zucchini squash (Wang *et al.*, 1992) and potato (Palta *et al.*, 1993) caused a compositional change in membrane lipids, a decrease in phase transition temperature, a rate of phospholipid degradation, a free sterol-phospholipid ratio, and an increase in unsaturation, all these changes demonstrated the relationship between membrane and CI (Marangoni *et al.*,1996).

Finally, in chill-resistant fruit such as apple, the changing permeability and microviscosity of membranes occurred near the beginning of cold storage at 0-5 C, paralleled with an increase in phospholipids content, this implied that a primary adaptive change to cold storage was an increase in phospholipids content (Lurie *et al.*, 1987), the increase in phospholipids as well as the increase in free sterols were also observed in apples by Bartley (1986).

All of the above informations indicated that the chilling injury could be considered as an accelerated senescence process since the process observed in chill-injuried fruit , such as ion leakage, lipid peroxidation, loss of phospholipid, increased of lipid saturation and apperance by score of chilling index, were the same as those discussed previously for senescence.

A generalized model for membrane injury as a result of chilling stress was presented in Figure 2.5, after exposure to low temperature, membrane lipids exhibited a modification of biophysical properties, probably resulting from enzyme action (Todd *et al.*, 1992), that could initiate the development of injuries, in the case of cold acclimation, membrane composition altered, in contrast, the biophysical properties would be maintained after exposure to the low temperature (Marangoni *et al.*,1996).

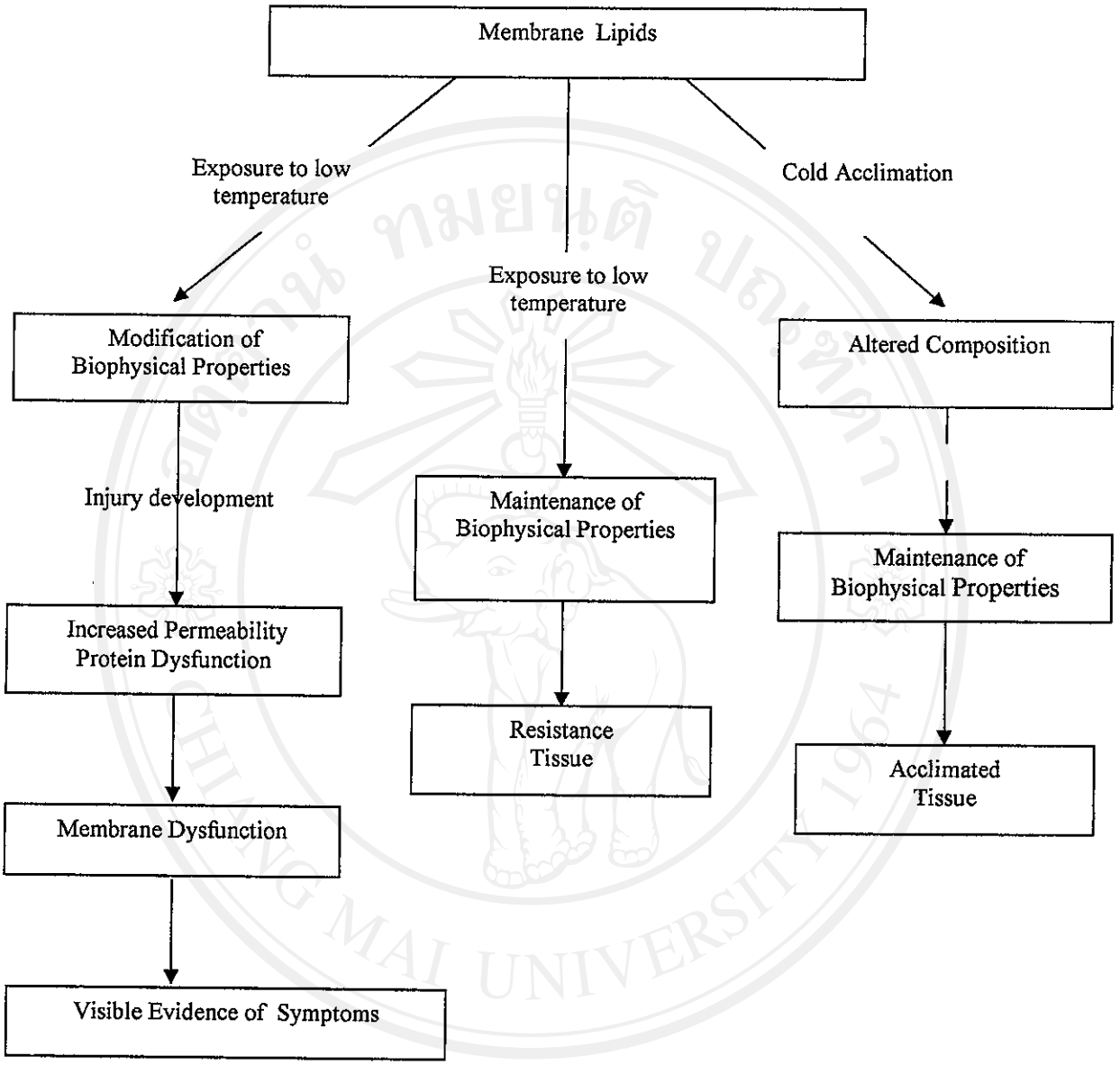

Figure 2.5 Membrane injury or acclimation resulting from chilling stress. (Shewfelt ;1992).

2.5.2 Symptoms of chilling injury

Symptoms of chilling injury generally develop after removal from the chilling temperature to non-chilling temperature, the development of symptoms is very slow and the symptoms are similar to those, which occur during other stress and injury. Some of more commonly occuring symptoms are listed below in successive order of their response time.

1. Surface lesion-pitting, large sunken areas, and discoloration.
2. Development of the symptom of pitting shows the important relationships that exist among the factors of commodity characteristics, the presence of mechanical damage, severity of chilling treatment, and the relative humidity of the atmosphere.
3. Surface lesion-pitting, large sunken areas, and discoloration.
4. Development of the symptom of pitting shows the important relationships that exist among the factors of commodity characteristics, the presence of mechanical damage, severity of chilling treatment, and the relative humidity of the atmosphere.
5. Water soaking of tissue – this disruption of cell structure and accompanying release of substrate, most favor the growth of microorganism.
6. Internal discoloration (browning) of pulp and vascular strands.
7. Breakdown of tissues.
8. Failure of fruits to ripen in the expected pattern under ripening conditions.
9. An accelerated rate of senescence, but with an otherwise normal appearance.
10. Increased susceptibility to decay, especially to organisms which are not usually found in healthy tissue.
11. Compositional changes, especially in relation to flavor and taste.

Altered metabolism and / or abnormal increase in a rate of production of carbon dioxide and ethylene occur sometimes during chilling, and very often after the chilled tissue being placed at warmer (non chilling) temperature (Moris, 1982).

2.5.3 Physiological and biochemical responses of plants to chilling stress.

The physiological changes may be considered primary or secondary injury. The primary injury is the initial rapid response that causes a dysfunction in the plant, but is readily reversible if the temperature is raised to non-chilling conditions.

Secondary injuries are dysfunctions that occurr as a consequence of the primary injury and that may not be reversible. The characteristic visual symptoms are the consequence of secondary chilling injuries (Wang, 1982,1990, 1991).

Primary response

The primary stage of chilling injury results in an increase in cytoplasmic calcium $[Ca^{2+}]_{cyt}$, changes in the cytoskeleton, or an alterration of membrane lipids (Woods *et al.*, 1984; Minorsky, 1985; Mercer and Smittle, 1992). Lyons and Raison (1970) suggested that an alteration in the state of membrane lipids from a liquid-crystalline state to a solid-gel structure, mediated by changes in the permeability of membranes.

Secondary response

1. Stimulation of ethylene production: Ethylene production in a number of plants is stimulated by chilling temperatures (Cooper *et al.*, 1969; Ichii and Hamada, 1978; Sfakiotakis and Dilley, 1974; Vine *et al.*, 1968; Wang and Adams, 1980; Wang *et al.*, 1971). The key enzyme, ACC synthase, which was stimulated by chilling, has been found to be a soluble enzyme and not membrane-bound (Boller *et al.*, 1979; Yu *et al.*, 1979). Cabrera and Saltveit (1990) reported that increased production of ethylene and CO_2 production of cucumber fruit under chilling condition.

2. Changes in respiratory activity: Increase in respiratory rate by chilling temperatures has been reported in many chilling-sensitive crops, including citrus

fruits (Eaks, 1960), cucumbers (Eaks and Morris, 1956), snap beans (Watads and Morris, 1966), and sweet potatoes (Lewis and Morris, 1956). The respiratory response has been suggested to be used as an index to measure the extent of chilling injury (Eaks, 1980; Eaks and Morris, 1956; Watada and Morris, 1966). The sustained increase in the respiratory rate after prolonged chilling exposure might be indicative of the irreversible metabolic disturbance and accumulation of the oxidizable intermediates (Eaks, 1980).

3. Increase in permeability and solute leakage: Chilling has been shown to increase permeability of the plasmalemma membrane, as measured by increased solute or electrolyte leakage (Creencia and Bramlage, 1971; Guinn, 1971; Wright and Simon, 1973). Calcium and postassium contents is another factor that has been implicated in chilling effects (King and Ludford, 1983; Cohen *et al.*, 1994). Lyons (1973) suggest that chilling injury is the result of ultrastructural membrane changes that permit electrolyte leakage and ion imbalance in the cell. Cohen *et al.* (1994) reported that changes in electrical conductivity of the flavedo tissues, total electrolyte leakage, and K^+or Ca^{2+} leakage were all inadequate predictors of chilling injury, appearing only after chilling injury was evident. Loss of electrolytes from chilled leaf tissues of *Scindapsus pictus* also was positively correlated with increasing exposure to chilling and with the progressive development of chilling symptoms (Smith and McWilliams, 1978). Wright (1974) also reported that the cracking of the cell membrane could be enhanced by dehydration and that a water deficit was an essential prerequisite for chilling induced solute leakage. The electrical conductivity can describe ion movement across a membrane (Whitlow *et al.*, 1992).

4.Changes in phenolic compounds and browning enzyme: Phenolic compounds widely exist in fruits and vegetables. This group of compounds mainly include hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, and isoflavonoids (Barden *et al.*, 1997; Ahmet Ayaz *et al.*, 1997). Zhang *et al.* (2000) reported that litchi peel contained mainly flavan-3-ol monomers and dimers were major phenolic compounds representing about 87.0% of the total phenolic compound detected. Tissue browning is dependent upon the concentration of phenolic compounds, the activity of polyphenol oxidase (PPO), oxygen and the concentration of antioxidants

(Nicolas *et al.*, 1994; Kader, 2002). PPO and its substrates varies and changes markedly in fruits and vegetables (Mayer and Harel, 1991). PPO is activated by moisture loss from the fruit (Lu *et al.*, 1992; Su and Yang, 1996). There are few reports on the major phenolic compounds, substrate specificity and affinity of longan fruit PPO. Jiang *et al.* (1999) reported that good substrates for PPO in longan cultivar Shixia were pyrogallol, 4-methyl cathecol, and catechol. Rhee and Iwata (1982) observed the tissues of eggplant and found that chlorogenic acid appeared to be the major phenolic substances in the internal parenchymal tissue and vasuclar tissues. Polyphenol oxidase was found in the same tissues. Polyphenol oxidase has been widely studied in various fruits such as apple (Harel *et al.*, 1964; Janovitz-Kalapp *et al.*,1990; Amiot *et al.*, 1992), banana (Galeazzi and Sgarbieri, 1981), grape (Harel and Mayer, 1971), litchi (Tan and Li, 1984; Jiang *et al.*, 1997), peach (Wong *et al.*, 1971), pear (Rivas and Whitaker, 1973), plum (Liu *et al.*, 1994), and longan (Jiang and Li, 2001; Tian *et al.*, 2002). The longan peel contained higher amounts of phenolic compound than pulp. This data supported by Hsu and Chyn (1991) as there found the phenolic compound in longan peel and seed were acetonylgeraniin A and B, and gallic acid which is further oxidized by polyphenol oxidase. Degree of discoloration in longan varied, depending upon activity of PPO and phenolic substance (Prapaipong and Rakariyatham, 1990). It is possible that the polymerization of tannic acid caused peel hardness and discoloration to bar the effective gas and water movement. PPO activity in longan peel and pulp was related to phenolic substance. The activity of PPO in peel sharply increased while phenol content decreased during storage.

5. Cytological responses: Relatively few studies have been conducted on the cytological responses of plants to chilling. Abe and Ogata (Abe and Ogata, 1978) found swelling of mitochondria and partials degradation of tonoplast in parenchyma cells of chilled eggplant fruits before any symptoms of pitting appeared on the surface. Similarly, the swollen mitochondria and ruptured tonoplast have been shown in chilled sweet potatoes (Yamaki and Uritani, 1972). Electron micrographs from tomato cotyledon cells indicated that membrane alterations preceded other cellular changes during chilling exposure (Ilker *et al.*, 1979). Other cytological responses to chilling in tomato cotyledons were loss of cell turgor, vacuolization, reduction in

volume of cytoplasm and vacuolar protein bodies, general disorganization of organelles, and a general loss of cytoplasmic structure. Patterson *et al.* (1979) recently found that chilling has a very rapid effect on cycloplasmic structure. The ultrastuctural changes, such as the partial disappearance of tonoplast in the cells of eggplant (Abe and Ogata, 1978), sweet potato roots (Yamaki and Uritani, 1972), and water convolvulus leaves (Hirata *et al.*, 1987; Hirata, 1987) were observed by electron microscopy during low-temperature storage. The tonoplast controls the contact between phenolic substances in vacuoles and polyphenol oxidase in plastids such as chloroplast. Disappearance of the tonoplast by chilling stress would accelerate the oxidation of phenolic substances, and the oxidation products may impair the function of other organelles or membranes, thus leading to visible symptoms of chilling injury (Wang, 1990). The microstructure may also affect disease incidence due to the decreased resistance of the fruit to pathogens. Pan (1994) observed that the mesocarp cells were the first to turn brown, followed by the endocarp. This browning spreads over the entire pericarp surface, mainly in the epicarp and outer layers of the mesocarp in Chinese cultivar (Qu et al., 2001).

2.5.5 Factors affecting chilling injury of horticultural crops

There are many factors that affect the occurrence of chilling injuries on tropical crops, foremost among these is a genetic diversity among the species and the cultivars within a sensitive species, which causes difference in symptom expression, another factor involved is the stage of physiological development (Yamada *et al.*, 1994), for example tomato fruits in their mature-green stage are susceptible to disrupt and fail to proceed the normal ripening process (Kader, 1992; Wang, 1982).

2.5.6 Postharvest techniques for reducing chilling injury.

Chilling injury is a huge trouble which has to be solved by postharvest physiologist in order to extend the shelf life and quality retention in commodities. The obvious way to avoid chilling damage is to avoid chilling temperature. If chilling

could not be avoids, treatments should be developed either to increase the tolerance of the tissue before chilling or to reduce the development of injury symptoms (Bramlage, 1982).

1. Chemical treatment

A number of chemicals have been shown to be effective in reducing chilling injury, for example, postharvest treated with $CaCl_2$ solution (1-10%) has been reported to reduce incidence of chilling injury in apple (Scott and Wills, 1975), okra (Ilker and Morris, 1975), avocado (Chaplin and Scott, 1980) and cantaloupe (Luna-Guzman *et al.*, 1999). However, calcium treatment also led to increase in chilling injury of papaya (Chen and Paull, 1986). Other chemicals such as sodium benzoate, othoxyquin, and mineral oil have been shown to be effective in reducing chilling injury (Wang, 1994a)

2. Growth bio-regulators application

Many growth regulators affect many biochemical and physiological processes in plant tissues, the effect on these processes may in turn alter the chilling tolerance of tissue. Abscisic acid (ABA) had been studied in relation to chilling injury. ABA application reduced chilling injury of grapefruit (Kawada *et al.*, 1979) and Zucchini squash (Wang, 1991); polyamine and methyl jasmonate had been also reported to reduce chilling in zucchini squash (Kramer and Wang, 1989, Wang, 1994c; Wang and Buta, 1994) and grapefruit (Wang and Buta, 1994).

3. Controlled atmosphere (CA) storage

Beneficial effect of controlled atmosphere in reducing chilling injury have been demonstrated in avocado (Spalding and Reeder, 1975; Pesis *et al.*, 1994), cucumber (Wang and Qi, 1997) and apple (Chu, 1999; Wang and Dilley, 1999), however, CA storage had no effect on reducing CI in some crops such as lemon, tomato (Wang, 1994a) and papaya (Chen and Paull, 1986), therefore, the efficiency of controlled atmosphere in ameliorating chilling injury symptoms is dependent upon the commodities, the concentration of O_2 and CO_2, duration and temperature of the treatment (Wang, 1982).

4. Waxing and packaging

Waxing and packaging have been applied to the surface of fresh fruits and vegetables, these methods had been reported to decrease the expression of chilling injury in grapefruit, chilling injury was also reduced by waxing in valencia, temple oranges (Davis *et al.*, 1973) and papaya (Chen and Paull, 1986), and by wrapping in cucumber (Wang and Qi, 1997) and mango (Pesis *et al.*, 2000). These methods were reduced chilling injury by modification the internal concentration of O_2 and CO_2, it was also reduced water loss (Morris, 1982), however, they had been shown to increase chilling injury in lime (Wardowski *et al.*, 1973).

2.6 Temperature management

2.6.1 Temperature preconditioning

Plant may be conditioned or hardened by exposure to a temperature slightly above the critical chilling range, for example, tomato seedlings conditioned at 12.5 °C for 3 hour were more resistant to subsequent chilling at 1 °C than seedling that were not conditioned. A 7 days exposure of grapefruit to 10 or 15 °C prevented or significantly reduced chilling injury during storage at 0 or 1 °C (Hatton and Cubbedge, 1982). Zucchini squash preconditioned at 10 or 15 °C for 2 days were found to suffer less chilling injury during subsequent storage at 2.5 or 5 °C (Kramer and Wang, 1989). Preconditioning papaya fruit for 4 day at 12.5 °C before storage for 14 day at 2 °C also reduced chilling injury (Chen and Paull, 1986), the decrease in chilling sensitivity in papaya with preconditioning treatment was thought to be associated with partial fruit ripening; allowing papayas to ripen at 24 °C has also been shown to decrease chilling injury during subsequent storage at 5 °C. Holding lemons for 3 days at 21 °C before storing at 1 °C for 21 days reduced chilling injury (McDonald *et al.*, 1985). Chilling injury of cucumber fruit was reduced by conditioning at 18 °C for more than 6 days prior to store at 5 °C (Hirose, 1985).

2.6.2 Intermittent warming

This method is the same as fluctuating or manipulating the temperature from low to high and then return back to low, one or more times for various periods of duration. Chilling injury can be avoided in many tissues, such as fruits, seeds and seedlings, if they are returned to warmer temperature before irreversible changes occur. Intermittent warming also shows potential in the storage of peach and nectarines, in peaches, control of chilling injury in the form of internal breakdown was obtained by removing the fruit to ambient temperature (23 to 25 °C) for 48 hours after 2 or 4 weeks stored at 0 °C. Peach and nectarines shifted to 0 °C after storage for 1 or 2 weeks at 5 °C developed less breakdown than those warmed for 2 days at 18.3 °C (Anderson and Penney, 1975). Chilling injury of grapefruit, stored at 0 and 2°C for 1 week and then removed to 21 °C for 1 day, and returned back to 0 °C for 2 weeks, could be lessened (Davis and Holfmanm, 1973). Moreover, intermittent warming showed potential in the storage of zucchini (Kramer and Wang, 1989), lemon (Cohen *et al.*, 1983; Cohen, 1988), cucumber (Cabrera and Salveit, 1990), tomato (Artes and Escriche, 1994), nectarine (Dawson and watkins, 1995) and mango (Vasanasong, 1988).

2.6.3. Heat treatment

Many chemical substances were developed effectively to control Insect diseases and physiological disorders of fruits and vegetables. At present, the consumers concern about chemicals used in postharvest treatment because they are harmful to human and environment (Lurie, 1997; Couey, 1989), therefore there should be other postharvest treatment that can be used to substitute the chemical treatment.

Postharvest heat treatment has been used for over century to disinfect, to control, to modify fruit responses to other stresses and to maintain fruit quality during storage (Armstrong *et al.*, 1989; Couey, 1989; Klein *et al.*, 1990; Paull, 1994a; Lurie, 1998; Mcdonald *et al.*, 1999), and it can also induce chilling tolerance in tropical

fruits and vegetables. Brooks and McCollum were the first who reported that pre-storage high temperature conditioning could reduce chilling injury in 1936, they found that by conditioning at 38 °C for 17 to 22 hours prior to store at 4.5 °C reduced pitting in grapefruit. Tomato that had been treated for 24 hours at 38 °C prior to 2 °C storage (Lurie and Klein, 1992) was later elucidated to involve heat-shock protein (Lurie *et al.*, 1996; Vlachonasis *et al.*, 2000), however, the same response was not observed in papaya (Paull, 1994b). For mango, each variety responsed differently to heat treatment, for example chilling injury in 'Keitt' was inhibited by pre-storage exposure to 38 °C for 24 hours (McColum *et al.*, 1993) but in 'Kensington Pride', the heat treatment employed (38-40 °C for 14 hours alone or followed by 10 min dip in 46-48 °C water, 10 min exposure to 46-48 °C hot air) did not cause substained reduction in chilling injury at 5 °C, although rate of ethylene production and physiological weight loss were reduced (Nair *et al.*, 2000). 'Nam Dok Mai' dipped in 50, 55, 60 °C water for 2 or 5 minutes before storage at 7 °C could reduced chilling injury (Angsooksri and Kalayanarat, 2003) which was the same as 'Nang Klang Wan' treated at 46° C for 15 min prior to cold storage, showed significant reduction in the chilling injury compared to non-heated fruits (Katawatchalakul, 2000), moreover, the heated mango could delay fruit ripening, the soluble solids, respiration rate, ethylene production, pectinmethylesterase activity and polygalacturonase activity were also decreased (Katawatchalakul, 2000; Angsooksri and kalayanarat, 2003; Yoo-a, 2003; Mitcham and McDonald, 1993; Ketsa *et al.*,1999) whereas the ripening of Philippine varieties 'Indian" and 'Carabao' or 'Super Manila' were not markedly affected (Acedo *et al.*, 2000). These contrasting results might be due to differences in treatment employed and in varietal response (Paull and Chen, 2000).

Transferring harvested fruit from ambient growth temperature to an elevated temperature induced stress, the severity of the induced stress is determined by both the temperature difference and the duration of exposure (Paull, 1994), for mango fruit, immersion in hot water at 42-49 °C had been reported to induce a range of external (lenticel spotting) and internal heat injuries in a number of cultivars (Table 2.3) (Jacobi *et al.*, 1996, 2001), skin damage, including skin scalding, lenticel damage and cavitation are commonly reported together with the retention of unripe, starchy areas

Table 2.3 Injuries to mango cultivars induced by disinfestation treatment, applied as hot water, vapor heat or forced hot-air treatments.

Mango cultivar	Heat injury	Heat treatment	Reference
'Tommy Atkins'	Darkend lenticels	HWT 46°C for 120 mins or HWT 49°C for 60 mins	Spalding *et al.* (1988)
'Keitt'	Darkend lenticels	HWT 46°C for 90 mins or HWT 49°C for 60 mins	Spalding *et al.* (1988)
'Kensington'	Skin scalding; uneven skin color development with ripening; starch retention in the form of layers and spots in ripe fruit; internal cavities	HWT 48°C for 7.5-30 mins	Jacobi and Wong (1992)
'Kensington', 'Irwin', 'Haden,'Tommy Atkins', 'Strawberry'	Skin scalding	HWT 48°C for 30-90 mins	Smith and Chin (1989)
'Kensington'	Internal cavities; starchy regions, retained in ripe mesocarp	HWT 47°C for 25 mins	Joyce *et al.* (1993)
'Keitt'	Internal cavities formed near the seed surrounded by hard unripe tissue	VHT 46°C for 3-4 h or VHT 48°C for 5 h	Mitcham and McDonald (1993)
'Tommy Atkins'	Peel pitting	FHAT 51.5°C for 125 mins	Miller *et al.* (1991)
'Carabao'	IB in inner mesocarp of ripe fruit; white starchy, tough lesion; fermented odour	VHT 46°C for 10 mins	Esguerra *et al.* (1991)

in the mesocarp as the fruit ripened. The severity and incidence depend upon mango variety, method of heat application and the level of stress suffered by the tissue (Jacobi *et al.*, 2001). A conditioning treatment of 40 °C for 8 hours before immersed in hot water at 48 °C to raise the fruit core temperature to 47 °C for 15 min was found to minimize heat injuries of 'Kensington' mango (Jacobi *et al.*, 2000). Previously, Joyce and Shorter (1994) had demonstrated that the severity of cavitation and the retention of starchy regions with heat-damage mesocarp of 'Kensington', could be reduced by a slow heating for 7 hours for the core to reach 37 °C. For avocado (Woolf *et al.*, 1995) and tomato (Hakim *et al.*, 1996), heat treatments in the range of 25-46 °C for 0.5-72 hours had also proved effective in raising heat tolerance.

There are three heating medium used in heat treatment, which are hot water, vapor heat and hot air. Hot water was originally used for fungal control, but has been extended to disinfestation of insects, vapor heat was developed specially for insect control, and hot air or forced hot-air heating has been used for both fungal and insect controls.

2.6.3.1 Hot water treatment (HWT)

Hot water treatments have been developed to disinfest mango fruit flies (Sharp *et. al.*, 1988, 1989a, b, c; Sharp and Picho-Martinez, 1990; Sharp and Spalding, 1984; Sharp, 1986, 1989; Nascimento *et al.*, 1992). In hot water immersion, the heat is transferred from water to the skin of the fruit, and from the skin through the flesh to the core, heat transfer from water to skin is faster than the skin to center, when fruit is immersed in hot water, the rate of heating of the skin and the outer mesocarp of the fruit is substantially faster than the one which is passed over by the air at the same temperature (Couey, 1989; Stewart *et al.*, 1990; Jordan, 1993).

In Central America and the United States of America, the commercial use of HWT for treating mangoes is widespread. Hot water dips for quarantine purposes usually consist of immersing fruits in water held at 43-46 °C, either in a batch or continuous process for 65-90 min depending on fruit size and shape; typically, cultivars with elongated fruit of less than 375 g in weight are heated to 46.1 °C for less than 65 mins, while cultivars weighing 375-570 g required 75 min to be heated to 46.1 °C (Anonymous, 1994).

2.6.3.2 Vapor heat treatment (VHT)

Vapor heat treatment is also referred as high humidity air heating. This process involves heating nearly saturated air and passing the air stream through the fruit, when the temperature of the mango fruit is at or below dew point of the air, condensation of atmospheric moisture occurs on the surface of the fruit, the heat from the fruit surface is transferred toward the fruit center by conductive energy transfer. (Jordan, 1993). Commercial facilities are operated in Okinawa, the Philippines, Thailand, The United States and Australia (Merino *et al.*, 1985; Unahawutti *et al.*, 1986; Armstrong, 1996; Heather *et al.*, 1997). The VHT disinfestations protocols are to heat the fruit core to 46-47 °C and held at these temperature for 10-30 min depending on cultivar and country of origin, for example 'Nam Dok Mai' of Thailand required 10 min holding time at 47 °C.

2.6.3.3 Forced hot-air heating (FHAT)

With forced hot-air heating, also known as non-condensing air heating, the heating of fruit is carried out by passing air held at a specified temperature through a bed of fruit. The heat transfer from the warm air to the fruit by convection via the skin, heat then transfer toward the center of the fruit, however, the transfer from the air to the skin is considerably faster than the transfer rate from the skin to the center of the fruit (Jordan, 1993).

The difference between VHT and FHAT is that the fruit surface treated by FHAT is dry, whereas the one treated by VHT has a moist surface (Armstrong, 1996). In an FHAT, the relative humidity of the air passed across the fruit can be as low as 30% and may fluctuate during treatment (Hallman and Armstrong, 1994), if the relative humidity is too low, fruit weight loss and shriveling may occurs, only papaya grown in Hawaii is treated by this mean before being exported to the mainland USA (Armstrong, 1996).

Compared with VHT and FHAT, HWT has a number of advantages which include a relatively ease of use by horticultural industries, short treatment time, reliable and accurately monitoring of fruit and water temperatures, plus the added benefits of killing surface decay organisms and cleaning plant exudates from

the fruit surface (Sharp, 1994), another important advantage of HWT from an economic point of view is that the cost of typical commercial system is approximately 10% that of a VHT system (Jordan, 1993).

2.7 Thermal properties

2.7.1 Specific heat

Specific heat (C_p) is one of the most important thermal properties associated with numerous food processing and engineering application. In food processing, engineers use Cp as a tool to design the process and equipment. In horticulture, C_p is used to calculate the heat load of fruits and vegetables for designing a refrigeration system.

Specific heat is a quantity of heat that is gained or lost by a unit weight of product to accomplish a unit change in temperature without change in state (Singh and Heldman, 1993), the unit of specific heat in SI unit is kJ/kg-°C. Polley *et al.* (1980) reported that the specific heat of apple (variety not specified) varied from 3,600-4020 J/kg °C with the moisture content between 75-80%. Alvarado (1991) developed a general correlation using 140 data points for apple pulp (no variety indicated) with three ranges of moisture fraction (wet basis) and found that the specific heat of apple pulp decrease exponentially from 3,640 to 2,680 J/ kg °C as the moisture fraction decreased from 0.876 to 0.497.

2.7.2 Relationship between specific heat and chemical composition

Specific heat of a food can also be predicted from its composition, there are several forms of equation for estimating specific heat of food as following:

Siebel (1982) cited in Stroshine and Hamann (1994): for food above freezing point

$$C_p = 0.837 + 3.349X_w \qquad (2.2)$$

Backstrom and Emblik (1965) cited in Dincer (1997): for food above freezing point

$$C_p = 1.20 + 2.990X_w \qquad (2.3)$$

Lamb (1976): for food above freezing point

$$C_p = 1.470 + 2.270X_w \qquad (2.4)$$

Leninger and Beverloo (1975)): for food above freezing point

$$C_p = \left(0.5X_f + 0.3X_s + X_w\right)4.180 \qquad (2.5)$$

Heldman and Singh (1981)): for food above freezing point

$$C_p = 1.424X_c + 1.549X_p + 1.675X_f + 0.837X_a + 4.187X_w \qquad (2.6)$$

Wang and Kolbe (1990): for fruit which moisture content between 0.25-0.90 at 0-90°C

$$C_p = 1.3767 - 0.003181T + 2.9293X_w \qquad (2.7)$$

Rapusas and Driscoll (1995) : for mango fruit which moisture content 0.81 at 10-40°C

$$C_p = 3.475 + 0.0051T \qquad (2.8)$$

Attapanyo (1995): for melon fruit which moisture content 0.886 at 10-45°C

$$C_p = 3.513 + 0.01T \qquad (2.9)$$

Where

X_f	=	mass fraction of fat
X_s	=	mass fraction of solids
X_w	=	mass fraction of water
X_c	=	mass fraction of carbohydrate
X_p	=	mass fraction of protein
X_a	=	mass fraction of ash

2.7.3 Thermal conductivity

Thermal conductivity provides a mean of quantifying the heat transfer properties of a solid material, the rate of heat transfer by conduction along a piece of material can be calculated from Fourier Law as (Singh and Heldman, 1993),

$$\frac{q}{A} = -k\frac{dT}{dx} \qquad (2.10)$$

Where

k	=	thermal conductivity, W / m-K or W / m-°C
q/A	=	heat flux (W/ m^2)
dT/dx	=	temperature gradient (K / m)

Thermal conductivity of biological materials is a function of cellular structure, composition and air content (Mohsenin, 1980), for most high-moisture foods, thermal conductivity is closed to that of water (Singh and Heldman, 1993). Thermal conductivity of a food is influenced by its composition, in the similar manner to C_p, there are many equations relating thermal conductivity to the chemical composition of food, some of the equations are:

Blackstrom and Emblik (1965) cited in Dincer (1997): for food above freezing point

$$k = 0.26 + 0.34X_w \tag{2.11}$$

Bowman (1970) cited in Lamb (1976): for food above freezing point

$$k = 0.056 + 0.567X_w \tag{2.12}$$

Comini et al., (1974): for food above freezing point

$$k = 0.26 + 0.33X_w \tag{2.13}$$

Choi and Okos (1983): for food above freezing point

$$k = 0.61X_w + 0.20X_p + 0.175X_f + 0.135X_a + 0.205X_c \tag{2.14}$$

Sweat (1994): for food above freezing point

$$k = 0.58X_w + 0.155X_p + 0.16X_f + 0.135X_a + 0.25X_c \tag{2.15}$$

Vagenas and Drouzas (1990): for fruit which moisture content between 0-0.96 and 0-80°C

$$k = 0.05 + 0.566X_w \tag{2.16}$$

Sweat (1974): for fruit and vegetables which moisture content >0.60 and 24-29°C

$$k = 0.148 + 0.493X_w \tag{2.17}$$

Vegenas *et al.*(1990): for fruit which moisture content between 0-0.96 and 0-80°C

$$k = 0.022 + 0.587X_w + 0.001924T \qquad (2.18)$$

Where

T = Temperature (°C)

2.7.4 Density

Density is one of the most important transport properties and so is widely used in process calculations. It is the unit mass per unit volume:

$$density = \frac{mass}{volume} \qquad (2.19)$$

SI unit of density is kg/m^3. There are three types of density in dealing with biological materials. Bulk density refers to the weight of mass of intact individual units of the material packed in a given volume by a specified method. This type of density includes the pore space within pack. Unit density or apparent density refers to the weight of each intact unit of the material divided by volume of the unit. This type of density includes the pore space within each unit of material. Finally, true density or solid density refers to the weight per unit volume of the solids within each unit of the material.

2.7.5 Thermal Diffusivity

The rate at which heat diffuses by conduction through a material depends on the thermal diffusivity and can be defined as:

$$\alpha = \frac{k}{\rho C_p} \qquad (2.20)$$

Where α is the thermal diffusivity (m^2/s), ρ is the density (kg/m^3), C_p is the specific heat (kJ/kg°C) at constant pressure and k is the thermal conductivity (W/m°C).

Thermal diffusivity of a food is influenced by its composition, in the similar manner to C_p and k, there are many equations relating thermal diffusivity to the chemical composition of food, some of the equations are:

Stroshine and Heldmann (1994)

$$\alpha = 0.146\times10^{-6}x_w + 0.100\times10^{-6}x_f + 0.075\times10^{-6}x_p + 0.082\times10^{-6}x_c \quad (2.21)$$

Riedel (1969) cited in Dincer (1997)

$$\alpha = 0.88\times10^{-7} + \left(\alpha_w - 0.88\times10^{-7}\right)x_w \quad (2.22)$$

Martens (1980)

$$\alpha = \left[0.057363x_w + 0.000288\left(T + 273\right)\right]\times10^{-6} \quad (2.23)$$

Chowdary (1988): for mango which moisture content 0.81 at 10-40°C

$$\alpha = 1.49x10^{-7} + 2.54x10^{-10}T \quad (2.24)$$

2.8 Heat transfer theory

Heat transfer is an operation that occurs repeatedly in the food industry. Whether it is called cooking, baking, drying, sterilizing or freezing, heat transfer is part of the processing of almost every food. An understanding of the principles that govern heat transfer is essential to an understanding of food processing.

Heat transfer is a dynamic process in which heat is transferred spontaneously from one body to another cooler body. The rate of heat transfer depends upon the differences in temperature between the bodies, the greater the difference in

temperature, the greater the rate of heat transfer.

Temperature difference between the source of heat and the receiver of heat is therefore the driving force in heat transfer. An increase in the temperature difference, increases the driving force and therefore increases the rate of heat transfer. The heat passing from one body to another travels through some medium which in general offers resistance to the heat flow. Both these factors, the temperature difference and the resistance to heat flow, affect the rate of heat transfer. As with other rate processes, these factors are connected by the general equation:

$$rate\ of\ heat\ transfer = \frac{driving\ force}{resis\tan ce} \qquad (2.25)$$

For heat transfer:

$$rate\ of\ heat\ transfer = \frac{temperature\ difference}{heat\ \ flow\ resis\tan ce\ of\ \ medium} \qquad (2.26)$$

During processing, temperatures may change and therefore the rate of heat transfer will change. This is called unsteady state heat transfer, in contrast to steady state heat transfer when the temperatures do not change. An example of unsteady state heat transfer is the heating and cooling of cans in a retort to sterilize the contents. Unsteady state heat transfer is more complex since an additional variable, time, enters into the rate equations.

Heat can be transferred in three ways: by conduction, by radiation and by convection.

In conduction, the molecular energy is directly exchanged, from the hotter to the cooler regions, the molecules with greater energy communicating some of this energy to neighbouring molecules with less energy. An example of conduction is the heat transfer through the solid walls of a refrigerated store.

Radiation is the transfer of heat energy by electromagnetic waves, which transfer heat from one body to another, in the same way as electromagnetic light waves transfer light energy. An example of radiant heat transfer is when a

foodstuff is passed below a bank of electric resistance heaters that are red-hot.

Convection is the transfer of heat by the movement of groups of molecules in a fluid. The groups of molecules may be moved by either density changes or by forced motion of the fluid. An example of convection heating is cooking in a jacketed pan: without a stirrer, density changes cause heat transfer by natural convection; with a stirrer, the convection is forced.

In general, heat is transferred in solids by conduction, in fluids by conduction and convection. Heat transfer by radiation occurs through open space, can often be neglected, and is most significant when temperature differences are substantial. In practice, the three types of heat transfer may occur together. For calculations it is often best to consider the mechanisms separately, and then to combine them where necessary (Earle, 1983).

2.9 Unsteady state heat transfer

Heat transfer is said to be steady state if the temperature at any point within a solid body does not change with time. If the rate of heat exchange between a solid product and its surroundings changes with time, the heat transfer is said to be transient or unsteady state and the temperature at any point and the heat content of the solid body both vary with time and distance. Heat transfer problems with time dependence are very common in engineering application. For examples, cooling or heating of particulate materials as in blanching and aseptic thermal processing and cooling or heating of food products. Calculation of unsteady heat transfer are complicated. It is convenient to express the variables in transient heat transfer as dimensionless groups. The initial and boundary conditions must also be specified.

Consider a product that is fresh harvested and exposed to a cold air flow. Heat is transferred by convection (and may be radiation) from its surface to the surroundings, conduction heat transfer takes place from the interior of the product to the surface, and its temperature at any point decreases until a steady state condition is reached. The numerical methods are the simplest equations to describe transient temperature distribution and heat transfer. Equations are:

Slab

$$\frac{\partial T}{\partial t} = \frac{k}{\rho C_p}\left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2}\right) \tag{2.27}$$

Cylinder

$$\frac{\partial T}{\partial t} = \frac{k}{\rho C_p}\left(\frac{\partial^2 T}{\partial r^2} + \frac{1}{r} \times \frac{\partial T}{\partial r}\right) \tag{2.28}$$

Sphere

$$\frac{\partial T}{\partial t} = \frac{k}{\rho C_p}\left(\frac{\partial^2 T}{\partial r^2} + \frac{2}{r} \times \frac{\partial T}{\partial r}\right) \tag{2.29}$$

where

T	=	temperature of product (°C)
t	=	time (sec)
x, y, z	=	distance from (m)
r	=	distance from radius (m)
k	=	thermal conductivity (W/m°C)
C_p	=	specific heat (kJ/kg°C)
ρ	=	density (kg/m3)

2.10 Finite Difference Method

The method consists in replacing each derivative in the equation by a discretization (usually truncated Taylor series). There are a lot of schemes, depending on the chosen discretization for each derivative. After the discretization, we can obtain explicit schemes - if there's no need to solve a system of equations, just to walk the grid nodes - or implicit if we have to solve a system of equations for each row of the grid.

Domain discretization have to be accomplished by means of quadrilaterals parallel to the X and Y axis. Usually quadrilaterals are of equal size.

If a finite differences scheme needs information of the n row to compute the $n+1$ row, it is called one step scheme. Those that need information about several rows, are called multi-step. A multi-step scheme using m steps needs the solution values in the first (m-1) levels, or they must be calculated using other method.

If the approximate solution that a method obtain converge to the true equation solution when the mesh spacing tends to zero, the scheme is convergent. A scheme is stable if the generated errors by the computation, such as the round or the truncation ones, vanish when the computation advances in the mesh. A scheme is consistent if the local truncation errors obtained when discretizing the Taylor series tend to zero when h, k and the elemental time interval tend to zero. The discretization error is a combination of the truncation error in the equation and the errors in the initial and boundary conditions.