THE EFFECTS OF HOT WATER TREATMENT ON KEY LIME (*Citrus aurantifolia*) IN CONTROLLING POSTHARVEST RIND DISORDER AND CHILLING INJURY INCIDENCE

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The effects of hot water treatment on key lime (Citrus aurantifolia) in controlling post harvest rind disorder and chillin
injury incidence / Fatin Fatma Mat Daud.

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By
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ENDORSEMENT

The project entitle THE EFFECTS OF HOT WATER TREAMENT ON KEY LIME (Citrus aurantifolia) IN CONTROLLING POST HARVEST RIND DISORDER AND CHILLING INJURY INCIDENCE
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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ABSTRACT

The beneficial effects of pre-storage hot water treatment (HWT) on post-harvest decay development has been shown in numerous temperate, sub tropical and tropical fruit, citrus fruit, vegetables and flowers. Key limes (*Citrus aurantifolia*) was treated with hot water dipping at 50°C and 55°C for 5 min and then stored at temperature of 5°C for 2 weeks and then for 1 weeks at 13°C for simulated shelf life to examine the use of hot water treatment (HWT) in controlling the post harvest decay incidence. HWT has no adverse effect on the physical and chemical properties of the limes (firmness, peel color, total soluble solid and pH value) during the storage. The percentage of decay incidence in treated limes at 50°C was low compared to the treated limes at 55°C and untreated fruit. The results confirmed that the HWT at 50°C is effectively reduce and controlling the decay incidence in key limes. HWT could be applied to the key lime (*Citrus aurantifolia*) in controlling the post harvest rind disorder and chilling injury incidence.

ABSTRAK

Kesan kebaikan rawatan air panas sebelum penyimpanan untuk ke atas pembentukan kerosakan selepas tuai telah ditunjukkan pada buah-buahan suhu sederhana, sub-tropika, tropika, sitrus, sayuran-sayuran dan juga bunga-bungaan. Limau nipis (*Citrus aurantifolia*) telah dirawat dengan rawatan rendaman air panas pada suhu 50°C dan 55°C selama 5 minit dan kemudiannya disimpan pada suhu 5°C selama 2 minggu dan dialihkan kepada suhu 13°C selama 1 minggu untuk merangsang hayat simpanan. Ini bertujuan untuk mengkaji keberkesanan rawatan air panas dalam mengawal kerosakan kulit dan kecedaraan pada suhu dingin pada limau nipis. Setelah berada di dalam penyimpanan selama 3 minggu, rawatan air panas didapati tidak memberi perubahan pada sifat fizikal dan kimia (kesegahan, jumlah pepejal terlarut dan nilai pH) pada limau nipis. Peratusan berlakunya kerosakan pada limau nipis yang dirawat pada suhu 50°C adalah rendah berbanding dengan limau nipis yang dirawat pada suhu 55°C dan limau nipis yang tidak dirawat (kawalan). Berdasarkan daripada keputusan kajian ini menunjukkan bahawa limau nipis yang dirawat dengan air panas pada suhu 50°C lebih berkesan untuk mengurang dan mengawal kerosakan kulit dan kecederaan pada suhu dingin. Rawatan air panas pada suhu ini sesuai untuk diaplikasikan ke atas limau nipis.

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°C	-	Degree celcious
g	-	gram
m	-	meter
mm	-	millimeter
cm	-	centimeter
Kj	-	kilo joule
s	-	second
%	-	percent
HWT	-	hot water treatment
CI	-	chilling injury

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background study

Positive effect of heat treatments on the storability of citrus fruit is well documented. Post harvest curing at 34 to 36°C for 48 to 72 hours controls effectively citrus decay and reduces its sensitivity to chilling injury (CI) (Del Rio et al., 1992). Shrink seal-packaging is essential for curing to protect the fruit from damage exerted by the high temperature (Ben-Yehoshua et al., 1987). However, the need to keep fruit for two to three days at high temperature complicates practical implementation of this method and compels a search for an effective short-term heat treatment to control both decay and chilling injury of citrus fruits. Hot water dip is one of the most easily-applied and environmentally safe fruit treatments. Its application for controlling brown rot of lemons was recommended by Fawcett in the first half of this century (Fawcett, 1936, pp. 419-420). In the eighties, hot water dip (49°C, 20 min) was tested as a possible means for quarantine treatment of grapefruit against Caribbean fruit fly (*Anastrepha suspensa hew.*), but exhibited a too high phytotoxicity (Sharp, 1985). To reduce the duration of quarantine treatment and thus prevent phytotoxicity, Gould (1988) proposed combining the hot water dip with subsequent cold storage of the fruit.

Wild and Hood (1989) reported that a short application of hot water (2 min, 53°C) or, even more effectively, hot thiabendazole(TBZ) (2-(4-thiazolyl)-benzimidazole; TE3Z) treatment reduces significantly the sensitivity of oranges (Wild and Hood, 1989) and grapefruit (Wild, 1990) to CI. According to McDonald et al. (1991) application of hot imazalil (1-[2-(2, 4-dichlorophenyl)-2(2-propenyloxy) ethyl] lH-imidazole) under the same conditions was even more effective than TBZ in reducing CI of grapefruit.

1.2 Problem statement

Citrus fruit are relatively non-perishable, and can normally be stored for long periods of 6 to 8 weeks. However, the development of various types of rind disorders limits the postharvest storage capability, and causes massive commercial losses. Post harvest rot and rind disorder are the major factor limiting the extension of storage life of many freshly harvested citrus fruit including *Citrus aurantifolia* cv. Key Lime. Thus export to many markets will require a disinfestations treatment. Heat treatment has been applied in other country such as Vietnam and Korea to reduce the infection and microbial loads in many fruit such as mango, apple, plum, stone fruit, cantaloupe and papaya.

1.3 Significance of Study

Locally, fruits are sold mainly for fresh consumption. The deterioration of physical appearance and damages due to disease attacks on fruits after keeping for a few days under ambient conditions could render losses in value and spoilage. These losses are costly to retailers for such a high valued fruit.

Citrus fruit are non-climacteric, with persistently low respiration and ethylene production rates, do not undergo any major softening or compositional changes after harvest and, therefore, can normally be stored for relatively long periods of 6 to 8 weeks (Kader, 2002). However, two major problems limit the long-term storage capability of citrus fruit: the first is pathological breakdown, leading to decay; the second is physiological breakdown, resulting in the appearance of various rind disorders. However, whereas it is practicable to solve the problem of decay development, either by the application of fungicides (Eckert and Ogawa, 1985) or by alternative environmental safe methods (Porat et al., 2002), there is not yet any reliable commercial method to alleviate the development of many kinds of rind disorder.

1.4 Objective of Study

This study is conducted to determine and observe the effects of hot water treatments on Key lime (Citrus *aurantifolia*) in controlling post harvest rind disorders and chilling injury incidence.

CHAPTER 2

LITERATURE REVIEW

2.1 Key lime (*Citrus aurantifolia* Swingle)

Limes (*Citrus aurantifolia*) are the fruit of tropical citrus tree closely related to lemons. This evergreen tree is in the Rue family, Rutaceae, which also includes citrus fruits such as oranges, lemons and kumquats. Limes are native to Southeast Asia, and probably originated in Indonesia or Malaysia. They made their way to the eastern Mediterranean with the Arabs, and to the western Mediterranean, with returning Crusaders, and eventually to the West Indies, when Columbus introduced citrus fruits there on his second voyage. These limes, used in most of the world, are what we call key limes. Key lime is the name used most often to refer to a primitive race of *Citrus aurantiifolia* cultivated and naturalized in the West Indies. It is also referred to as Mexican lime, West Indian lime, lima, limón criollo, limón agria, limón boba, and citron (Little and Wadsworth, 1964).

Key lime is believed to be native of eastern Malaysia. It was introduced to the Asian mainland early in historical times and carried by Arab traders to the Middle East and eventually came to Europe during the Crusades (Burkill, 1997). The species was introduced to the West Indies by Columbus during his second voyage (Ehler, 2002). Key lime has been planted throughout the tropics and has naturalized in at least Puerto Rico, the Virgin Islands (Little and Wadsworth 1964), and the Florida Keys (Nelson, 1996).

Key lime is an evergreen, spiny shrub or small tree to 6 m in height. The plant has single or multiple stems and irregular branches covered with smoothish brown to gray bark. The twigs are quadrangular (when young), green, and bare sharp axillary spines 3 to 17 mm long (Figure 2.1). The leaves are yellow-green to dark green, with 5 to 28mm winged petioles and elliptic to oval leathery 4 to 13cm long blades with edges that have minute rounded teeth. The crushed foliage has a strong, distinct, spicy (citrus) odor and taste. The four- to five-petaled white flowers occur in few-flowered axillary clusters. The fruits (hesperidiums) are ellipsoidal, 3 to 5 cm in diameter, have juicy, greenish-yellow flesh, and are yellow at maturity. They contain a few white, pointed seeds about 1 cm long (Liogier, 1988).

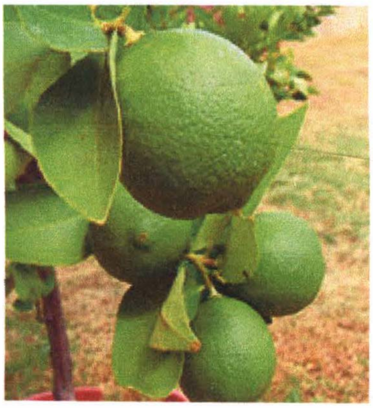

Figure 2.1: *Citrus aurantifolia*

Table 2.1: Nutritional values of lime

Nutritional Values					
Preparation	**Serving Size**	**Carbs**	**Fiber (g)**	**Fat (g)**	**Energy (kj)**
Lime (raw)	1 whole, peeled (45g)	0.5	1	0	40
Lime Juice	1 tablespoon	2	0	0	25

2.2 Hot Water treatment

Fruit and vegetables are an important source of carbohydrates, proteins, organic acids, vitamins and minerals for human nutrition. When humans use plants or plant parts, whether for food or for aesthetic purposes, there is always a post-harvest component that leads to loss (Kays, 1997). All fresh harvested commodities need to be free of disease agents, insects, and synthetic chemicals, and cleaned of any dirt or dust before being packed for export. The susceptibility of fresh harvested produce to post-harvest diseases increases during prolonged storage, as a result of physiological changes that enable pathogens to develop in the fruit (Eckert and Ogawa, 1988). However, since there are very few, or, in many cases, no registered post-harvest fungicides for control of decay-causing agents (How, 1991), post-harvest rot is the major factor limiting the extension of storage life of many freshly harvested fruit and vegetables. In a time of increased awareness among consumers that many of the chemical treatments of fruit and vegetables to control insects, diseases, and physiological disorders are potentially harmful to humans, there is an urgent need to develop effective, non-damaging physical treatments for insect disinfection and disease control in fresh horticultural products (Lurie, 1998). Although irradiation (Marquenie et al., 2002), use of materials that are generally regarded as safe (GRAS) (Larrigaudiere et al., 2002), hypobaric treatment (Romanzzi et al., 2001) or modified atmosphere packaging (Rodov et al., 2001), are non-pesticide technologies that are being investigated to extend the storage and shelf life of fresh produce, heat treatment appears to be one of the most promising means for post-harvest control of decay (Lurie, 1998).

The beneficial effects of pre-storage hot water immersion treatment (HWT) to prevent rot development has been shown in numerous temperate, sub tropical and tropical fruit, vegetables and flowers (Schirra et al., 2000). This treatment has a number of advantages which include relative ease of use, short treatment time, reliable monitoring of fruit and water temperatures and the killing of skin borne decay causing agents. (Lurie, 1998). However, the physiological responses of cultivars of different fruit or flower species to heat treatments can vary by season and growing location (Schirra et al., 1997). The reason for the variation in response between production regions may arise from differences in climate, soil type, season, production practices, and fruit maturity at harvest (Jacobi et al., 2001).

In the first decades of the 20th century, post-harvest 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 those (Lichter et al., 2000).

Post-harvest heat treatment has attracted recent research interest as a promising alternative to replace or to reduce the use of toxic chemicals during storage (Fallik, 2004), in the form of hot water dips, hot dry air, or vapor heat. Vapor heat treatment has been applied mainly for insect control, while hot air dry has been used for both fungal and insect control (Lurie, 1998a and Lurie, 1998b). Hot water treatments were first reported in 1922 to control decay on citrus fruit (Fawcett, 1922) but their use has been extended to insect disinfestations (Lurie, 1998b). Pre-storage heat treatments to control decay are often applied for a relatively short time (minutes), because the target pathogens are found on the surface or in the first few cell layers under the skin of the fruit or vegetable. Hot water is preferred for most applications since water is a more efficient heat transfer medium than air. In addition to hot water immersion, a new technology based on a brief hot water rinse and brush to clean and disinfect freshly harvested produce has been developed (Fallik, 2004). Post harvest heat treatments can also be used to induce fruit tolerance to cold temperatures and to reduce the development of chilling injury (CI) symptom during cold storage and cold quarantine (Schirra et al., 2004). Mitcham and McDonald (1993a) reported an increase in ethylene production as a response to heat stress. Increase in ethylene production might stimulate ripening processes. However, this stimulus could be limited because of the effects of elevated temperatures on the enzymes of the ethylene biosynthetic pathway (Paull and Chen, 2000).

Physical treatments to eliminate organism of quarantine concern, especially fruit flies (*Anastrepha fraterculus* and *Ceratitis capitata*) were developed as alternatives to chemical treatments, which face severe restriction, if not prohibition because of their negative environmental effects (Couey, 1989). For mangoes, hot water dips have been approved. These treatments allow mango shipments out of areas where fruits flies are endemic (Mitcham and McDonald, 1993b). The use of hot water as disinfestations treatment has spread because of its efficacy and the low incidence of damage to the treated fruit (Jacobi et al., 1995). Nevertheless, some peel disorders as well as quality losses have been observed (Jacobi and Wong, 1991). Among those is accumulation of starch grains in sub-epidermal tissues, probably resulting from heat deactivation of starch hydrolases (Jacobi and Wong, 1992). Negative effects on fruit color were also reported (Joyce et al., 1993). All these effects were more pronounced on mangoes harvested at the mature green ripeness stage than at later stages (Jacobi and Wong, 1992). Besides their use as quarantine treatments, hot water dips are also effective in the control of anthracnose, a critical postharvest pathogen that can cause severe losses in mango (Coates et al., 1993). To control postharvest pathogens, the use of higher temperatures has been recommended, though for shorter exposure times in order to avoid damage to treated fruit.

Hot water dip is one of the most easily-applied and environmentally safe fruit treatments. Its application for controlling brown rot of lemons was recommended by Fawcett in the first half of this century (Fawcett, 1936). In the eighties, hot water dip (49°C, 20 min) was tested as a possible means for quarantine treatment of grapefruit against Caribbean fruit fly (*Anastrepha suspensa hew.),* but exhibited a too high phytotoxicity (Sharp, 1985). To reduce the duration of quarantine treatment and thus prevent phytotoxicity, Gould (1988) proposed combining the hot water dip with subsequent cold storage of the fruit. Wild and Hood (1989) reported that very short application of hot water (2 min, 53°C) or, even more effectively, hot thiabendazole (2-

(4-thiazolyl)-benzimidazole; *TE3Z)* treatment reduces significantly the sensitivity of oranges (Wild and Hood, 1989) and grapefruit (Wild, 1990) to CI. According to McDonald et al. (1991) application of hot imazalil (1-[2-(2,4-dichlorophenyl)-2(2-propenyloxy)ethyl] lH-imidazole) under the same conditions was even more effective than TBZ in reducing CI of grapefruit. In this work, Wild and hood reported the evidence that short-term (2 to 3 min) water dips at 53°C may control effectively both CI and decay of various citrus fruits (grapefruit, oroblanco, lemon, and kumquat) during extended storage. Interaction of this treatment with sealing and with fungicide addition is considered. The effect of hot dips is compared with that of long-term heat treatment (curing) at different storage temperatures.

Heat treatments are also promising non-chemical means to control fruit pathogens. A number of post-harvest heat treatments, including curing at 36°C for 3 days, hot water dip for 2 min at 52°C and a hot water brush for 20 s at 62°C all were found to decrease both natural decay and chilling injury following 8 weeks of storage at 2°C (Porat et al., 2000). Furthermore, heat treatments have also been developed for insect disinfestations. A hot forced air treatment has been developed for use against Mexican fruit fly (*Anastrepha ludens* (Loew)) in oranges, tangerines and grapefruit (Mangan et al., 1998). The heating time varied depending on the size of the fruit and the temperatures were 45 to 46°C. This treatment is much shorter than cold quarantine (hours instead of days) and might be suitable to 'Oroblanco' fruits.

2.3 Post-harvest Decay

Post-harvest decay is the major factor limiting the extension of storage life of many fresh harvested commodities. All fresh fruits 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 post-harvest diseases increases during prolonged storage as a result of physiological changes that enable pathogens to develop in the fruits (Fallik, 2004). The concept of killing pathogenic fungal spores by heat treatment is not new. In the early 1930s, fruits 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 mould control (Lemessa et al., 2004). Heat treatments, however, not only affect the pathogen but can have beneficial effects on the fruit. Research in Israel has shown that if citrus fruit are held at 35°C in a humid environment (95-99% RH), mould infection does not occur and prevents decay (Fallik, 2004). This is due to the enhanced formation of lignin, which is a related compound that prevents invasion by mould spores. With citrus fruits, hot water dip treatments were reported to control post-harvest decay several decades ago (Smoot and Melvin, 1965).

2.4 Post-harvest rind disorder.

Generally, the various rind disorders in citrus fruit can be divided into two main groups: chilling injury (CI) that develop following storage at low sub-optimal temperatures; and other rind disorders, not related to chilling, that develop during storage at optimal non-chilling temperatures (Kader and Arpaia, 2002). Chilling damage in citrus fruit may appear in various forms, such as browning of the flavedo (the outer pigmented layer of the peel) (Figure 2.3) as in oranges, browning of the albedo (the inner white layer of the peel) as in lemons, appearance of dark sunken areas of collapsed tissue (pitting) (Figure 2.4) as in grapefruit, and 'watery breakdown' as in oroblanco (Porat, 2003). Other peel disorders, which are not related to chilling, include rind breakdown (Figure 2.2), stem-end rind breakdown (SERB), and the shriveling and collapse of the stem-end button that indicates aging. Several postharvest horticultural treatments, such as intermittent warming, application of heat treatments, high and low temperature conditioning, etc., have been developed to reduce chilling injury disorders in citrus fruit (Porat, 2003). Nevertheless, to the best of our knowledge, no commercial treatments are yet available to reduce the development of other rind disorders that occur under optimal, non-chilling temperature conditions.

Figure 2.2: Rind breakdown.

2.5 Chilling injury

Chilling injury is primarily a disorder of crops of tropical and subtropical origin, although certain physiological disorders will appear in temperate crops only when they are stored at low temperatures. Chilling injury is not the same as freezing injury, which is a result of damage from ice crystals formed in tissues stored below their freezing point. The minimum safe temperature for chilling sensitive commodities will be well above their freezing point. The critical temperature for chilling injury varies with the commodity, but it generally occurs when produce is stored at temperatures below 10°C to 13°C. Therefore, crops which are susceptible to chilling injury often have a short storage life as low temperatures cannot be used to slow deterioration and pathogen growth. Chilling injury may occur in the field, in transit or distribution, in retail or home refrigerators. The effects of short periods of chilling may be cumulative in some commodities. The primary cause of chilling injury is thought to be damage to plant cell membranes. The membrane damage sets off a cascade of secondary reactions, which may include ethylene production, increased respiration, reduced photosynthesis, interference with energy production, accumulation of toxic compounds such as ethanol and acetaldehyde and altered cellular structure. As plant structures differ in both susceptibility to damage and ability to repair these membranes, symptoms vary greatly between commodities.

Citrus fruits are susceptible to a series of physiological disorders of the epicarp and endocarp when they are stored at temperatures between 0°C and 15°C for longer than 3 to 4 weeks. The degree of sensitivity to chilling injury varies according to species and cultivar. The etiology of the disorders is still not clearly understood, however, a likely explanation is the is the cytological effect of chilling stress, causing and increase in cell and sub-cell membrane permeability until the membranes split with loss of electrolytes and metabolic distress due to changes in some enzymatic processes. The altered biochemistry gives rise to an accumulation of intermediate metabolites including ethanol and acetaldehyde. Over a certain concentration these become toxic and damage the cell structure, first reversibly then irreversibly (Lyon and Breidenbach, 1987). Chilling injury is thus correlated with the duration of the undesirable temperature, which would explain the reduced incidence of these injuries in fruits subjected to temperature cycles. As we know, chilling injury can lead to the post-harvest rind disorder. Chilling damage in citrus fruit may appear in various forms, such as browning of the flavedo (the outer pigmented layer of the peel) (Figure 2.3) as in oranges, browning of the albedo (the inner white layer of the peel) as in lemons, appearance of dark sunken areas of collapsed tissue (pitting) (Figure 2.4) as in grapefruit, and 'watery breakdown' as in oroblanco (Porat, 2003).

Figure 2.3: Browning of flavedo

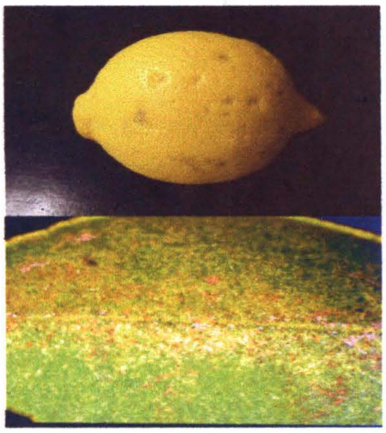

Figure 2.4: Pitting

Based on the previous study, heat treatments have been found to reduce the susceptibility of some fruits to chilling injury. This phenomenon has been reported in hot air-treated avocado (Woolf et al., 1995) tomato (Lurie and Klein, 1991) mango (McCollum et al., 1993) and persimmon (Cowley et al., 1992), and in hot-water-treated avocado (Woolf et al., 1996), cucumbers (McCollum and McDonald, 1993) and oranges (Wild and Hood, 1989). From the reported preliminary studies, suggest that chilling injury in cold-stored persimmons might also be inhibited by hot-water treatments, thus extending post-harvest life.

Previous studies on citrus fruit reported that post-harvest hot water dipping inhibited or reduced pathogen development (Rodov et al., 2000), improved fruit resistance to chilling injury (Schirra et al., 2004) and greatly increased the efficacy of fungicides applied in post-harvest treatments (Schirra and Mulas, 1995). However, information is scarce regarding the beneficial effects of post-harvest heat treatments on satsuma mandarin as an efficient pretreatment to improve its storage stability. In previous study, satsuma mandarins of an early harvesting cultivar were treated with hot water dips under varying conditions and then stored at low and ambient temperatures to examine the feasibility of such a heat treatment as an environmentally benign method to maintain commodity quality during post-harvest storage and marketing. Chilling injury can lead to many post-harvest decay in citrus fruit. This has been proved by several previous studies.

Symptoms of cold damage can appear after about 3-4 weeks of cold storage, varying according to species and stage of the disease. Cold pitting, pox manifest with round or irregular spots usually sunken, light brown becoming darker. It is most frequently seen in oranges and grapefruits but often appears also on other citrus fruit species. Another common symptom is oleocellosis (rind oil spot). This disorder, manifesting as darkening of the inter-glandular tissue, is found frequently in oranges and lemons

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

Key limes (*Citrus aurantifolia*) were purchased at Pasar Tani, Kota Bharu. The limes were in the maturity index of 2 depends on its color. The limes then were pre-cooled in refrigerator because it can not be immediately transfer to the laboratory due to the distance problem.

3.2 Methods

The fruits were randomly chosen and grouped into 3, which each group containing 3 replicates of key lime. Treatment include were control, hot water treatment at 50°C for 5 min and hot water treatment at 55°C for 5 min. Two water baths at different temperatures, 50°C and 55°C was set up and the temperature constantly monitored with thermometer. Limes in each of the group were then dipped into the water bath at 50°C and 55°C for 5 minutes. After the dipping, the limes were left at room temperature for 2 hours before packing into Low Density Polyethylene plastic (LDPE). The reason limes were left at room temperature for 2 hours is to cool down the limes before packaging and storage to avoid sudden increase in temperature that might lead to other mechanical and chemical properties damage. Similar procedure as above mentioned also prepared fro the control fruit. After packing, the limes were then stored in chiller at 5°C for 2 weeks. The observations were made on 0, 3, 6, 9, 12, 15, 18 and 21. Zero day is denote to before the heat treatments applied. After 2 weeks of storage in cool room, the limes were transferred to the ripening room at 13°C to stimulate the retail shelf life period of the limes.

3.3 Post-harvest parameter

3.3.1 Physical analysis

Fruit firmness was determined by a Texture Analyzer with a needle probe (P/2N). Lower deformation value will indicates higher fruit firmness.

Peel color was measured at three equally spaced sites of the marked fruit around the equator using a colorimeter (CR-200, Minolta Co., Japan), and average scores were recorded in terms of CIE-$L^* a^* b^*$, where L^* is the lightness, a^* is the changes of color from green to red and b* value is the changes of color from blue to yellow. The measurement will be taken before the heat treatment and after the storage. The samples of fruits from each treatment were marked for color measurements.

Decay incidence in each treatment was visually assessed in terms of rind disorder (rind breakdown) and chilling injury incidence (pitting, and browning of flavedo). Decay data were presented as average total percentage of fruit showing such decay and infection.

3.3.2 Chemical analysis

Chemical analyses were performed following the treatments and after the storage on fruits in each treatment. The fruit were crushed in a blender and filtered through several layers of gauze to extract the juice. pH of the juice were measured using a pH meter (MP-220, Mettler-Toledo Co., UK). Total soluble solid (TSS) in the juice was determined with a digital handheld refractometer (PR-32, Atago Co., Japan) at room temperature.

3.3.3 Statistical analysis

All data were subjected to the analysis of variance using SPSS software version 16. Treatments mean were further separated by Tukey test for least significant different at $P \leq 0.05$.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Firmness

Fruit firmness of key lime was not affected with hot water treatment (HWT) (Appendix A). There are no significant differences of changes in firmness within the treated or untreated limes. However all treatment showed a decreasing trend after been stored for 6 days at in storage (Figure 4.1). In general the firmness of the citrus fruit often depends on the turgidity and weight loss of the fruit itself (Hong et al., 2006). However the firmness of the key lime treated with hot water treatment at 55°C showed a higher dropping in the firmness on day 18 after the changes of temperature storage from 5°C to 13°C (Figure 4.1). It is because the transpiration and weight loss rate increased because of the warmer temperature (Cohen et al., 1994). So, when the transpiration rate of the citrus fruit increased the firmness of the citrus fruit will decreased. Mechanical damage will definitely occurs on the surface of severely chilling-injured citrus fruits. Thus, it will affect the rate of the weight loss due to the transpiration through the opening cell wall on the surface. In addition, decreased in fruit firmness may be ascribed to the decrease in weight loss. However the firmness of the citrus fruits depends on the type of cultivars.

Hot water treatment can either increase or decrease water loss and firmness of fruit, depending on the treatment and the commodity has been reported (Hong., 2007) . Hot water treatment on citrus fruit during storage has been reported in various fruit include an increased of weight loss in 'Fortune' mandarins (Schirra and D'hallewin, 1997) and blood oranges (Schirra et al., 2004) and a decreased of weight loss in kumquat and 'Marsh' grapefruit (Rodov et al., 1995). 'Valencia' oranges hot waterdipped at 45°C for 42 min became firmer, whereas the fruit at 53°C for 12 min showed an increased weight loss and decreased firmness (Williams et al., 1994). 'Oroblanco' treated with hot water at 53°C for 2 min had higher fruit firmness than controls also been reported (Rodov et al., 1995).

In the present study, the key lime treated with hot water at 55°C for 5 min resulted in higher firmness during storage at 5°C as compare to other treatments. However, key lime treated with hot water treatment at 50°C retained firmness after the storage temperature change to 13°C. Limes that treated at 55°C showed a decrease in rate of softening after day 6 of storage (Figure 4.1). The decrease in the rate of softening may be due to inhibition of the synthesis of cell wall hydrolytic enzymes such as polygalacturonase (Lazan et al., 1989) and a- and b-galactosidase (Sozzi et al., 1996). This inconsistency of the firmness in each fruit related to the different reaction of the fruits toward the hot water treatment. There are many factors related to such responses toward the hot water treatment. The response of a particular fruit to the heat treatment results from a combination of factors including the host, physiological age of the commodity, time and temperature of exposure, treatment methods, and storage temperature (Lydakis and Aked, 2003).

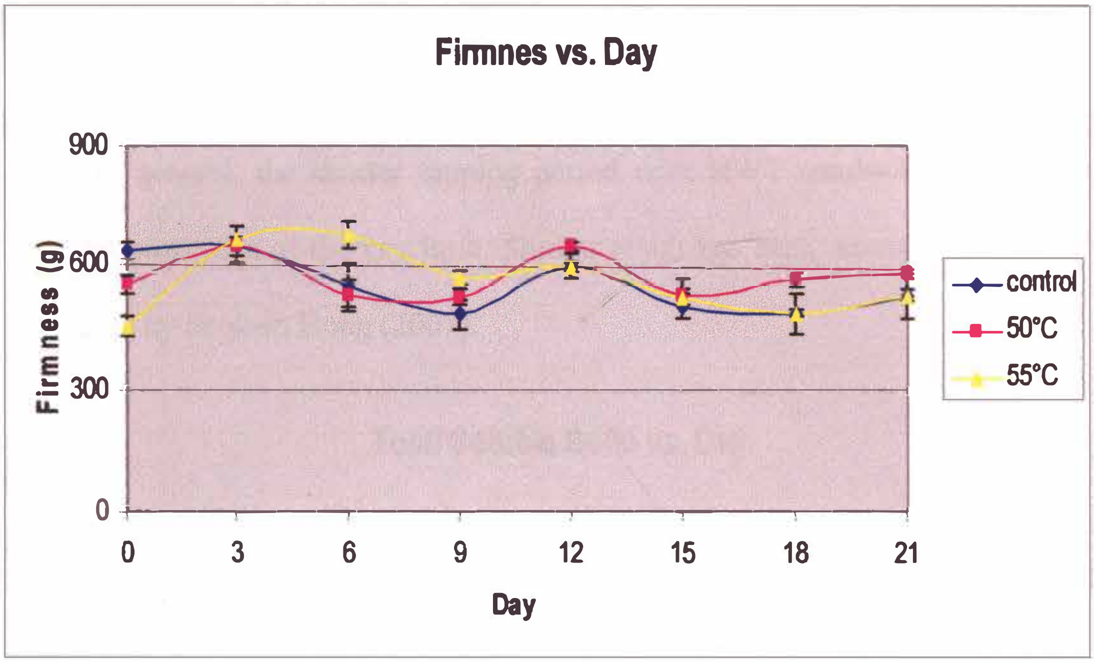

Figure 4.1: Changes of firmness in untreated and treated limes at 50°C and 55°C during storage. Vertical bars represent standard deviation (SD).

4.2 Chemical Properties of Key Lime.

Hot water treatment (HWT) did not significantly affect the chemical properties of the key lime including Ph (Appendix B) and Total Soluble Solid (TSS) during storage (Appendix C). However, TSS of key lime showed an increasing trend after been treated with HWT. TSS of treated lime at 55°C increased during day 9 of the storage period (Figure 4.2). The increasing trend of the TSS in the key lime maybe ascribed to the increased of solute concentration due to water loss (Hong et al, 2007) and also might be due to the conversion of starch into sugar (Kramer., 1983).

The pH value of the key lime treated with HWT at 50°C and 55°C showed significant differences only on day 9, 15 and 21 (Appendix B).

In general, the shorter dipping period with HWT results in no apparent on the chemical properties of the key lime. Similar result has been reported earlier on Satsuma mandarins by Seok-In Hong (2007).

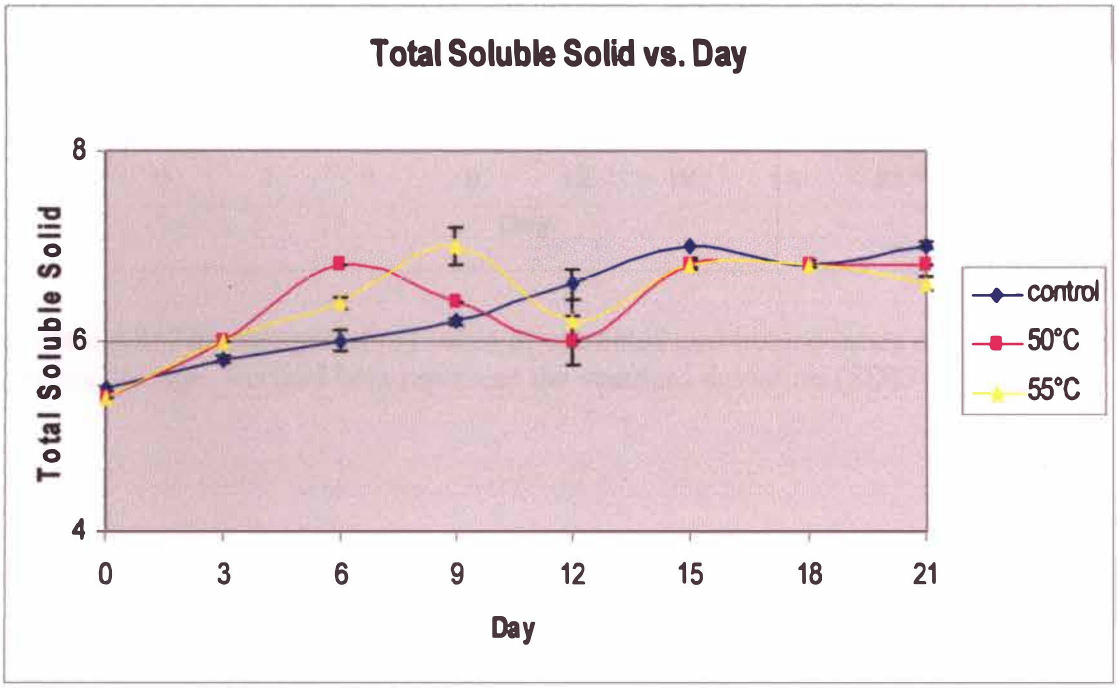

Figure 4.2: Changes of total soluble solid in untreated and treated limes at 50°C and 55°C during storage. Vertical bars represent standard deviation (SD).

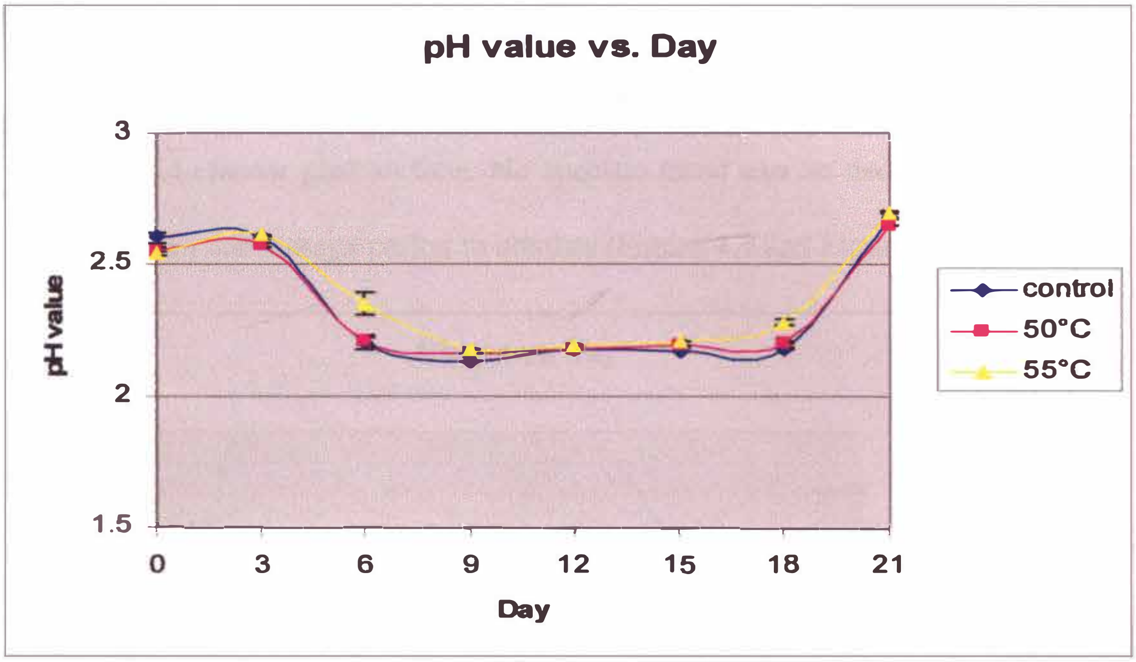

Figure 4.3: The changes of pH value in untreated and treated limes at 50°C and 55°C during storage. Vertical bars represent the standard deviation (SD).

4.3 Physical Properties of Key lime

There is no significant effect of changes in peel color of the untreated and treated key lime during the storage (Appendix E and F). Hot water treatment somehow did retain the greenish color of the limes until day 9 (Figure 4.5). The retention of green color could be attributed in part to inhibition of fruit senescence by heat shock (Rodov et al., 2000). Heat treatments disrupt ripening and inhibit ethylene formation in various fruits (Paull, 1990). In addition, heat can exert a direct inactivating effect on chlorophyll-degrading system. The enzyme chlorophyllase plays an important role in citrus fruit degreening (Amir-Shapira et al., 1987). There is also no significant effect in the lightness of the untreated and treated key limes (Appendix D). Even though there is no significant value in the peel color changes through the storage period, the treated limes with HWT showed a glossier and cleaner peel surface. No specific trend can be deduced as the peel color fluctuated from one storage period to another (Figure 4.4 and Figure 4.5).

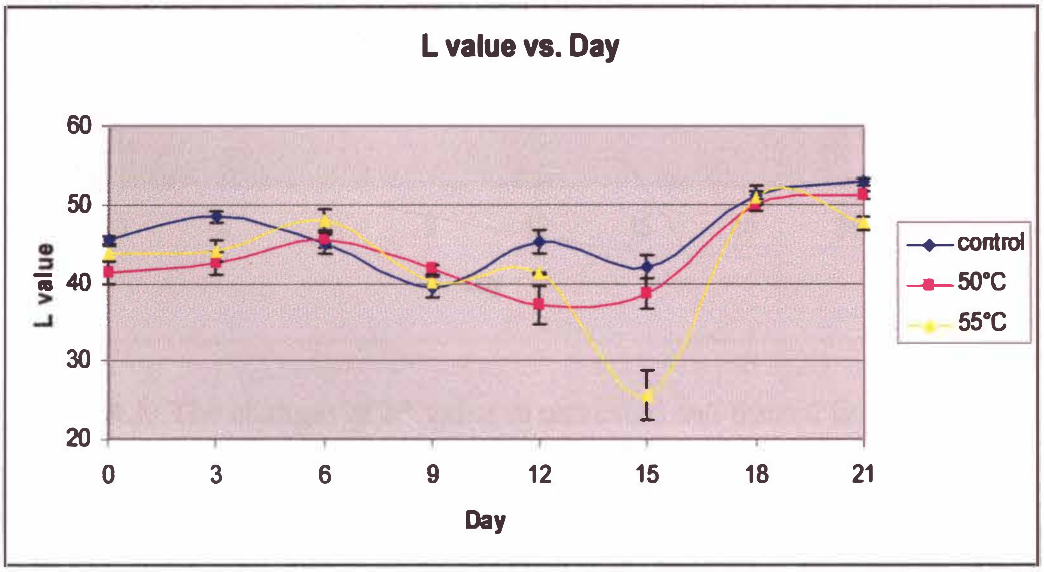

Figure 4.4: The changes of L* value in untreated and treated limes at 50°C and 55°C during storage. Vertical bars represent standard deviation (SD).

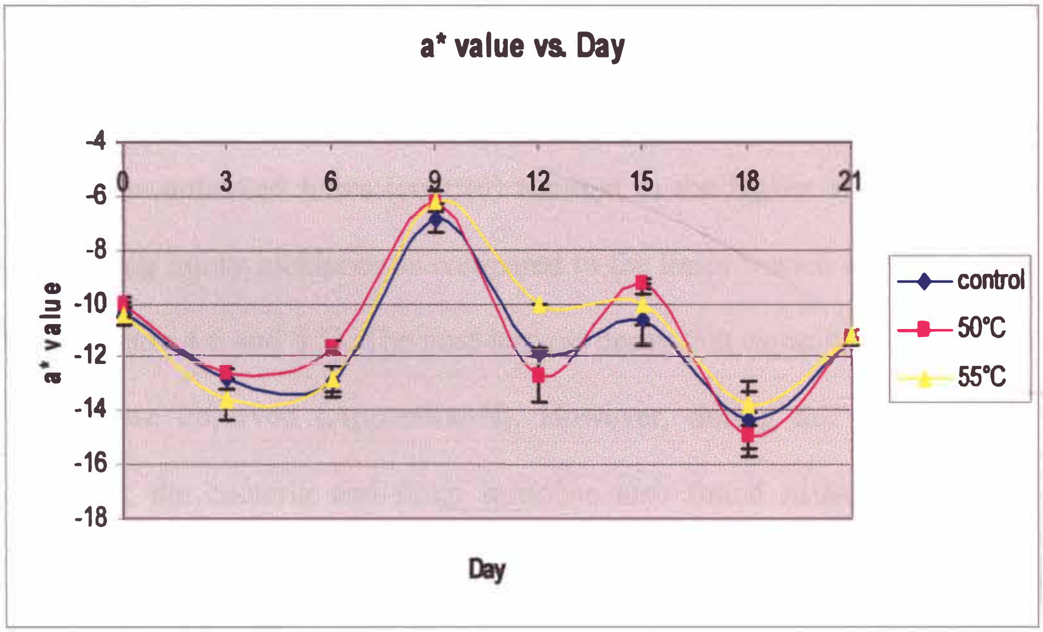

Figure 4.5: The changes of a* value in untreated and treated limes at 50°C and 55°C during storage. Vertical bars represent standard deviation (SD).

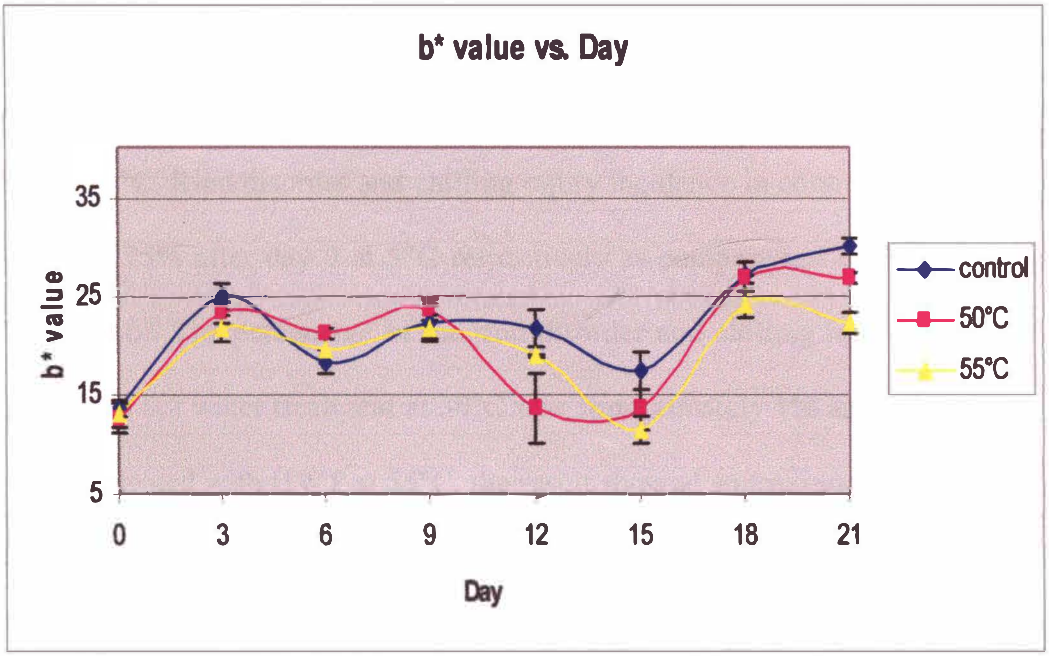

Figure 4.6: The changes of b* value in untreated and treated limes at 50°C and 55°C during storage. Vertical bars represent standard deviation (SD).

4.4 Decay incidence

The untreated limes (control) resulted in the higher appearance of rind disorder and chilling injury incidence as compared to the limes treated with hot water at 50°C and 55°C (Figure 4.6 and 4.7). The post-harvest decay that cause by the rind disorder, chilling injury were observed (Appendix H). However, during the observation of the decay incidence, the bacteria and fungi infection also found in treated and untreated limes (Figure 4.6). The decay and the infection start to develop on day 9. The untreated limes showed a high percentage of decay and infection followed by limes treated at 55°C. Following all those treatment, the decayed and infection increase throughout the storage period and are likely to increase more during the changes of storage temperature from 5°C to 13°C. Rind disorder and chilling injury incidence in control showed approximately 15% and 25% after day 9 at 5°C respectively as compared to other hot water treatment (Figure 4.6). The percentage of the rind disorder and chilling injury incidence in treated limes with hot water treatment at 50°C was approximately 1% and 3% respectively while in limes treated with HWT at 55°C, the result showed approximately 11% and 10% of the occurrence subjected to rind disorder and chilling injury respectively (Figure 4.6). In case of the bacteria and fungal infection after day 9, the untreated and treated limes with hot water treatment at 50°C and 55°C showed percentage of the bacteria and fungi infection, which is approximately 10%, 1%, and 3% respectively. Short-term hot water dips reduce sensitivity of cold-stored citrus fruit to chilling injury (Wild, 1990).

After day 15, the untreated and treated limes were then transfer to the cool room at 13°C. The observation was once again done on day 21. The percentage of the rind disorder and chilling injury in untreated limes was approximately 20% and 80% respectively (Figure 4.7). The occurrence subjected to bacteria and fungi infection was approximately 50%. In treated limes at 50°C, percentage of the occurrence of rind disorder and chilling injury are 3% and 5% respectively while 5% in bacterial and fungal infection (Figure 4.7). Furthermore, the percentage of the rind disorder and chilling injury in treated limes at 55°C approximately 35% and 30% respectively and the occurrence subjected to bacteria and fungi infection is 10% (Appendix G). Hot water treatment were found to reduce the sensitivity to the chilling injury in both treated limes at 50°C and 55°C compared to the untreated limes (control). Reduction in sensitivity to chilling injury by hot-water treatment has also been reported in avocado (Woolf et al., 1996), cucumbers (McCollum and McDonald, 1993) and oranges (Wild and Hood, 1989).

The reduction in decay development in post-harvest citrus treated with hot water treatment is considered to be mainly due to the host–pathogen interactions modulated by the treatments and partly to the reduction in the epiphytic microorganism population, compared to untreated fruit (Smilanick et al., 2003). The primary post-harvest pathogen of citrus fruit in many places is *Penicillium digitatum*, a wound pathogen. Wounds are mostly made and inoculated when the fruit are harvested and hot water treatment affects the control of the pathogen inside these wounds. The effects of hot water treatment on citrus fruit may be associated with melting and redistribution of natural epicuticular wax on the fruit surface, plugging numerous microscopic cuticular cracks and stomata to improve physical barriers to pathogen penetration (e.g., *Botrytis cinerea* whose spores can germinate and penetrate the surface of fruit) and transpiration (Lydakis and Aked, 2003). In fact, natural openings and barely visible cracks in the epidermis of treated fruit were partially or entirely sealed with rearranged natural wax components present on the cuticle, thus limiting sites of fungal penetration into the fruit (Fallik, 2004). This mechanism can prevent weight loss via transpiration and the development of decay and CI, and can potentially assist in maintaining the good appearance and taste in fruit.

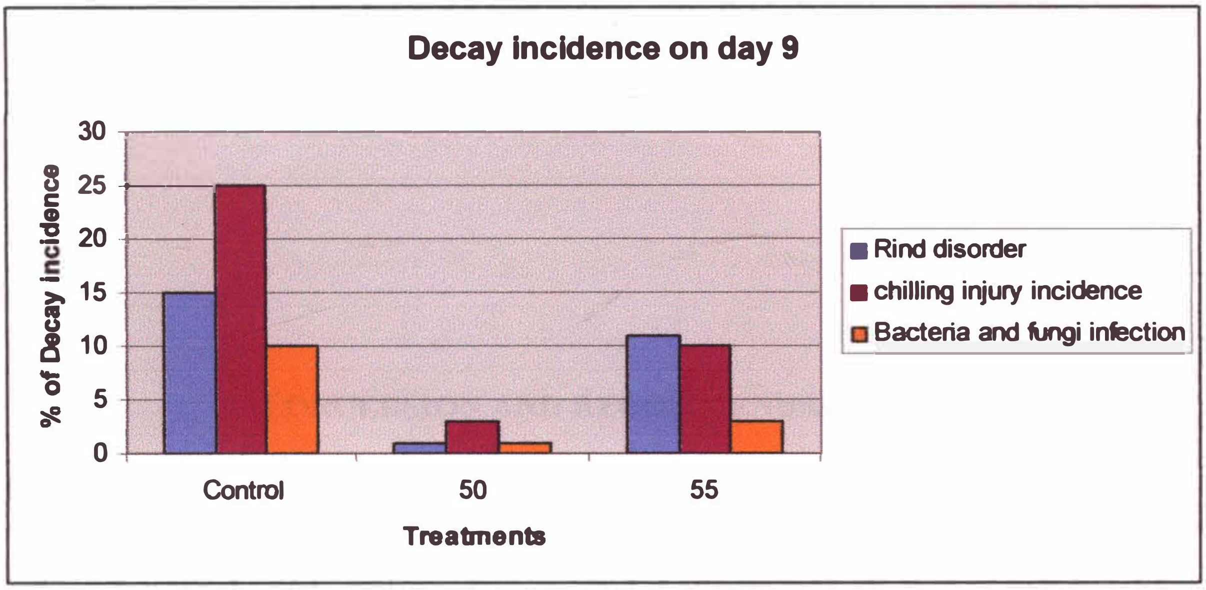

Figure 4.7: The incidence of post-harvest decay on day 9 affected by different hot water treatment.

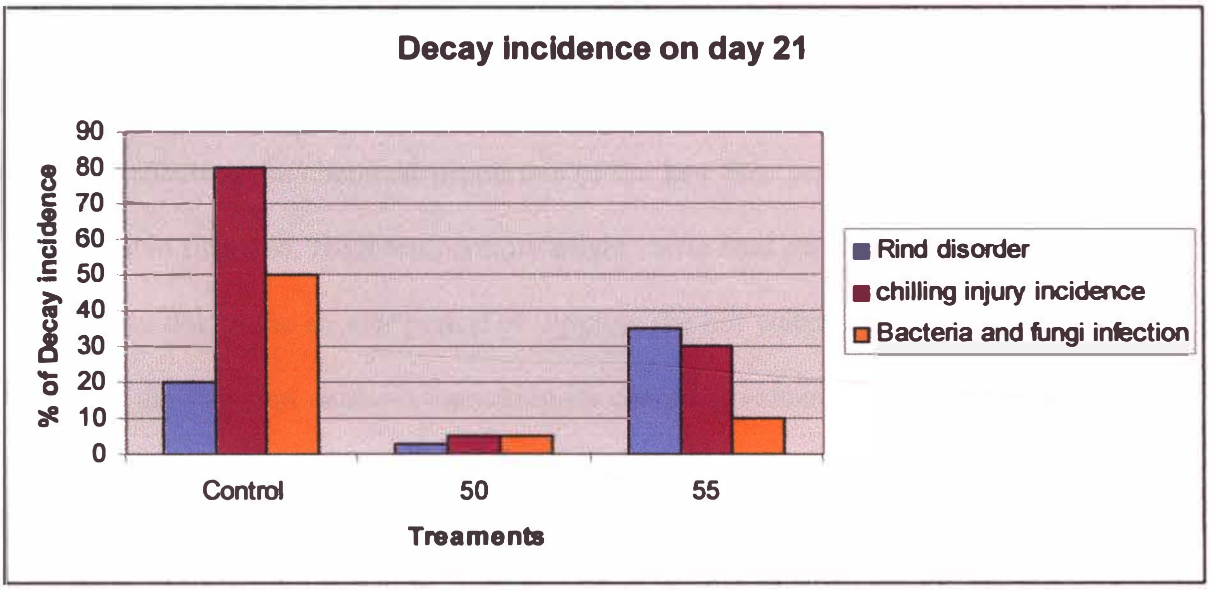

Figure 4.8: The incidence of post-harvest decay on day 21 affected by different hot water treatment.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the hot water treatment controls the post-harvest decay without adversely affecting the chemical properties of the key lime particularly pH, total soluble solid and also firmness. High temperature might cause heat damage to the sensitive citrus cultivars but due to the shorter period of dipping, the hot water treatments only affect the surface of the key lime without damaging its chemical values. Thus, the temperature of the HWT and the dipping period depends on the thickness of the citrus skin. The untreated key limes exhibit the highest percentage of occurrence subjected to rind disorder, chilling injury and bacteria/fungi infection while the key limes treated at 50°C exhibit the lowest percentage of decay incidence.

Therefore, hot water treatment at 50°C for 5 min can be recommended as an effective approach in reducing and controlling the post-harvest decay incidence in key lime.

5.2 Recommendations

This study can be further conducted in order to determine the type of the bacteria and fungi that affected the key lime.

In further study, hot water treatment with the combination of fungicide or other chemical can be used to determine the effect of heat treatment on key lime as a post-harvest decay. Many chemical that has been reported before in previous study has been use together with hot water to reduce post-harvest decay in citrus fruits. The application of hot water treatment and other chemical can be used to replace the use of fungicide that also has been reported on previous study.

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APPENDICES

APPENDIX A

Firmness of untreated and treated limes at 50°C and 55°C during storage.

Treatment	0	3	6	9
control	638.88 ± 67.1240^a	651.97 ± 147.8020^a	553.06 ± 165.6910^a	483.27 ± 115.6400^a
50	559.02 ± 72.5285^a	653.72 ± 70.3145^a	529.43 ± 118.9600^a	526.25 ± 64.5452^a
55	455.46 ± 72.9283^a	666.9 ± 111.9890^a	679.54 ± 98.2695^a	573.29 ± 49.0447^a

Treatment	12	15	18	21
control	604.21 ± 92.8679^a	504.29 ± 101.4270^a	484.36 ± 161.0180^a	532.81 ± 181.1760^a
50	651.82 ± 40.8143^a	527.77 ± 63.5333^a	573.12 ± 62.3209^a	589.46 ± 34.1951^a
55	601.96 ± 40.0208^a	523.99 ± 155.7110^a	487.23 ± 25.5397^a	554.54 ± 35.0659^a

APPENDIX B

pH value of the untreated and treated limes at 50°C and 55°C during storage.

Treatment	0	3	6	9	12	15	18	21
control	2.64±0.0529^{a}	2.61±0.0153^{a}	2.25±0.0643^{a}	2.14±0.0058^{a}	2.20±0.0173^{a}	2.17±0.0577^{a}	2.19±0.0116^{a}	2.69±0.100^{a}
50°C	2.59±0.0458^{a}	2.59±0.0265^{a}	2.26±0.0557^{a}	2.16±0.0000^{b}	2.19±0.0173^{a}	2.20±0.0116^{b}	2.21±0.0116^{a}	2.66±0.100^{b}
55°C	2.56±0.0265^{a}	2.62±0.0577^{a}	2.48±0.1358^{a}	2.18±0.0000^{c}	2.20±0.0577^{a}	2.21±0.0000^{b}	2.32±0.0404^{b}	2.71±0.005^{b}

APPENDIX C

Total soluble solid value of the untreated and treated limes at 50°C and 55°C during storage.

Treatment	0	3	6	9	12	15	18	21
control	5.57±0.0577^{a}	5.93±0.1155^{a}	6.40±0.3464^{a}	6.27±0.1155^{a}	7.07±0.5033^{a}	7.00±0.000^{a}	6.80±0.0000^{a}	7.13±0.115^{a}
50°C	5.40±0.0000^{a}	6.00±0.0000^{a}	6.80±0.0000^{a}	6.40±0.0000^{a}	6.73±0.8083^{a}	7.00±0.200^{a}	6.87±0.1155^{a}	6.80±0.000^{a}
55°C	5.40±0.0000^{b}	6.00±0.0000^{a}	6.60±0.2000^{a}	7.53±0.6110^{b}	6.87±0.7024^{a}	6.87±0.115^{a}	6.87±0.1155^{a}	6.87±0.230^{a}

APPENDIX D

L* value of untreated and treated limes during storage

Treament	0	3	6	9
control	47.03± 1.3551^{a}	50.66± 2.5800^{a}	48.14± 3.6983^{a}	43.75± 4.4962^{a}
50	44.53± 4.5431^{a}	47.17± 4.4487^{a}	47.84± 2.9193^{a}	44.36± 2.3191^{a}
55	46.27± 3.2200^{a}	48.49± 4.2274^{a}	51.16± 4.2012^{a}	42.95± 2.3791^{a}

Treament	12	15	18	21
control	50.58± 4.6976^{a}	47.38± 4.6980^{a}	54.38± 4.0072^{a}	56.64± 1.8243^{a}
50	46.14± 7.8227^{a}	43.58± 6.2727^{a}	51.94± 2.2448^{a}	52.62± 1.9005^{a}
55	41.87± 0.5357^{a}	37.20± 10.1077^{a}	52.62± 2.3028^{a}	50.74± 2.6938^{a}

APPENDIX E

a* value of untreated and untreated limes during storage

Treatment	0	3	6	9
control	9.88± 0.6260^{a}	11.62± 1.1853^{a}	10.96± 1.7954^{a}	5.79± 1.7305^{a}
50	8.94± 1.0710^{a}	12.23± 0.7015^{a}	10.76± 0.8733^{a}	5.75± 0.5118^{a}
55	9.27± 1.4339^{a}	10.83± 2.4641^{a}	11.15± 1.4445^{a}	5.11± 1.1610^{a}

Treatment	12	15	18	21
control	11.66± 0.2811^{a}	8.42± 3.0710^{a}	12.02± 3.3120^{a}	10.86± 0.4860^{a}
50	9.41± 3.1365^{a}	8.61± 0.6176^{a}	12.15± 2.6001^{a}	10.56± 0.8617^{a}
55	9.94± 0.1384^{a}	7.64± 2.4035^{a}	10.71± 2.7033^{a}	10.75± 0.5138^{a}

APPENDIX F

b* value of untreated and treated limes during storage.

Treatment	0	3	6	9
control	15.50± 2.2498^{a}	29.19± 4.1342^{a}	22.05± 4.1420^{a}	26.71± 5.8648^{a}
50	15.75± 4.6266^{a}	25.65± 3.2492^{a}	22.39± 1.5088^{a}	27.35± 3.3121^{a}
55	16.65± 3.9830^{a}	25.51± 4.3134^{a}	25.29± 6.9437^{a}	25.72± 3.6994^{a}

Treatment	12	15	18	21
control	28.03± 5.7006^{a}	22.82± 6.0085^{a}	30.61± 4.6994^{a}	31.74± 2.4660^{a}
50	25.91±11.0892^{a}	17.88± 6.2861^{a}	28.42± 1.4950^{a}	27.96± 1.7319^{a}
55	19.19± 0.2937^{a}	15.62± 4.2062^{a}	27.32± 4.5015^{a}	26.17± 3.4486^{a}

APPENDIX G

Observation of decay incidence on day 9

Lime at control temperature

Lime treated at 50°C

Lime treated at 55°C

APPENDIX H

Observation of decay incidence on day 21

Limes at control temperature

Limes treated at 50°C

Limes treated at 55°C

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Awards : Dean list (Dec 08/09)
Dean list (Jul 09/10)

THE EFFECTS OF HOT WATER TREATMENT ON KEY LIME (CUTRUS AURANTIFOLIA) IN CONTROLLING POST HARVEST RIND DISORDER AND CHILLING INJURY INCIDENCE - FATIN FATIMA BINTI MAT DAUD