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QUALITY, CHILLING INJURY, AND ANTIOXIDANT ENZYME ACTIVITIES OF *CARICA PAPAYA* L. CV. 'FRANGI' AFTER HOT WATER TREATMENT AND STORAGE

NASIM SHADMANI

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By

NASIM SHADMANI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Chairman: Siti Hajar Ahmad, PhD

Faculty: Agriculture

The newly introduced 'Frangi' papaya (*Carica papaya* L.) fruits are in demand worldwide. The fruits are highly perishable and susceptible to chilling injury and insect pest and disease infestations. Currently, there is a demand to reduce postharvest chemical insecticides and fungicides by using safe physical treatments such as heat treatments. Double-dip hot water treatment (hot water dip) has the potential to meet the above-mentioned demands for many countries. In addition, an optimum storage temperature may provide fresh and high quality papaya, despite the long transportation duration. There is a need to apply cost effective, sustainable and safe postharvest methods to extend storage and shelf life of the papaya fruits. The objectives of this study were to investigate the effect of heat treatment and storage temperatures on postharvest

quality characteristics, chilling injury, peel electrolyte leakage and antioxidant enzyme activities of Frangi papaya during three weeks of storage. The experiment was conducted using a randomized complete block design (RCBD), in a factorial arrangements of treatments (2 heat treatment levels x 2 storage temperatures x 4 storage duration), with three replications of three fruits per replication.

The fruits were double-dipped in hot water (30 min at 42 °C, 20 min at 49 °C) and stored at 6 and 12 °C for three weeks. Non hot water dip fruits acted as the control treatment. Physical and chemical quality characteristics, such as fruit color, weight loss, firmness, titratable acidity, pH, ascorbic acid, soluble solids concentration, and ethylene and carbon dioxide production rates, were measured at weekly intervals for three weeks. Chilling injury, peel electrolyte leakage and antioxidant enzymes (catalase, ascorbate peroxidase and superoxide dismutase) activities were also determined at weekly intervals during the three weeks of storage. The fruit quality characteristics such as color values, titratable acidity, ascorbic acid and soluble solids concentration were not significantly affected by double-dip hot water treatment. Treated fruits had a lower weight loss and higher firmness after week 2 of storage compared with control fruits. Ethylene production rate fluctuated during storage and was not inhibited by hot water dip at week 1 of storage. Fruits stored at 12 °C showed lower firmness and higher weight loss but normal peel colour progression compared to fruits stored at 6 °C. Inhibition of the normal ripening processes during the three weeks of storage at 6 °C was evidenced by the high pulp firmness and impaired peel color progression.

Double-dip hot water treatment alleviated the Frangi papaya chilling injury incidence. Peel and pulp chilling injury incidence were affected by hot water treatment. However, at weeks 1 and 2 of storage, peel chilling injury incidence was higher in hot water dip fruits compared with control fruits. The peel electrolyte leakage percentage of heat treated samples was significantly lower than control fruits. Hot water dip fruits which were stored at 6 °C had significantly lower peel chilling injury incidence than control fruits. It could be concluded that hot water dip was capable of reducing peel chilling injury at 6 °C storage.

The fruits characteristics at the molecular level were monitored at optimum storage temperature (12 °C) and chilling temperature (6 °C). Peel electrolyte leakage was significantly and positively correlated with catalase activity. The increase in peel chilling injury incidence resulted in the increase of catalase activity which functions to reduce the active oxygen species. The catalase activity was lower in treated fruits and a significant increase in catalase activity was observed in control fruits during week 2 of the storage. Ascorbate peroxidase activity was higher in hot water dip fruits.

Similarly hot water dip fruits stored at 12 °C had higher ascorbate peroxidase activity compared with control fruits. Frangi papaya stored at 12 °C during three weeks storage showed higher superoxide dismutase activity than 6 °C. The results of the present study are indicative of the fruits' active antioxidant defense mechanism in response to hot water dip. Thus, by the application of hot water dip on Frangi papaya, it is possible to capture a greater share of the international market and simultaneously maintain the fruits quality

characteristics, extend storage life and reduce peel and pulp chilling injury during a long storage period. As hot water dip is a physical treatment, the processes involved are environment friendly while fruits produced are safe for consumption.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KUALITI, KECEDERAAN DINGIN DAN AKTIVITI ENZIM ANTIOKSIDAN BAGI *CARICA PAPAYA* L. 'FRANGI' SELEPAS RAWATAN LEPASTUAI AIR PANAS

Oleh

NASIM SHADMANI

Ogos 2013

Pengerusi: Siti Hajar Ahmad, PhD

Fakulti: Pertanian

Carica papaya L. 'Frangi' merupakan varieti betik yang baru diperkenalkan di Malaysia, dan ianya mendapat permintaan yang tinggi di seluruh dunia. Buah ini cepat rosak dan mudah mengalami kecederaan dingin dan serangan serangga perosak serta penyakit. Bagi mengatasi masalah tersebut, rawatan lepastuai secara fizikal seperti haba lebih selamat digunakan berbanding rawatan secara kimia dengan menggunakan racun serangga dan kulat. Rawatan dua kali rendaman air panas merupakan keadah rawatan lepastuai yang sesuai digunakan bagi mengatasi masalah kecederaan dingin, serangan serangga dan penyakit. Di samping itu, suhu penyimpanan yang rendah diperlukan bagi memastikan buah betik sentiasa segar dan mempunyai kualiti yang tinggi sepertimana yang dikehendaki oleh pasaran luar negara, walaupun melalui tempoh pengangkutan panjang.

Disebabkan itu, kaedah lepastuai yang kos efektif, lestari dan selamat diperlukan untuk melanjutkan tempoh penyimpanan dan jangka hayat buah betik.Kajian ini bertujuan untuk mengkaji kesan rawatan rendaman air panas dan suhu penyimpanan ke atas ciri kualiti lepas tuai serta kecederaan dingin, kebocoran elektrolit pada kulit buah dan aktiviti enzim antioksidan bagi buah betik Frangi sepanjang tiga minggu penyimpanan. Eksperimen telah dijalankan menggunakan reka bentuk blok rawak lengkap, dalam susunan rawatan faktorial (2 tahap haba x 2 suhu penyimpanan x 4 tempoh penyimpanan), dengan tiga replikasi, tiga biji buah betik setiap replikasi.

Buah betik telah di rendam di dalam air panas (suhu 42 °C selama 30 min, diikuti dengan suhu 49 °C selama 20 min) dan disimpan pada suhu penyimpanan pada 6 °C dan 12 °C selama 3 minggu. Buah betik yang tidak direndam di dalam air panas bertindak sebagai kawalan. Ciri kualiti fizikal dan kimia, seperti warna kulit dan warna isi buah, kehilangan berat, kekerasan, asid tertitrat, pH, asid askorbik, kandungan pepejal larut, dan kadar penghasilan gas-gas etilena serta karbon dioksida, diukur pada setiap minggu selama 3 minggu. Selain itu, kecedaraan dingin, kebocoran elektrolit pada kulit buah dan aktiviti enzim antioksidan (katalase, askorbat peroksidase dan superoxide dismutase) dianalisis setiap minggu dalam tempoh 3 minggu penyimpanan. Ciri kualiti buah betik yang dinilai seperti warna kulit dan warna isi, asid tertitrat, asid askorbik dan kandungan pepejal larut adalah tidak memberikan kesan signifikan dengan rawatan air panas. Buah betik yang dirawat dengan air panas mempunyai berat yang lebih rendah dan kekerasan yang lebih tinggi selepas 2 minggu penyimpanan berbanding dengan buah betik kawalan. Kadar pengeluaran gas etilena berubah-ubah semasa penyimpanan dan tidak terganggu

disebabkan oleh rawatan air panas walaupun selepas seminggu penyimpanan. Buah betik yang disimpan pada 12 °C menunjukkan kekerasan yang lebih rendah dan kehilangan berat yang lebih tinggi tetapi mengalami perubahan warna kulit buah yang normal berbanding buah betik yang disimpan pada 6 °C. Proses peranuman buah betik yang normal berlaku dalam tempoh 3 minggu penyimpanan pada suhu 6 °C telah dapat dibuktikan dengan kadar kekerasan isi buah yang tinggi dan perubahan warna kulit buah yang berkurangan.

Rawatan rendaman air panas sebanyak dua kali terhadap buah betik Frangi dapat mengurangkan insiden kecederaan dingin. Rawatan ini dapat memberi kesan terhadap insiden kecederaan dingin pada kulit dan isi buah betik. Walaubagaimanapun, hasil kajian menunjukan bahawa simpanan pada minggu pertama dan minggu ke-2, insiden kecederaan dingin pada kulit dan isi buah betik yang dirawat dengan rendaman air panas adalah lebih tinggi berbanding dengan buah betik kawalan. Peratusan kebocoran elektrolit pada kulit buah betik yang dirawat dengan rawatan rendaman air panas adalah lebih rendah berbanding dengan buah betik kawalan. Buah betik yang direndam air panas dan disimpan pada suhu 6 °C mempunyai lebih rendah insiden kecederaan dingin pada kulit buah. Secara kesimpulannya, rawatan rendaman air panas mampu mengurangkan insiden kecederaan dingin pada kulit buah yang disimpan pada suhu

6 °C.Ciri buah betik pada peringkat molekul dipantau pada suhu penyimpanan optimum (12 °C) dan suhu penyejukan (6 °C). Kebocoran elektrolit pada kulit buah adalah sangat ketara dan berkolerasi secara positif dengan aktiviti katalase. Peningkatan dalam insiden kecederaan dingin pada kulit buah menyebabkan peningkatan aktiviti katalase, katalase

berfungsi untuk mengurangkan oksigen teraktif yang meningkat disebabkan oleh insiden kecederaan dingin pada kulit buah. Aktiviti katalase adalah lebih rendah dalam buah betik yang dirawat dan peningkatan paling ketara dapat diperhatikan pada buah betik kawalan yang disimpan dalam tempoh 2 minggu. Aktiviti peroxidase ascorbate adalah lebih tinggi dalam buah betik yang dirawat dengan rendaman air panas. Buah betik yang dirawat dengan rendaman air panas dan disimpan pada suhu 12 °C juga mempunyai aktiviti askorbat peroksidase yang tinggi berbanding dengan buah betik kawalan. Betik Frangi yang disimpan pada 12 °C selama 3 minggu penyimpanan turut menunjukkan aktiviti superoxide dismutase yang tinggi berbanding buah betik yang disimpan pada suhu 6 °C.

Keputusan kajian ini menunjukkan antioksidan menjadi aktif kerana ianya bertindak sebagai mekanisme pertahanan dalam tindak balas buah betik terhadap rendaman air panas. Oleh yang demikian, kaedah rendaman air panas terhadap betik Frangi boleh digunakan sebagai rawatan lepastuai untuk mengekalkan kualiti buah, memanjangkan hayat penyimpanan dan mengurangkan kecederaan dingin kulit dan isi semasa tempoh penyimpanan yang lama. Kaedah rendaman air panas merupakan rawatan lepastuai secara fizikal dan ianya adalah salah satu rawatan lepastuai yang mesra alam manakala buah yang dihasilkan daripada rawatan tersebut adalah selamat untuk dimakan oleh pengguna.

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I certify that a Thesis Examination Committee has met on 23 August 2013 to conduct the final examination of Nasim Shadmani on her thesis entitled "Quality, Chilling Injury, and Antioxidant Enzyme Activities of *Carica papaya* L. cv. Frangi after Hot Water Treatment and Storage" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Yahya bin Awang, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mahmud bin Tengku Muda Mohamed, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Azizah binti Osman, PhD
Professor
Faculty of Food science
Universiti Putra Malaysia
(Internal Examiner)

Elhadi Yahia, PhD
Professor
Autonomous University of Queretaro
Mexico
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 September 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

Siti Hajar Binti Ahmad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Nazamid Saari, PhD
Professor
Faculty of Food science
Universiti Putra Malaysia
(Member)

Phebe Ding, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NASIM SHADMANI

Date: 23 August 2013

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LIST OF ABBREVIATIONS

AA : Ascorbic acid

ACC : 1-aminocyclopropene-1-carboxylic acid

ACO : 1-aminocyclopropene carboxylic acid oxidase

AOS : Active oxygen species

APX : Ascorbate peroxidase

C* : Chromaticity

CA : Citric acid

CAT : Catalase

C_2H_4 : Ethylene

CI : Chilling injury

CO_2 : Carbon dioxide

RCBD : Randomized complete block design

DHA : Dehydroascorbic acid

EFE : Ethylene forming enzyme

FID : Flame ionization detector

FW : Fresh weight

GC : Gas chromatography

h : Hour

h^o : Hue

HA : Hot air

HPO_3 : Metaphosphoric acid

HSP : Heat shock protein

HT : Heat treatment

HWRB : Hot water rinsing and brushing

HWT : Hot water treatment

H_2O_2 : Hydrogen peroxide

kD : Kilodalton

L* : Lightness

LSD : Least significant difference

NaOH : Sodium hydroxide

ns : Non signifiant

O_2 : Oxygen

PAL : Phenylalanine ammonia lyase

PEL : Peel electrolyte leakage

POD : Peroxidase

PPO : Polyphenol oxidase

RH : Relative humidity

ROS : Reactive oxygen species

r^2 : Correlation coefficient

R^2 : Regression coefficient

SAS : Statistical analysis system

SSC : Soluble solids concentration

SOD : Superoxide dismutase

TA : Titratable acidity

TSS : Total soluble solid

VHT : Vapor heat treatment

CHAPTER 1

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most important tropical fruit crops, planted in tropical and subtropical countries. Because of its high nutritional quality, sweet taste and pleasant aroma it become popular all around the world. Papaya is planted commercially in more than 20 countries and the amount of total world papaya production is 11.8 million metric tons (Food and Agriculture Organization (FAO), 2011).

It is necessary to develop postharvest handling methods, due to the chilling susceptibility characteristics of tropical and subtropical fruits. Several postharvest methods have been reported to reduce chilling injury (CI) and decay incidence such as high temperature treatments, intermittent warming and radiation. The main high temperature treatments are: vapor heat treatment (VHT), hot water treatment (HWT), and hot air treatment (Fallik, 2004). Postharvest heat treatments (water or vapor) are considered as safe and non-polluting physical treatments and they are able to control the disease and insect disinfestations during the storage and marketing of fresh fruit (Lurie, 1998a). Heat treatments are proficient to reduce CI, diseases or insects and could affect the papaya fruit quality. Internal color, total soluble solids, weight loss, and β-carotene and lycopene concentrations did not change after heat treatment (Ali et al., 2000). A two stage hot water dip (42 °C water for 30 min, followed by 49 °C water for 20 min) was applied for disinfecting papaya from tephritid fruit fly eggs in Hawaiian papayas (Couey and Hayes, 1986). Fruits (at ripening stage 2) were immersed in hot water, and then hydro-cooled with water spray at room temperature (Couey and Hayes, 1986).

The effect of double dip hot water treatment on ripening is influenced by the maturity stage (20% of yellow skin) at harvest (Moy, 1993). Cold storage is the common storage method but several fruit varieties harvested at higher temperatures are normally CI susceptible during cold storage (Saltveit and Morris, 1990). The injury symptoms during cold storage are the reaction of many fruit varieties to temperatures at or near freezing. Nonfreezing temperatures below 10-12 °C caused CI of many tropical and subtropical fruit species (Saltveit and Morris, 1990). The CI symptoms are often revealed when the chilled products have been removed from cold storage to a warmer temperature (Paull and Chen, 1983). Common CI symptoms include scald or skin discoloration, skin desiccation, pitting, internal breakdown, uneven ripening, progression of large sunken regions, poor flavor, and poor color manifestation (Paull and Chen, 1983).

Physiological responses may appear as low ethylene production, impaired photosynthesis, acetaldehyde accumulation, ethanol, proteolytic enzymes activation, and programmed cell death after extended cold storage (Wang, 1990). The CI first appear in the fruit pericarp then induces a loss in marketing quality (Watkins et al., 1995). The CI symptoms are the results of oxidative stress in the tissues. The CI occur when active oxygen species (AOS) such as hydrogen peroxides, superoxide and hydroxyl radicals are in overload of the scavenging capacity in fresh tissue (Hodges et al., 2004). The role of antioxidant enzymes in regulation of AOS can be assessed by measuring the activity of guaiacol peroxidase and catalase (CAT) through postharvest storage (Sala, 1998). Pre-conditioning fruit treatments with hot water (Schirra and D'Hallewin, 1997, Fallik, 2004) probably induce chilling tolerance by modulating antioxidant organizations and avoiding the AOS accumulation (Sala and Lafuente, 2000).

In order to assess the effects of hot water dip on 'Frangi' papaya, fruits stored at 6 and 12 °C temperatures for three weeks. The activities of antioxidant enzymes including catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) were measured each week during three weeks of storage. The aim of this study was to evaluate the effects of storage temperature, storage duration and hot water dip on 'Frangi' papaya. Fruit quality parameters such as changes in color, weight loss, respiration, ethylene production, firmness, soluble solids concentration (SSC), ascorbic acid (AA), pH, titratable acidity (TA), CI incidence, peel electrolyte leakage (PEL) and antioxidant enzymes activities were evaluated during three weeks of storage.

CHAPTER 2

LITERATURE REVIEW

2.1 Papaya

Papaya (*Carica papaya* L.), a member of the *Caricaceae* family, is a herbaceous crop and widely cultivated in the tropical and subtropical countries like India, West Indies, Sri Lanka, Philippines, Malaysia, Bangladesh and other countries in Tropical America (Chan and Baharuddin, 2008). Papayas have been reported to originate in Nicaragua and South Mexico but now the papaya has spread extensively all over the tropics, mostly in Africa and Asia (Chan and Paull, 2008). The main commercialized papaya varieties around the world are 'Solo' in Brazil, 'Maradol', originally from Cuba and Mexico, 'Sekaki' also named as 'Hong Kong' and 'Eksotika' cultivated in Malaysia, and 'Khack Dum' from Thailand (Chan and Paull, 2008).

The major exporting countries are Mexico, Brazil and Belize, while European regions and the United Sates are the major papaya importers (Chan and Paull, 2008). Papaya grows all the year round, with a variety of sizes, peel colors and pulp softness (Fagundes and Yamanishi, 2001, Fuggate et al., 2010). Papaya is a valuable source of calcium and vitamins A, B_1, B_2 and C. In addition, it contains minerals like potassium, magnesium, copper, and boron (Paull et al., 1997, Wall, 2006). Amount of protein in papaya is relatively small (5%) and the nutritional characteristics of the fruit is related to the growing conditions, variety, and ripeness (Sankat and Maharaj, 1997).

Red flesh cultivars such as ‘Maradol’, ‘Pococi’, ‘SunUp’ and ‘Sunrise’, have more nutritional qualities related to the high lycopene level (1.4-3.7 mg/100 g), a kind of powerful antioxidant that protects cells from reactive oxygen species (ROS) (Gayosso-García Sancho et al., 2011). Papaya contains high amounts of phytonutrients and phytochemicals (vitamins C and E, carotenoids and polyphenols) (González-Aguilar et al., 2008b). In addition, papaya variety ‘Eksotika’ has the highest flavonoid content among all exotic Mauritian fruits (Luximon-Ramma et al., 2003). These immense nutritional characteristics made papaya a highly demanded fruit globally. The most important export constraint is related to the short postharvest life and disease susceptibility during transport and marketing. Physiological disorders cause limitations of fruit exportation, production losses and a negative economic impact on the papaya production chain (Campostrini et al., 2010).

Papayas are extremely perishable and, therefore, postharvest researchers concentrated on developing storage potentials and adequate fruit transport facilities to reduce market losses (Kechinski et al., 2012). These are requirements to maintain the fresh fruit quality characteristics during the market chain and also to reduce storage chilling injury (CI). Proper strategy is able to eradicate all the pathogens and inhibit additional infection (Conway et al., 2004). *Carica papaya* is an economically essential fruit grown in Malaysia with an export value of about RM100-120 million per year (Rabu and Mat, 2005). A new variety, an F1 hybrid named ‘Frangi’ commercially known as ‘Paiola’ has been planted for export. It has improved flavor and storage life compared to the Eksotika. It has good customer acceptance in domestic and international market such as

Singapore and London. Frangi has the potential to place Malaysia as a significant international papaya exporter, with an expected daily volume of 80 metric tons of fruit, especially during peak production of the crop (Chan and Baharuddin, 2008). By the use of alternative postharvest methods, it is possible to extend papaya postharvest storage life during the shipment and marketing (Ali et al., 2011). Measurement of physico-chemical characteristics, with regards to the structural changes, will expand the understanding of ripening process reactions. The reactions include physiological and biochemical alterations which change fruit composition (Pereira et al., 2009). Among the most apparent symptoms of papaya ripening are reduction of pulp firmness, chlorophyll degradation, and carotenoid synthesis that occurred in the fruit's skin (Lazan et al., 1989).

2.2 Postharvest storage

2.2.1 Postharvest storage of tropical fruits

Tropical fresh products are extremely vulnerable to qualitative and quantitative decay and losses, like sensorial, microbial and nutritional. Fungicides application and heat treatment followed by low temperature storage are postharvest treatments that have been utilized to extend the tropical fruits' shelf-life (Gonzalez-Aguilar et al., 2010). Fruits and vegetables encounter quality alterations after harvest. There are three important indices of fresh products qualities. First, the sensorial qualities consisting of color, firmness and aroma are important. Product safety including deteriorative microorganisms and pathogens are the second index. Third factor is nutritional qualities, which includes the

bioavailability and amount of bioactive compounds in the fresh products (Gonzalez-Aguilar et al., 2010). Main deterioration causes are CI, fungal decay, and quick maturation which fastened fruit senescence (Chan and Tian, 2006). Chilling sensitivity and limited marketing period cause development of postharvest handling methods to control ripening and reduce losses of postharvest diseases and disorders (Singh and Pal, 2008). Papaya fruit stored at temperatures higher than 15 °C tend to turn yellow and ripen quickly which result in decay incidence. Storage at high temperatures could accelerate water loss and shriveling and fruit softening, whereas papaya stored below 15 °C could lead to CI (Chan, 1988).

The intensity of papaya fruit CI symptoms when subjected to chilling temperatures are related to the storage temperature, fruit maturity at harvest, cultivar, and the length of exposure to CI temperature (Chen and Paull, 1986). Papaya fruit stored as low as 7 °C for about 14 days at the color-turning ripeness will be capable of ripening normally when moved to room temperature. The CI symptoms appeared after 14 days at 7 °C for mature-green fruit, and after 21 days for 60% yellow fruit (Chen and Paull, 1986). In addition to the quality and fruit maturity at harvest, the application of an optimum temperature during transportation, distribution, and retailing is a key factor to determine the quality of the fresh papaya fruit (Proulx et al., 2005). Decay, mechanical injuries, and firmness reduction were the principle postharvest defects of papayas shipped from Hawaii to the mainland U.S (Hernández et al., 2006). Low temperature storage extends postharvest life of papaya, as well as maintains quality indices like texture, flavor, aroma and nutritional composition during the supply chain. Storage at low temperature has

direct effects on the fresh papaya respiration rate. Fruit susceptibility rate and respiration rate would double for every 10 °C increase in temperature (Rivera et al., 2007). Many enzymes are involved in synthesis of ethylene, organic acids, carbohydrates, volatile compounds, and carotenoids (Sivakumar and Marisa, 2012).

2.2.2 Postharvest storage of papaya

The responsible enzymes will be inhibited at lower temperatures, and consequently, ripening-related modifications in color, texture, aroma, and flavor are retarded. Thus, 10 °C storage is necessary for papayas to extend their shelf life (Sivakumar and Marisa, 2012). Papaya will ripen to 60% -70% yellow skin colors in 4-6 days, when fruits were kept at 25-28 °C. Papayas could be stored at 10 °C for 14 to 21 days with proper postharvest disease control. Market life will become very short if the papaya is harvested at advanced maturity stages, for example 5-6 days for three-quarter ripe fruit (Sivakumar and Marisa, 2012). Harvesting papayas at a lower maturity stage will result in a CI progression when handled at less than 15 °C. 'Kapoho Solo' papayas, at mature-green stage, had improved CI symptoms following storage at 22 °C compared to fruits which were previously stored for 7, 10 and 20 days at 0,2, and 7.5 °C, respectively. 'Hawaiian' papayas stored at 5 °C for four days and then moved to 24 °C, showed a dark, dull olive discoloration emerging on the equatorial fruit surface (Chan et al., 1985). Harvested Kapoho Solo papayas at 60% yellowing can be stored for 17 days at 2 °C without progression of CI symptoms. Fruit color improved faster when fruit were transferred to 25 °C from subsequent storage at 10 °C for 5, 10, and 15 days, when the fruits firmness

were considerably reduced (Ali et al., 1993). Harvesting of Eksotika papaya at 5% yellow and storage at 10 °C for 20 days entirely inhibited the peel color development, without firmness reduction (Chen and Paull, 1986). In the supermarket shelve, papayas frequently showed very poor quality, with CI symptoms, mechanical injuries, diseases, shriveling, or combinations of these factors (Nunes et al., 2006). High relative humidity (RH) maintenance is also necessary because papaya fruits are susceptible to shriveling. It has been recommended to store the fruit at 90-95% RH to avoid skin dryness and high weight loss. On the other hand, high RH may develop decay incidence, particularly if moisture condenses on the fruit surface over a long time, when transportation temperatures fluctuated. For efficient papaya marketing along the supply chain, it is essential to be aware of optimum temperature and RH to maintain overall fruit quality (Sivakumar and Marisa, 2012). In summary, low storage temperature prior to heat treatments methods could be useful to reduce the fruit CI incidence during the storage and postharvest handling.

2.3 Postharvest pests and diseases

Postharvest diseases reduced the fruit quality and caused severe losses. Improper storage temperature or high RH can increase the postharvest diseases incidence of fruits. Fungal pathogens enter through the cuts or wounds on the fruit surface. Insect injuries can become an ideal infection sites for many other postharvest diseases causing micro-organisms. Therefore, it is necessary to remove damaged fruit from the packing line during the grading process (Snowdon, 1991). Postharvest rot is the main factor which

limited the extension of storage life in many freshly harvested fruits and vegetables. Anthracnose is one of the most important and common fungal disease of papaya which is caused by *Colletotrichum gloeosporoides* (Paull et al., 1997). Field inoculums of *C. gloeosporioides* in the form of conidia originated from dying infected petioles of the lower leaves and caused infection during postharvest storage. The disease is more extreme during wet weather and conidia distributes by splashing rain. They are moved by air currents to the developing fruit. If moist condition last for a few hours, the conidia will develop appressoria and infect the fruit. Infections stayed latent and symptoms become apparent only when the fruit ripens during the postharvest storage (Chau and Alvarez, 1983). Another pathogen that infects and restricts the papaya postharvest life is *Rhizopus stolonifer*. The infection happened during harvest and handling and induced a soft and watery rot. The fungus spreads quickly to the adjacent healthy fruit. It caused widespread decay under optimum conditions of storage (Snowdon, 1991).

Stem-end rots are caused by *Lasiodiplodia theobromae*, *Mycosphaerella caricae*, and *Phomacaricae papayae*. These fungi become stable during fruit ripening and remain inactive under the cuticle. Some fungi go through the mechanical damages, such as the broken peduncle during harvesting. The spots and rots existence can degrade external quality and caused market rejections (Sivakumar and Marisa, 2012). Fruit diseases markedly increased in severity and prevalence during refrigerated storage (González-Aguilar et al., 2003). Decay control is usually obtained by hot water treatment and chemical fungicides. Heat treatment increased softening and chemical applications can induce fruit damages, like hard lumps, that reduce postharvest fruit

quality (Couey et al., 1984). In papaya, 8-12 °C has been suggested as an optimum storage temperature (Paull et al., 1997). The limiting factor in the storage of papaya cultivar 'Tainung No. 1' at 16 °C for 17 days was fungal decay, while storage at 10 °C and less is identified to cause CI. Generally, low temperature storage at 10-12 °C can delay decay progression in fresh papayas (Maharaj and Sankat, 1990). In addition, strict preharvest disease control and orchard sanitation must be applied to avoid postharvest diseases.

2.4 Postharvest heat treatment

Postharvest heat treatment is applied for disinfestations and disinfection of crops, like fresh flowers, fruits, and vegetables (Lurie, 1998a). In addition to disease control and insect disinfestations, fruit postharvest heat treatment have been applied to increase fruit tolerances to other stresses as well as to keep fruit quality during storage (Lurie, 1998a, Paull and Chen, 2000). The aims of heat treatment, mostly used for fruits, are to prevent insect infestations, manage diseases, modify tissue reactions to other kinds of stress and keep fruit quality indices during storage (Paull and Chen, 2000). To bring this theory into practice, time-temperature combinations and the botanical source of edible parts such as fruits, leaves, stems, buds, petioles, inflorescences must be examined in advance. Furthermore, the optimum temperature and time combination selected to extend quality of fresh product during storage are affected by the maturity stage, cultivars, growing conditions and size (Fallik, 2004). Treatments like heating in dry air, steam or water will be selected according to the product specifications.

Before packaging for export, all freshly harvested products should be free of disease pathogens, insects, synthetic chemicals, and also without any dirt or dust (Fallik, 2004). Many methods are applied to preclude fruit diseases and CI extension during postharvest life. Chemical products are employed for perishable products to extend their shelf life (Vicente et al., 2002). Some of non-pesticide methods that are being examined to expand the fresh products storage and shelf life are irradiation, modified atmosphere packaging (Rodov et al., 2000), application of materials that are generally regarded as safe (GRAS) (Larrigaudiere et al., 2002) and hypobaric treatment (Romanazzi et al., 2001). Heat treatment was found to be one of the most promising method for postharvest decay control to eliminate or reduce the use of chemical application (Lurie, 1998b, Couey et al., 1984).

Application of heat treatment is to decrease the use of chemical treatments and increase the application of safe practices against moulds and pests (Mulas and Schirra, 2007). However, the critical obstacle to the extensive use of heat is the vulnerability of many fruits to the high temperatures needed for effective treatment (Yang et al., 2009b). Ripening inhibition in different fruits will occur if they are exposed to temperatures more than 35 °C (Lurie, 1998a, Paull, 1990). Pre-storage heat treatments for controlling decay are often utilized for a short time (minutes) because the main pathogens are detected on the surface or maybe under the fruit or vegetable skin, in the first cell layers (Paull, 1990). Heat may restrict pathogen spread by prompting defense mechanisms in the epicarp outer layer (Ben-Yehoshua et al., 1997). The cytological changes induced by high temperature exposure (more than 45 °C) include coagulation of the cytoplasm,

cytolysis, nuclear alternations and altered mitosis. Protoplasmic streaming is prevented, followed by increased protoplasmic viscosity and a reduction of membrane permeability. Moreover lower temperatures (less than 40 °C) have no influence on cytology (Cheng et al., 1988). Heat could be applied in several ways to fruits and vegetables: by hot water treatments, vapor heat, or hot dry air (Lurie, 1998b) or by hot water rinsing and brushing (HWRB) (Fallik et al., 2001a). These methods are the commercial practices used to decrease decay and prevent ripening, but can cause flesh mealiness in some fruits (Paull and Chen, 2000, Fallik, 2004).

It has been determined that heat treatment is able to inhibit ethylene synthesis and postpone softening in plums when combined with controlled atmosphere storage, and heat treatment can efficiently decrease internal breakdown (Serrano et al., 2004). Postharvest exposure to temperatures higher than 40-42 °C sometimes enhance the storage life and improve the fruit flavor (Lurie, 1998a). The symptoms of higher temperature (more than 45 °C) exposure are skin scald, failure to completely soften, or softening at a reduced rate (Paull and Chen, 1990). Fruit exposure to high or low temperatures could be applied as useful methods for non-chemical disinfestations or as a postharvest method to reduce CI at low storage temperatures (Woolf et al., 2004). For example, hot air treatment is able to reduce chilling damages in 'Hass' avocados (Woolf et al., 1995). Some results are in contrary with the efficacy of heat treatment, for example 'Blood' oranges were not able to tolerate heat treatment to a fruit center temperature of 44 °C for 100 min or 46 °C for 50 min because of the harmful effects on fruit quality (Mulas et al., 2001).

The hypotheses to explain the heat injury concepts are disruption of protein synthesis, failure of membrane integrity and protein denaturation. Protein denaturation at detrimental temperatures are regarded as irreversible, meanwhile lower storage temperatures will result in a reversible inactivation (Levitt, 1980). Heat treatments which are able to enhance chilling tolerance are thought to function throughout the induced synthesis and accumulation of specific proteins called heat-shock proteins (HSPs) (Sabehat et al., 1996). These proteins induced thermo-tolerance on the tissue in the way they are produced, so exposure to lethal temperatures does not cause injury (Krishnan et al., 1989).

2.4.1 Hot water treatment (hot water dip)

Hot water treatments were first mentioned in 1922 as a method to manage the decay on citrus fruits (Fawcett, 1922), and their application has been expanded to insect disinfestations (Lurie, 1998b). It was reported that treating with hot water has become more and more commercially accepted, and a significant progression occurred with the addition of brushing (Ferguson et al., 2000). One of the advantageous effects of prestorage hot water immersion treatment is to avoid rot development, which has been displayed in lots of temperate, sub-tropical and tropical fruits, vegetables and flowers (Lurie, 1998b, Schirra et al., 2000). Hot water treatment has some benefits, such as the relative ease of application, short treatment time, safe observation of fruit and water temperatures, and eradication of skin-borne decay-causing agents (Couey, 1989, Lurie, 1998b). The reasons for the different responses between the production areas may be

due to differences in season, climate, production practices, soil type and fruit maturity stage at harvest (Jacobi et al., 2001, Schirra and D'Hallewin, 1997). Couey (1989) divided fruits into two groups: heat-tolerant and heat-sensitive to hot water treatment. The first group includes papayas, bananas, cantaloupes and peppers. The obstacles with the classification are that thermo-tolerance is associated with cultivars, fruit size, season, maturity and postharvest handling (Paull, 1995, Ferguson et al., 1998). Hot water immersion of papayas at 48 °C for 20 min has been a standard treatment to control postharvest diseases such as stem-end rot and anthracnose. The efficiency of Thiabendazole (TBZ) fungicide treatment increased with hot water treatment (Schirra et al., 2000). An integrated treatment with HW-spraying and TBZ decreased the prevalence of papaya anthracnose and stem-end rot (Couey et al., 1984). Moreover, TBZ is also found to be an efficient fungicide to control CI in cold stored papaya (Pérez-Carrillo and Yahia, 2004).

Heat treatment [dry (50%) hot air (48.5 °C) for 4 h] in 'Maradol' papaya did not cause changes in fruit quality characteristics. Moreover, the interaction between heat treatment and storage temperatures affected sugar concentration (Pérez-Carrillo and Yahia, 2004). The double dip hot water treatment only eradicates eggs and early instars larvae near the fruit surface. Livable larvae of oriental fruit fly were seen after treatment in fruit with blossom-end defects (Zee et al., 1989). In some cases, double dip hot water quarantine treatment caused uneven ripening at some maturity stages, with unusual fruit softening and surface scalding (Armstrong and Mangan, 2007). Hot water treatment showed better results than air treatment in CI reduction and decay of tomatoes (Lurie et al., 1997).

Heated tomatoes had higher phospholipids, lower sterol contents and less saturated fatty acid in the outer pericarp tissue in comparison with unheated fruits (Lurie et al., 1997). The two major commercial hot water treatments are hot water rinsing and brushing (HWRB) and hot water immersion. Normally, some main apparatus of hot water immersion units are treatment tank, water circulation system, heat exchanger part, and temperature checker. The apparatus is simple to assemble, easy to manage, and the price is reasonable (Tsang et al., 1995). Heat injury was observed in HWRB method, at above 60 °C, and the best results were achieved when the brushes rolled at a speed of 60 rpm (Ben-Yehoshua et al., 2000).

Hot water dip for the aim of quarantine normally consist of fruit immersion in water and kept at 43-49 °C for several minutes to 2 h. However, exposure time in hot water depends on the fruit size, the larger the fruits, the longer would be the required exposure time. This treatment gave 99.99% quarantine security against Mexican fruit fly, without any negative effects on fruit market quality (Jacobi et al., 2001b). Hot water treatment at 49 °C for 20 min was accepted by the United States Department of Agriculture, Animal and Plants Health Inspection Service (USDA-APHIS) for some tropical fruits, like papaya, litchi or carambola, from Hawaii (USDA-APHIS, 1997). The USDA-APHIS (1997), heat treatment can sometimes result in heat injury development in mangoes, both at external and internal areas. Heat treatment increased ripening in mango varieties like 'Keitt' (Jacobi and Giles, 1997) and 'Tommy Atkins' (Talcott et al., 2005). Heat-injured fruit showed an increase in water loss and fruits failed to reach the optimum peel color (Joyce et al., 1993). Moreover, internal injury includes abnormal softening, development

of internal cavities, lack of starch breakdown and poor color development. Some areas of the flesh remain hard while others soften (Jacobi et al., 2001b). Hot water immersion of apples, followed by 7 or 10 weeks of cold storage at 5 °C was realized to be efficient in controlling leaf roller pests *Epiphyas postvittana* (Walker). The occurrence of hot water treatment-related injuries varied between locations, harvest date and orchards. Apples from early harvest showed lower levels of damage in comparison with mid and late harvest (Smith and Michael, 2000). Hot water immersion as a means of quarantine treatment showed effective results, at 49 °C for 12-15 min, against banana aphids, ants and mealy bug-infested red ginger (Chantrachit and Paull, 1998).

The HWRB in organic crops affected by high decay incidence has been reported as an effective method to reduce rot development (Porat et al., 2000a). The total fruit quality, of fruits heated at optimum hot water temperatures and exposure times, was obviously better than the untreated ones (Lurie, 1998b, Fallik et al., 2001b). Citrus immersion in 50-53 °C water for 2-3 minutes was suggested to control *Penicillium* spp. or *Alternaria* spp. that cause rots in grapefruit, oranges or lemons (Nafussi et al., 2001, Couey, 1989). Hot water treatment of 40 and 41 °C for 25-30 min decreased the body rots caused by *Colletotrichum* in avocado, while 42 °C for 30 min increased rots during one season. These treatments with cold disinfestations also improve maintaining of avocado internal and external fruit quality (Hofman et al., 2002). Hot water treatment of mature-green tomatoes in a short duration at 39-45 °C is an efficient, cost benefit and environmentally harmless method to reduce decay, but 48 °C dip was found to be destructive to tomato qualities and conditions (McDonald et al., 1999).

Guava (*Psidium guajava* L.) when subjected to hot water immersion for 35 min at 46.1 °C raised susceptibility of fruits to decay, but postponed fruit ripening for 2 days (McGuire, 1997). Dipping bell pepper at 53 °C for 4 min before storage showed effects on decreasing decays after 14 and 28 days of storage at 8 °C (González-Aguilar et al., 2000). Melons profited from HWRB at 59 °C for 15 seconds (Fallik et al., 2000) and 55 °C for 20 second had benefits on Litchi and apple (Lichter et al., 2000, Fallik et al., 2001b). The optimal HWRB treatment for citrus fruit was 62 °C for 15 s to reduce the fruits decay progression (Porat et al., 2000a), and 52 °C treatment for 15 s considerably reduced the progression of decay in red tomatoes (Ilic et al., 2001). The HWRB for grapefruit at 62 °C for 20s was found to be effective in disease resistance improvement against *Penicillium digitatum* (Porat et al., 2000b).

2.4.2 Vapor heat treatment

Vapor heat treatment (VHT) was mostly used to control insects, while hot dry air has been utilized for both fungal and insect control (Lurie, 1998b). Water is the preferred medium for many applications, since it is a more resourceful heat transfer medium than air (Fallik, 2004). VHT facilities work in many regions, although mostly they are applied for subtropical fruits quarantine treatments (Lydakis and Aked, 2003). VHT process consist heating air which is almost saturated with moisture and the air stream passed all across the fruit. VHT utilized moist hot air to kill interior feeding pests and is a substitute to chemical fumigation with methyl bromide(Lurie, 1998a). During VHT process, fruit core temperature is increased to 47.2 °C over a period of 6-8 h.

Finally, the fruits are cooled to less than 30 °C with water (Moy, 1993). This treatment is adequate to kill both larvae and eggs of fruit fly. VHT was applied commercially in Hawaii for papayas exported to Japan (Armstrong and Mangan, 2007). In mango fruit when the temperature is at or below the dew point, the atmospheric moisture condensation appear on the fruit surface (Jacobi et al., 2001b). By this method, fruit are heated through conductive energy transfer. From the fruit surface heat was transferred into the fruit center (Jordan, 1993). Research on table grapes grey mould control showed that VHT at 52.5 °C for 21-24 min and 55 °C for 18-21 min could be satisfactory treatments, but as these temperatures are near to the fresh fruit tolerance threshold, it can caused undesired effects on grapes quality (Lydakis and Aked, 2003).

2.4.2 Forced hot air treatment

Forced hot-air treatment (dry heat) is a modification of VHT to kill papaya melon fly, Mediterranean fruit fly, and oriental fruit fly eggs and larvae (Armstrong et al., 1989, Armstrong et al., 1995). In forced hot air treatment, papayas were heated for 4-7 h until the fruit center reached 47.2 °C, with the RH between 45-65%. The efficiency of this treatment depends on the physiological variability, thermal conductivity of fruit and fruit size (Cavaletto, 1998). Forced hot-air treatment has been applied commercially for Hawaiian and Belize papayas exported to the U.S. mainland, as well as from the Cook Islands, Fiji, Samoa and Tonga to New Zealand (Armstrong and Mangan, 2007). The slow heating of the forced hot-air treatment induced a preconditioning affects that leads to better fruit quality.

It is better than the fast heating of the two-stage hot water treatment (Chan, 1991). Hot air treatment at 38 °C for 3 days was mentioned to alleviate banana fruit CI during cold storage at 8 °C (Chen et al., 2008). The occurrence of papaya postharvest diseases decreased when the forced hot-air quarantine treatment was combined with TBZ or hot water immersion at 49 °C for 20 min (Yahia, 2006). The incidence of disease was not significantly affected by the sequence application of hot air or hot water treatment. However, when hot water was done prior to hot air treatment, the symptoms of pitting and scalding improved but degreening and incidence of internal lumpiness (hardened lumps of ripe flesh) increased (Yahia, 2006). High temperature treatments is capable of changing the pattern of ripening-related alteration in papaya, including respiration and ethylene production, activity of ethylene-forming enzyme, skin color, 1-aminocyclopropane-1-carboxylic acid amount, and internal carotenoid synthesis (da Silva et al., 2007).

In citrus fruits quarantine treatment using high-humidity hot air is efficient for medfly disinfestations, when the fruit center temperature is at 44 °C for 100 min or at 46 °C for 50 min (Schirra et al., 2004). The treatment includes passing the forced 48.5 ± 0.5 °C hot air with 40-60% RH over the papayas till the fruit core temperature reached to 47.2 °C (Armstrong et al., 1995). Mango quarantine efficacy against the West Indian fruit fly, *Anastrepha obliqua* (Macquart), was attained by using a forced hot-air system through running air temperature at 50 °C with air speed at about 0.4 m^3/s, and elevating humidity until the dew point (Mangan and Ingle, 1992). In papaya fruit, forced, hot-air at 48.5 °C for 3-4 h treatment for fruit flies did not significantly decrease postharvest

diseases incidences in comparison with hot-water treatments or fungicide (Nishijima et al., 1992). In Hawaii papaya, 'Kapoho Solo' forced hot air treatment was reported to control Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), melon fly, *Bactrocera cucurbitae* (Coquillett), and oriental fruit fly, *Bactrocera dorsalis* (Hendel), eggs and larvae.

2.5 Physiological response to heat treatment

2.5.1 Heat shock proteins (HSPs)

HSPs are produced as a result of high temperature exposure (Lee and Vierling, 2000a). These proteins inhibit irreversible protein denaturation, which cause detrimental effects on the cell. In plants, this activity may be increased by small HSPs (Lee and Vierling, 2000a). HSPs that induced thermo-tolerance have been reported for papaya (Paull and Chen, 1990), and apples (Ferguson et al., 1998). These proteins were induced within 30 min after treatments at temperatures that ranged from 34-42 °C. A role was reported for HSPs in cellular operation during stress in high temperature. The smaller molecular weights HSPs protect cellular proteins from thermal aggregation and prevent protein folding activity (Lee and Vierling, 2000a). Other HSPs have specific functions, for example, reactivation of proteins that already have aggregated or support the degradation of misfolded proteins (Murakami et al., 2000). Heat shock may induce reactive oxygen species (ROS) that can result in a membrane and protein damage. The development of oxygen radical scavengers, like sueroxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are developed by heat shock (Holmberg and Bülow, 1998).

It has been reported that the effect of ROS to scavenge the toxic by-products is supported by HSPs production (Altschuler and Mascarenhas, 1982). The ROS and HSPs mechanisms operate more efficiently at higher temperature when it is increased slowly (3 °C each hour) in comparison with the quick increase in temperature (Altschuler and Mascarenhas, 1982). The papaya fruit softening disorders are related to the injurious heat treatment and are decreased or inhibited by a pretreatment at 42 °C for 1h or different pretreatment time at 38 °C. These treatments should be followed by 3 h at 22 °C (Paull and Chen, 1990). Papaya VHT for 8 hours at 44 °C was designed to decrease subsequent damages (Seo et al., 1974). It was expected that VHT duration provide the necessary conditions to induce HSPs associated with thermo-tolerance. Preconditioning cucumber with 24 hours treatment at 32.5 °C could improve the subsequent tolerance for 50 min to hot water treatment at 46 °C (Chan and Linse, 1989). However, 32.5 °C was normally lower than the acquired temperature to induce HSPs. Longer duration (24 h) can possibly induce HSPs and thermo-tolerance (Chan and Linse, 1989).

Heat treatment prohibited biochemical pathways in some fruits and vegetables which are associated with ripening and other processes (Paull and Chen, 2000). Ripening pears subjected to 40 °C heat treatment resulted in a protein synthesis disruption via polysomes loss. This response (protein synthesis disruption) is reversible and demonstrates a close correlation between protein synthesizing mechanism and the ripening progress (Romani and French, 1977). HSPs synthesis at 39 °C in pear cells is more prominent compared to 40 °C, when the treatment had been extended up to 8 h (Ferguson et al., 1994). Protein synthesis in papaya fruit changed when they are exposed

to 38 °C for 2 h. There was no protein accumulation in control fruit held at 22 °C for 10 h. Translated polysomal RNA showed that new polypeptides were synthesized following heat shock induction. Thermo-tolerance are reduced with continuance fruit exposure to 42 °C (Paull and Chen, 1990). Paull and Chen, (1990) mentioned also that 42 °C was the maximum temperature to induce the heat shock tolerance and increase the protein degradation, as mentioned earlier for pear cells. Persistent exposure to 42 °C may induce damages but heat shock polypeptide is still synthesized. These results were similar with tomato fruit treatment for a long period at 35 °C (Picton and Grierson, 1988).

Apple fruit held 4 days at 38°C showed a change in protein synthesis profiles (Lurie and Klein, 1990). The results from gel electrophoresis showed new protein bands with low molecular weight (14-22 kDa) and high molecular weight (68 and 92 kDa) protein ranges (Lurie and Klein, 1990). The obtained results corresponded with HSP locations and they were similar with the results observed in papaya (Paull and Chen, 1990). The higher rate of protein degradation continued with a higher temperature. If the heat threshold was exceeded, the protein degradation will lead to disruption through the pathways of metabolic fluxes (Callis, 1995). The HWRB led to the accumulation of 21, 22 and 25 kDa proteins that cross-reacted with citrus and tobacco chitinase antibodies (Pavoncello et al., 2001). In addition, 38, 42 and 43 kDa proteins also accumulated that cross-reacted with citrus and tobacco β-1, 3-glucanase antibodies. The increase in glucanase and chitinase proteins accumulation is part of fruit disease resistance complex mechanisms which is induced by HWRB. The mode of action in hot water treated fruits is to reduce the lemon fruit decay.

The resistance mechanism in inoculated and hot-dipped fruit resulted in the production of lignin-like material at the inoculation site, followed afterwards by accumulation of the phytoalexins scoparone and scopolitin (Nafussi et al., 2001). Two-dimensional gel electrophoresis of the boiled soluble protein fraction indicated two new polypeptides that are connected in heat-induced chilling tolerance, but it is not certain that these polypeptides are the so called HSPs (Zhu et al., 2003). Grapefruit exposure (for a short time) to high temperature with HWRB protection mechanism may consist of the induction and accumulation of 105 kDa protein. The protein cross-reacted with an antibody increased against a bovine HSP and 18 and 21 kDa proteins that cross-reacted with HSP18 and HSP21 pea antibodies (Porat et al., 2000b, Pavoncello et al., 2001). An understanding of the necessary conditions to produce HSPs and thermo-tolerance are important to optimize heat treatment applied for insect disinfestations and disease control (Paull and Chen, 2000).

2.5.2 Antioxidant enzymes

Postharvest treatments are applied to preserve fresh produce quality with a major focus on keeping freshness and preventing microbial growth and decay. However, development of antioxidant systems as a secondary reaction under certain environmental stress situations have been monitored, with regards to some stress types applied as postharvest treatments (Gonzalez-Aguilar et al., 2010). Most postharvest treatments induce the changes in fruit natural circumstances to achieve longer postharvest life. For example, high O_2 atmospheres and irradiation caused damage to some vital molecules of

food deteriorative microorganisms, with changes in fruit biochemical procedures (Charles et al., 2009). Some postharvest treatments act as a secondary response induced mechanisms that affect the metabolic activities of the treated products such as stimulating the antioxidant system in the fruit. The antioxidant system stimulation as a response to postharvest stress could be able to improve the antioxidant status in tropical fruits, however the mechanisms that postharvest treatments triggered these types of responses have not been illuminated (Gonzalez-Aguilar et al., 2010). Heat treatment application, as a commercialized method, has become common knowledge over the past two decades and has been applied for disinfection and disinfestations of tropical fruits, such as papaya, mango and citrus fruits (Jacobi et al., 2001b). Many fruit ripening processes are influenced by heat treatment, such as ripening control, pigment metabolism, fruit softening, disease development, carbohydrate metabolism and volatile production (Jacobi et al., 2001b, Talcott et al., 2005, Zhang et al., 2009).

Heat treatment also had effects on fruit ripening stages such as cell-wall metabolism and softening, respiration and ethylene synthesis (Zhang et al., 2009). Several studies have reported the relation of heat tolerance with the increase in antioxidant enzymes and phyto-chemicals such as phenolic compounds and carotenoids (Gonzalez-Aguilar et al., 2010). Proper heat treatment leads to the induction of chilling tolerance in plant tissue and this fact is in accordance with the 'cross-adaptation' scenario especially experienced with a kind of sub-lethal stress, like osmotic or heat stress. The scenario was associated with different stresses that stimulate the same secondary messengers, such as ROS (Wang et al., 2012). Heat stress can cause a sharp increase in H_2O_2 rates and induced a

strong rise in H_2O_2 rates in *Arabidopsis thaliana* suspension cell. This increase is about 2.3-fold at 37 °C and 2.5-fold at 44 °C in 1 h treatment (Volkov et al., 2006). It has been mentioned that the increase of reactive oxygen levels in plant tissue could be related to NADPH oxidase which catalyses the superoxide anion production (Vignais, 2002). The accumulation of H_2O_2 ROS can lead to membrane lipid peroxidation. The POD enzyme facilitates the unsaturated fatty acids oxidation by singlet oxygen and formation of malondialdehyde. The study by Ghasemnezhad et al. (2008) signified an increase in CAT, SOD and POD activities after hot water treatment in mandarins. The same results on mango fruit showed an increase in vitamin C content and total carotenoids in hot water treated fruits (Djioua et al., 2009). These results demonstrated that heat treatment have the capability to extend postharvest life of several fruits and promote their bioactive compounds (Gonzalez-Aguilar et al., 2010).

The SOD enzyme has a main role in defense procedures against oxidative stress. SOD exist in all aerobic organisms and in most of the cellular parts that generate ROS (Mittler, 2002). These enzymes dismutate the superoxide radical ($O_2^{\bullet -}$) to form H_2O_2 and molecular oxygen, so as to inhibit the superoxide radicals to produce hydroxyl radicals. The CAT depleted the hydrogen peroxide produced in peroxisomes through β-oxidation of fatty acids, glyoxylate cycle and purine catabolism (Yoshimura et al., 2000). Carotenoids and some terpenoids are the major secondary metabolite compounds increased by heat treatments and involved in the cell photo protection because of their capacity to store the energy (Peñuelas and Munné-Bosch., 2005). These molecules interacted with membrane lipids, induce membrane thermo-stability and decrease the

lipid peroxidation vulnerability under elevated temperatures (Peñuelas and Munné-Bosch., 2005). Carotenoids also, have an essential function to protect fruit tissue by scavenging ROS. In addition, the alterations in the fruits oxidative metabolism by heat treatment application increased the activity of CAT, SOD and ascorbate-glutathione cycle (Ghasemnezhad et al., 2008). Antioxidant enzymes inhibit lipid peroxidation in foods during processing and storage (Cisneros-Zevallos, 2003).

2.6 Chilling injury, symptoms and mechanism

Low temperature storage is usually used to expand fruits postharvest life and maintain their quality. Tropical and subtropical fruits are often injured when exposed to low temperatures (Jiang et al., 2002). The CI is a physiological disorder that could affect consumers' preference. The CI severity is related with several intrinsic and extrinsic factors (Luengwilai et al., 2012). Intrinsic factors include cultivar, prior growing conditions, and prior exposure to stress. Extrinsic factors include surrounding relative humidity, sanitation, temperature, duration of exposure, and degree of mechanical injury (Luengwilai et al., 2012). The CI concept can be divided into two procedures (Orr and Raison, 1990); a primary event is temperature-dependent and commences when the temperature falls beneath a threshold temperature for a particular duration, and induces some metabolic dysfunction. The secondary event is time-dependent and includes a large amount of metabolic procedures. It can be negatively affected as a consequence of the primary event, and resulted in the progression of measurable symptoms attributed with CI (Orr and Raison, 1990).

Generally, indications of CI in papaya are characterized by skin pitting and scald and development of sunken spots and peel discoloration. Uneven ripening, hard lumps, inferior fruit flavor, and increased susceptibility to postharvest pathogens occurred in pulp. The specifications are flesh water-soaking and abnormal ripening with blotchy pulp discoloration (Ali et al., 1993). The CI severity is related with cultivar, maturity stage and exposure time. Less mature harvested papayas or less ripe fruit are more vulnerable to CI than mature fruit (Chen and Paull, 1986). The CI symptoms will appear after moving the chilled fruit at 25 to 28 °C (ambient temperatures) during marketing. Precise CI symptoms assessment on mature-green 'Solo' papaya surface showed the progression of dark olive spots of 1-2 mm around the fruit diameter (Paull, 1999).

These spots joined together with severe injury to create scald-like regions. However, moderate bruises became more evident during ripening and showed up as green areas (Paull, 1999). Sometimes green areas enclosed with a pitted part of the skin causing a blemished appearance on the fruit. In a more yellow fruit, progression of light brown spots that normally combined together is related to the collapsed tissue. Internal CI symptoms at mature-green stage are indicated by hard white regions that do not soften even when transferred to a ripening temperature. In severely chilled fruit, off-flavor and odor are associated with skin and tissue injury (Chen and Paull, 1986). Pre-storage heat treatment and irregular warming have been applied to reduce CI in papayas stored at low temperatures (Huajaikaew et al., 2005). Papaya exposure at 42 °C for 6 h prior to storage at 5 °C cause CI symptoms alleviation (Huajaikaew et al., 2005). Warmed papaya at 15 or 20 °C for 3 days, and held at 5 °C for 2 days (intermittent warming) improved CI

symptoms (Huajaikaew et al., 2005). Fruits stored constantly at 5 °C revealed a greater pitting extent and when moved to ambient temperature, fruit remained unripe (Huajaikaew et al., 2005). Moreover, papaya stored at 5 °C developed CI symptoms more severe and quicker in comparison with intermittently heated fruit. Intermittently warmed fruit at 20 °C showed higher yellow color index than fruit warmed intermittently at 15 °C (Huajaikaew et al., 2005). Some abiotic stresses, such as heat-shock, cold-shock, osmotic shock, ethanol, and salinity, used before chilling storage tend to enhance chilling tolerance (Erkan et al., 2005, Rivera et al., 2007).

Postharvest heat-treatment decreased CI incidence of citrus (Rodov et al., 1995), mangoes (Mohammed and Brecht, 2002), and maintained storage quality of avocado (Hofman et al., 2002), banana (Promyou et al., 2008) and mandarin (Ghasemnezhad et al., 2008) during cold storage. Temperature has a key role in fruits and vegetables metabolism (Marangoni et al., 1996). Different kind of stresses like CI will lead to degradation of the membrane, such as membrane phospholipids hydrolysis of free fatty acids, and peroxidation of constituent polyunsaturated fatty acids with a corresponding free radicals production (Palma et al., 1995). CI include swollen cells, chloroplasts and mitochondria disorganization, and cytoplasm membranes vesiculation (Murphy and Wilson, 1981). The first exhibition of CI is chloroplast swelling, distortion, swelling of thylakoids, and diminution in volume and number of starch granules (Kratsch and Wise, 2000). Due to the activity of lipoxygenase, CI is accompanied by lipid degradation, and their common substrates are linoleic and linolenic acids (Maalekuu et al., 2006). The degradation of such polyunsaturated fatty acids produces malondialdehyde and peroxide

ions, the products of oxidation damage. Polyphenol oxidase (PPO) seems to be the main enzyme in tissue browning of cold-injured fruit and vegetables (Leja et al., 2003). It has been concluded that heat-induced chilling tolerance is dependent on the incidence of HSPs (Sabehat et al., 1998). Moreover, heat-induced chilling tolerance increased PAL, CAT, ascorbate peroxidase (APX) and SOD activities (Sanchez-Ballesta et al., 2000).

2.6.1 Peel electrolyte leakage

The membrane ion leakage is a sign of membrane integrity reduction, which result from membrane damage (Azevedo et al., 2008). The development in the intracellular electrolyte leakage resulted mostly from an increase in membrane permeability, and it is due to the damages caused, for example, by salt (Amor et al., 2006) or water stress (Pimentel et al., 2002). Senescence or ripening of detached plants' biological structure also coincides with the membrane integrity loss, caused by cell collapse (Azevedo et al., 2008). Membranes of chilling vulnerable products undergo changes in biophysical attributes related to their composition, while membranes of chilling tolerant varieties sustain their liquid-crystalline condition (Marangoni et al., 1996). The primary alteration of CI includes changes in membrane composition and structure and resulted in a reduction of metabolic dysfunction and permeability control (Valdenegro et al., 2005). The structures of constituent membrane lipids can influence the membranes fluidity, with unsaturated fatty acid, including lipids, being more fluid than saturated lipids (Marangoni et al., 1996). Electrolyte leakage is a parameter that has been applied to signify physical injury to the plasmalemma caused by low-temperature stress (Parkin

and Kuo., 1989). There was a growing increase in tomato electrolyte leakage within a few days of chilling stress (Saltveit, 2002), but in kiwifruit, an increase up to 15% have been observed when the fruit is exposed for 40 h at -2 °C (Gerasopoulos et al., 2006). Low-temperature breakdown is a disorder which resulted in significant quality losses in kiwifruit during prolonged cold storage, and seems to be related with factors that affect membrane functions (Gerasopoulos et al., 2006). Heat injury causes electrolyte leakage and induces the heat stress in cellular membranes (Inaba and Crandall., 1988). A sigmoid curve expressed electrolyte leakage of tomato discs which is caused by raising the temperature. The threshold exposure times for heat treatment that caused tomato leakage were 34 min at 55 °C, 105 min at 50 °C and 166 min at 45 °C, but tomatoes did not demonstrate visible heat injury (Inaba and Crandall., 1988). There was a 50% higher electrolyte leakage isolated from apple fruit discs after being held at 38 °C for 2 days (Lurie and Klein, 1990).

2.6.2 Chilling injury and antioxidant defense mechanism

Plants have developed complicated defense mechanism to save them against pathogen attack like bacteria, fungi, viruses and moreover against the environmental stress situations (Campo et al., 2004). One of these mechanisms involved ROS production like singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), H_2O_2 and hydroxyl radical ($^{\bullet}OH$) (Campo et al., 2004). The production of ROS and/or free radicals is the expected result of the electron transport included in respiratory and photosynthetic pathways that normally occur in plant tissues (Toivonen, 2004). Stress procedures, such as fruit ripening,

enhanced the ROS production in plant tissues and resulted in tissue injury. The SOD activity in cold storage steadily increased in mume fruit stored at 1 °C. After 15 days of chilling exposure at 1 °C, SOD activity was elevated by 1.6 times (Imahori et al., 2008). Excessive production of ROS, like hydrogen peroxide and superoxide anion, could be a considerable factor in tissue damage (Halliwell, 2006). Cells contain secondary metabolic compounds such as ascorbate, carotenoids, flavonoids and glutathione (Halliwell, 2006). In addition, cells produce several enzymes like APX, SOD, CAT, dehydroascorbate reductase, glutathione reductase, water-soluble antioxidants (ascorbic acid and glutathione) and lipid soluble antioxidants like α-tocopherol and β-carotene (Mittler et al., 2004). These compounds turned the ROS into products with lower toxicity (Kang and Saltveit, 2001). The ROS are considered as cellular prerequisite, because they are included in signaling pathways to induce the antioxidant molecules.

Plant antioxidant pathways are usually sufficient to keep them from oxidative damage during normal growth periods and moderate stress (Kang and Saltveit, 2001). The main components of this cycle contain glutathione reductase, APX, and antioxidant compounds like ascorbate and glutathione. The capability of this cycle to protect tissue is related with antioxidant concentration and the activity of the mentioned enzymes (Noctor and Foyer, 1998). Accelerating oxidation, ascorbate degradation and glutathione pools with extensive and/or severe stresses and damage are related with senescence and cell death (Noctor and Foyer, 1998). However, at high concentrations, ROS distract the normal metabolism by oxidizing proteins, nucleic acids, carbohydrates, or lipids and affect the cell membrane integrity and deactivate key cellular operation (Campo et al.,

2004). The ROS are the main mediators of oxidative injuries in plants, and are involved in fruit senescence (Hariyadi and Parkin, 1991). Phenolic compounds probably act co-operatively with the ascorbate–glutathione-dependent pathway (Kähkönen et al., 1999). Phenolics are able to scavenge ROS according to their redox characteristics. Phenolics contribute as reducing agents, hydrogen donors, oxygen scavengers, and metal chelators (Kähkönen et al., 1999). The ROS have been related with CI progression during low temperature storage of different fruits such as mango (Ding et al., 2007b), loquat (Cao et al., 2009), zucchini squash (Wang, 1995), and mume (Imahori et al., 2008). Postharvest treatments which are stress-types have been protected fruits (Lim et al., 2007). These stresses are able to trigger some enzymatic and non-enzymatic antioxidant mechanisms of the fresh produce, thus, contributing to an adaptation procedure to stressful situations and consequently to fruit quality preservation and better antioxidant potential (Lim et al., 2007).

2.7 Postharvest characteristics

2.7.1 Color

Color measurement is an important way of quality evaluation of food and fruit products (Shewfelt, 2009). Fruit color indices may result from a combination of many forms of anthocyanins presented together, as well as the conditions of pH and ions present in the vacuole. The external fruit color is an essential factor in consumers' preference (Shewfelt, 2009). The principal pigments in many fruit are carotenoids and anthocyanins, which are synthesized through the terpenoid and phenylpropanoid pathways, respectively (Shewfelt, 2009).

However, color of fruits and vegetables is an external expression of the structure and form of plant pigments (Shewfelt, 2009). Fruit ripening process and vegetable yellowing normally involve the uncovering of yellow to orange xanthophylls and carotenes due to chlorophyll disappearance (Chen and Ramaswamy, 2002). Assessment of the changes in pigments is necessary to understand the ripening and senescence physiology. In addition, it is also important to detect physical changes in fruit appearance (Voss, 1992).

McGuire and Reeder (1992) demonstrated that, in a circle color chart L* represent lightness of the color in the circle diameter ranging from black 0 to white 100. Chroma is the indicator of color purity with the variety from rich and vivid colors in circle circumference to the dull and grayish colors in the center $C= (a^{*2}+b^{*2})^{1/2}$. Hue angle was referred to show the purity in color spectrum. It is a belt around the circle and showed the "color name" h°=arc tan b*/a*. 0° is a definition for red-purple color, 90° for yellow, 180° bluish-green and 270° stands for the blue region. a* is a measure of redness (or –a* of greenness) and b* of yellowness (or –b* of blueness) on the hue-circle (McGuire and Reeder, 1992). The golden yellow color in ripe Banana (*Musa acuminata* AAA group) fruit is because of chlorophyll breakdown, which causes carotenoid pigments in the plastids to become visible (Blackbourn et al., 1990). However, during ripening process at temperatures higher than 24 °C, bananas did not develop a completely yellow peel color and remained green. The green-ripe fruits are supposed to have low market quality and consequently catch a lower market price. In tropical regions, it is very common that this green-ripe phenomenon appears to be abnormal, in comparison with many other fruits, which degreen quickly at higher 24 °C (Blackbourn et al., 1990).

Chlorophyll retention could be the consequence of partial maintenance of granular-thylakoid membranes (Ding et al., 2007a). In addition, a new protein, assigned as senescence-inducible chloroplast stay-green protein (SGR, STAY-GREEN), could be one of the upstream elements regulating the chlorophyll degradation pathway at the gene expression stage (Yang et al., 2009b). It is also reported that sugar, considered as a plant development and senescence signal, was the main factor regulating chlorophyll degradation (Yang et al., 2009a). Disruption of tomato, apples and pear fruit ripening happens when fruit are subjected to temperatures more than 30 °C for 48 h or more. This disruption is characterized by a failure to improve normal pigmentation, little softening, and marked reduction in ethylene production (Yoshida et al., 1984, Picton and Grierson, 1988).

2.7.2 Weight loss

Weight loss is caused by the water loss, which occurred throughout the stomata, cuticle and stem scar (Paull and Chen, 1989). In several fresh commodity, the quality reduction in appearance and texture was related to the water loss (Paull, 1999). The water loss content depends on the thickness of cuticle and varies with maturity stage and cultivar. The 8% weight losses make small 'Solo' type papaya unacceptable for marketing. Weight loss of 4-6% also caused rejection of large size 'Red Lady' papaya (Nunes et al., 2006). The water loss occurrence during papaya handling tended to cover actual SSC, TA and AA losses that are expressed on the basis of fresh weight. The compositional data were explained on a dry weight basis to demonstrate the real losses occurred in

chemical constituents regardless of the concentration effect imposed by water loss (Proulx et al., 2005). The HWRB removes soils, dust and fungal spores from the fruit surface. Fruit heating changed the structure of the cuticle through decreasing the cracks number in apples (Lurie et al., 1996a) and cuticle permeability in peaches (Phillips and Austin, 1982). In some cases, heat treatment increased the grapefruit water loss (Porat et al., 2000c), though without significant changes for oranges (Williams et al., 1994).

2.7.3 Firmness

Fruit softening, during ripening is an essential characteristic that causes fruits to be desirable for consumption. Simultaneously, it is associated with postharvest shelf life reduction and susceptibility to microbial infection. The economic importance of textural changes caused interests to find the biochemical mechanism for it (Handa et al., 2012). Usually, fruit softening is the result of structural weakening, which involved a synchronized series of modifications to the polysaccharide and protein components of the primary cell wall and middle lamella (Brummell, 2006). Developmental regulations of cell wall stabilizing and depolymerizing proteins and also protein glycosylation have been determined during ripening of fruit (Negi and Handa, 2008). Changes of papaya fruit firmness during ripening are related with cell wall dissociation, which is under the control of many enzymes (Lazan et al., 1995). Fruit softening is the result of pectin depolymerization in the cell walls, by which high molecular weight insoluble pectin is transformed into soluble polyuronides (Lazan et al., 1995). In carambola cv. B10 and in papaya cv. Eksotika, pectin depolymerization caused hemicellulose modification (Lazan

et al., 1995, Chin et al., 1999). Fruit ripening lead to softening and it is catalyzed by enzymes such as pectin methylesterase (PME), β-galactosidase, polygalacturonase (PG), and cellulose (Pérez et al., 2010). There is a positive relationship between PG and xylanase activity and fruit softening (Paull and Chen, 1983). Pectins keep cell wall components attached to each other and when cell-to-cell links become weak, the tissue rigidity and firmness will be reduced. Sometimes, flesh softening will be delayed following fruit exposure to 38-40 °C (Evangelista et al., 2002, Salvador et al., 2007). Disinfestations at 45-50 °C, will increase or delay softening (Paull and Chen, 1990).

Apple fruit exposure at 38 °C for 4 days led to the decrease of soluble and increased of insoluble pectins (Shalom et al., 1996). Treated tomatoes after 96 h at 40 °C had less soluble polyuronides, galactose and arabinose (Mitcham and McDonald, 1992). The softening disorder has been related to the diminution of cell wall hydrolytic enzymes. However, heat disorder of cell wall breakdown has been considered as the reason of delayed or poor softening. Reports on different cell wall degrading enzymes confirmed that the disorders could be associated with mRNA synthesis and stability, or protein synthesis and degradation (Lashbrook et al., 1998). In tomato fruits, temperatures between 35-60 °C caused 87% loss of purified glucanase activity. Moreover, exposure at 50 °C for 5 min resulted in 50% loss of glucanase activity (Pressey, 1983). The pectinesterase activity were suppressed in tomatoes held at 33 °C , though there was slight difference in the apple pectin esterification degree after treatment at 38 °C for 4 days (Klein et al., 1995). PG activity returned after 6 days lag when fruits were exposed to 25 °C (Yoshida et al., 1984).

A similar reversibility was found in apple fruit softening held at 38 °C for 4 days (Lurie and Klein, 1990). It has been concluded that the failure of heated fruit to soften could be result of (a) transcribe breakdown, (b) loss of mRNAs coding for wall softening enzymes, or (c) failure to translate mRNAs. Moreover, heating is able to activate or increase enzyme turnover causing cellular injuries directly or indirectly through destruction of cellular compartments (Callis, 1995).

2.7.4 Soluble solids concentration

Sweetness is one of the major characteristics to assess fruits and vegetables quality and marketability (Georgelis, 2002). Sweetness is utilized as a ripening index for determining the ripeness stage and for quality standards of papaya. Mostly, it depends on the type and structure of sugars present. Sugar amount is also dependent on total solids, pH, titratable acidity (TA) and fruit size (Georgelis, 2002). In many fruits, glucose and fructose are the major amount of soluble sugars (Baldwin et al., 1991). The major carbohydrates in papaya fruit are glucose, sucrose, and fructose (Zhou and Paull, 2001). The highest amount of carbohydrate during the initial development stage is glucose (Zhou and Paull, 2001). Sucrose content was elevated during ripening process and varies between 2 to 6 g/100 g fresh weight in ripe fruit (Chan et al., 1989). It was recommended that the sweet taste of papaya is the result of fruit firmness reduction and textural alternations (Gomez et al., 2002). The changes in skin and flesh color and the increased total soluble solids (TSS) in pulp are related to ripening. Therefore, TSS, skin color, flesh firmness and carotenoid amount, could be regarded as ripening

characteristics to determine papaya ripening (Schweiggert et al., 2011). In a few wild varieties of tomato and melons, sucrose is the main sugar. The TSS amount changes with fruit maturity, expressing a maximum rate at ripening (Cho et al., 1993). For example, in tomato fruit tissues, the sugars accumulation was found to vary between the mesocarp and locular parts. Furthermore, sugar content is different with plant nutrition, climate, soil and storage conditions (Mounet et al., 2009). Fruit sugar content and its impact on taste are determined by several methods. The TSS-to-titratable acid ratio and the total sweetness index are general measurements (Grierson and Kader, 1986).

Soluble solid concentration (SSC) is a refractometric parameter that specify the ratio (%) of dissolved solids in a solution (Beckles, 2012). In tomato, because of the acid effects on sweetness, the ratio of TSS-to-titratable acid could be another useful taste marker (Grierson and Kader, 1986). Hot water treatment at 41 °C considerably increased the SSC ratio in 'Navel' oranges as compared with the control fruits (Bassal and El-Hamahmy, 2011). In other reports, hot water treatment was not effective to maintain SSC content of Blood (Schirra et al., 2004) and 'Valencia' oranges (Erkan et al., 2005). The SSC percentage of Satsuma mandarin indicated a slight increase that is consistent with water loss. SSC was highest at 50 °C for both 2 and 5 min of hot water treatments. SSC tended to be lower in damaged Satsuma mandarin, but there was only a small impact of treatment on the SSC (Ghasemnezhad et al., 2008).

2.7.5 Ascorbic acid

Vitamin C is the most important vitamin for nutrition found in fruits and vegetables. More than 90% of this vitamin needed in human diet is provided by fruits and vegetables. Vitamin C is described as the generic name for all compounds that demonstrated the biological activity of L-ascorbic acid (AA). L-AA is the main active form while L-dehydroascorbic acid is an oxidation outcome, and demonstrates the biological activity (Lee and Kader, 2000b). In several horticultural products, L-dehydroascorbic acid corresponds to less than 10% of total vitamin C, but it tends to increase during storage.

Vitamin C is so vulnerable to destruction when the products are exposed to adverse handling and storage situation. Losses of fresh fruits and vegetables are increased by prolonged storage, higher temperatures, lower RH, physical injuries, and CI (Wills et al., 1984). Vitamin C, a water-soluble vitamin which naturally exists in fruits and vegetables, is usually applied as food additive and antioxidant (Pénicaud et al., 2010). L-AA is an efficient antioxidant related to its high electron-donating ability and ready conversion back to the active reduced form (Valente et al., 2011). L-AA is simply oxidized, particularly in aqueous solutions, and significantly favored by the existence of oxygen, heavy metal ions, such as Fe^{3+},Cu^{2+}, Ag^{+}, high temperature and alkaline pH (Parviainen and Nyyssonen, 1992). Ascorbate oxidase has been found to be the main enzyme in charge of AA enzymatic degradation. Ascorbate oxidase is a copper-containing enzyme which oxidizes AA to L-dehydroascorbic acid with the existence of

molecular oxygen (Saari et al., 1995). Ascorbate oxidase is related with fast growing areas in the plant and is bound to cell walls as well as a soluble protein in the cytosol. Under stress conditions, like pathogen or chemical exposure, ascorbate oxidase levels increased (Loewus et al., 1987). A delay of 24 h at 30 and 40 °C between tomato harvesting and processing led to 5 and 12% reduction in AA content, respectively (Lee and Kader, 2000b). A loss in vitamin C was faster in citrus fruits stored at higher temperatures than fruits stored at lower temperature (Nagy, 1980). The extent of reduction in AA amount in response to increased temperatures was more in vegetables compared to acidic fruits (citrus) because AA stability is higher under acidic condition (Nagy, 1980). The AA concentration in fresh green asparagus kept at 4 °C was reduced 2 days after harvest.

The CI accelerate losses in sensitive crops, such as sweet potatoes, bananas, and pineapples (Esteve et al., 1995). Hot water treatment in Navel and Valencia oranges at 50 °C significantly increase the AA content compared with control (Bassal and El-Hamahmy, 2011). Spectrophotometric, colorimetric, fluorometric, voltammetric, potentiometric, titrimetric, spectrofluorimetric, and chromatographic are analytical quantitative methods for AA measurement in foodstuffs (de Quirós et al., 2009, Pénicaud et al., 2010). In fresh-cut papaya, L- AA amounts were calculated on a dry weight basis because weight losses varied among samples and consequently, L-AA content on a fresh weight basis did not show an actual L- AA amount (Argañosa et al., 2008). Vitamin C amount could be increased during maturity and ripening, and reduced during storage and also is affected by the climate, soil type, production practices and

harvest season. The carotenoids and vitamin C concentrations, respectively, were correlated (r^2=0.98 to 0.99) with antioxidant capacity (Tripathi et al., 2011).

2.7.6 Titratable acidity and pH

The different kinds of organic acids in fruits are obtained from different sources, such as biochemical processes or microorganisms (bacteria or yeasts) activities (Hernández et al., 2009). The carboxylic acids indicate fruits pH and total acidity. They act as metals chelating agents that block oxidation and chemical precipitation. In addition, the non-volatile organic acids affects the fruits' sensorial characteristics, such as aroma, taste and color (Hernández et al., 2009). It is also used to determine fruit development or ripening (Avenoza et al., 2006). In Solo papaya, citric, malic and α-ketoglutaric acids were the main identified and quantified acids (Brekke et al., 1971). The existence of oxalic, citric, galacturonic, ascorbic, L-malic, quinic, succinic, fumaric and D-malic acids has been reported in Sunrise papaya (Cano et al., 1994).

Citric acid (335 ± 32 mg/100 g FW) was the main organic acid in ripe papaya followed by L-malic acid (209 ± 12 mg/100 g FW) (Hernández et al., 2009). According to the Hernández et al. (2009), oxalic, tartaric, quinic, succinic and fumaric acid contents in papaya were 10, 13, 52, 55 and 1.2 mg/100 g FW, respectively. High TA was maintained in hot water dip (50 or 55 °C for 5 min) Valencia orange and 'Marsh' grapefruit, compared with control fruits (Mohamed et al., 2002, Birla et al., 2005). Hot water treatment did not show significant effects on TA content of Blood oranges

(Schirra et al., 2004). An earlier study showed the significant ($P \leq 0.05$) reduction of pH value from the original pH 5.7-5.8 of cut papaya at 6±1 °C during 60 days storage (Chauhan et al., 2006). The organic acid content in hot water-treated Satsuma mandarin was reduced sharply after harvest, but there was no effect on total acid amount after 8 weeks storage (Ghasemnezhad et al., 2008). At harvest, pawpaw fruit tissues had a pH value of 6.54 and the pH considerably increased during 4 to 72 h of ripening but declined during storage (Galli et al., 2008). Reduction of organic acid levels in fruit during cold storage may demonstrate the changes in energy metabolism, pH stability, and defense compounds that inhibit or repair injury induced by chilling temperature (Maldonado et al., 2004).

2.7.7 Ethylene and carbon dioxide production rate

Fruit maturity is a complicated developmental process that involves many specific biochemical changes in cellular metabolism. There are some physico-chemical issues that have been used to determine fruit ripening stages (Azevedo et al., 2008). Ethylene is a plant hormone in a gas form which plays a serious role in physiological processes during plant development; involving a massive metabolic shift during the ripening of climacteric fruits (Bapat et al., 2010). Climacteric fruits exhibited increase in respiration rate and biosynthesis of ethylene at the beginning of ripening. It has been suggested that it is associated with coordination of the fruits ripening process (Handa et al., 2012). However, there are extensive proofs showing different stress-causing factors, such as freezing, salt stress, chilling, wounding, and pathogen attack that stimulate the ethylene

production (Kacperska, 1997). The elevation in ethylene synthesis rate may occur soon after the stress, depending on the stress-causal factor. The respiration rate of ripening fruit was primarily enhanced by exposure to higher temperatures (Mitcham and McDonald., 1993). Fruits' reactions to temperature fluctuations varied with the physiological age of the tissue (Inaba and Crandall., 1988). After heat treatment, the respiration rate was reduced to near or under the level of the non-heated control fruit. Storage at approximately 33 °C for 12 days suppressed the respiration rate in tomato fruit and did not totally reverse after returning the fruit to ambient temperature (Inaba and Crandall., 1988). Heat treatment has an effect on the consequent increase of the climacteric respiratory. The rise between the preliminary pre-climacteric level and the climacteric peak in avocado fruit was 25% at 25 °C, and 30% at 30 °C (Biale and Young, 1971). During heat treatment at 43-48 °C, papaya fruit ripening initially indicate an elevated respiration rate (Paull and Chen, 1990) and in mango the rate was reduced to similar level or below that of the control (without heat treatment) fruits (Mitcham and McDonald., 1993).

The amount and timing of the primary peak in papayas are related with RH of the heated air. The climacteric peak in heat treated fruits were postponed for about 2 days (Mitcham and McDonald., 1993). Likewise, the respiration rate in apple fruit was lower upon returning of heated fruits to the ambient temperatures in comparison with the non-heated fruits (Klein et al., 1990). At higher temperatures, the ethylene synthesis is also reversibly inhibited (Dunlap et al., 1990). Fruit exposure to the long high temperature periods rapidly recovers their ability of ethylene synthesis (Dunlap et al., 1990).

The 1-aminocyclopropane-1-carboxylic acid (ACC) conversion to ethylene is obviously so susceptible to heat injury above 30 °C. Papaya fruits exposure to short temperature period of 40 °C or higher caused 75% loss of ACC oxidase (Paull and Chen, 1990, Ketsa et al., 1999). The ACC oxidase inactivation by heat, in cucumber and papaya, is biphasic with both phases following first order kinetics. In papaya, ACC oxidase heat resistance is about 25% of total ACC oxidase activity. Recovery ability of the ethylene production requires protein synthesis. It seems that the protein loss is due to the reduction in 1-ACC oxidase mRNA and synthesis, rather than protein denaturation (Lurie et al., 1996b). Temperatures higher than 35 °C resulted in an accumulation of endogenous 1-ACC content in tomato and apple tissue and decreased the ethylene production in both fruits (Klein, 1989, Atta-Aly, 1992). The ACC accumulation did not occur in fruits exposed to higher temperatures or for longer period. ACC synthase is less sensitive to losses due to heat stress than ACC oxidase in apple and tomato (Klein, 1989, Atta-Aly, 1992). 1-ACC synthase activity is absent in mangoes and only partially recovers when returned to the optimum temperatures (Ketsa et al., 1999).

The differences between the oxidase and the synthase responses could be related to differences in turnover rates (Ketsa et al., 1999). Heat treatment causes injury-induced wound ethylene production 1-2 days after treatment, with a subsequent advance ethylene climacteric peak four days later. The ethylene peak occurred earlier in fruits which were not subjected to the injurious heat treatment (Paull and Chen, 1990). The ethylene synthesis pathway is well recognized in higher plants, and the regulatory control is obtained in two steps: the composition of, 1- ACC from S-adenosyl- LL -Methionine

(SAM) and the alteration of this intermediate into ethylene. The ACC synthase catalyzes the first level, while ACC oxidase catalyzes the second level of ethylene synthesis (Kende, 1993). A papaya fruit respiration rate at 20 °C at color break is 9-18 mg CO_2 $kg^{-1}.h^{-1}$ and at the ripe stage is 70-90 mg $CO_2.kg^{-1}.h^{-1}$. In the ripening fruit, ethylene production ranged from 7 to 10 $\mu g.kg^{-1}\cdot h^{-1}$ (Paull, 1993). In fruits and vegetables, gases are transported by means of the wax layer on the epidermis and through pores, like stomata and lenticels in the skin of the produce. Gases are mainly transported through the intercellular spaces in the tissues (Terashima et al., 2001).

Intercellular area is interconnected with narrow capillary tubes and supplies resourceful means of ventilation in fruit tissues (Burton, 1982). However, permanence, distribution, and free volume have impacts on the effectiveness of such spaces in the diffusion procedure (Burton, 1982). The molecules diffusion process in plant tissues is due to the morphology, such as size and channels number for aeration and the fruit metabolism. It is also expected that the diffusion attributes of fruit are varied and depends on the harvest time, cultivar, and section in the fruit. In addition, maturity might have an influence on diffusion properties (Ho et al., 2006). During ripening, the cell membranes breakdown, and the intercellular spaces in the tissues are filled with the cell components that induced a barrier to gas diffusion (Rajapakse et al., 1990).

CHAPTER 3

QUALITY CHARACTERISTICS OF CONTROL AND HEAT TREATED *CARICA PAPAYA* var. 'FRANGI' DURING THREE WEEKS OF STORAGE

3.1 Introduction

Papaya *(Carica papaya L.)* cultivation is extensive in tropical and subtropical regions. The United States, Europe and Japan import large volumes of this fruits because of its highly nourishing qualities. However, the fruits are chilling susceptible and highly perishable. Thus, it does not belong to a major class of traded fruits. In addition, long-time storage with a low temperature may cause physiological fruits injuries (Maharaj and Sankat, 1990).

The Frangi papaya or Paiola hybrid is a new variety with bright yellow skin, developed and promoted by a local exporter, the Malaysian Agrifood Corporation Berhad (Chan and Baharuddin, 2008). 'Frangi' papaya is in small size, has a long shelf life and takes 8 to 10 days to ripen completely after harvest. It has a sweet taste and pleasant aroma, with an attractive commercial market (Ong et al., 2013).Transporting of fresh papaya between countries has brought a risk of introducing insects and pests to new regions. Therefore, for export, fresh papaya should receive an approved quarantine treatment to avoid the distribution of exotic pests (Sivakumar and Marisa, 2012). Surface pests such as mites, trips, scales, aphids, whiteflies, or leafhoppers on exported fruits may cause

fruits rejections and hinder shipments by port inspectors (Paull and Chen, 2000). To overcome these challenges, fruits undergo postharvest heat treatments to control insect disinfestations, decay and to delay ripening and modification of fruits due to other stresses (Lurie, 1998a, Paull and Chen, 2000). Heat treatments, such as hot-water immersion, vapor heat, or forced hot air, are quarantine treatments approved for fruit flies and surface pests on papayas (Lurie, 1998a). Postharvest heat treatments are non-polluting and physically safe for control of diseases and insect disinfestations during fresh fruits postharvest storage and marketing (Lurie, 1998a). In a few countries, hot water treatment is used commercially as an effective decay control method (Jacobi et al., 1995). Fruit flies, such as the Asian papaya (*Bactrocera papayae*) and papaya (*Toxotrypana curvicauda*), are quarantine insects that limit papaya marketability (Pantoja et al., 2002, Yahia, 2006). Hot water treatment opportunities would allow commodities to be exported by less expensive sea transport, rather than by air (Fallik, 2004).

Reducing transport temperature slows down the ripening process. Tropical fruits experience chilling injury (CI) when stored at 10 °C and less (Lurie, 1998b). However, low-temperature storage (10-12 °C) can delay decay incidence in fresh papaya. An and Paull (1990), demonstrated a relationship between papaya postharvest life during storage and the maturity stage at harvest. Papaya harvested at mature green stage (less than 10% yellow) could be stored 2-3 weeks at 8-10 °C (An and Paull, 1990). Hot water treatment prevents rot development and kills skin-borne decay-causing agents as well as CI reduction (Lurie, 1998b, Ben-Yehoshua et al., 2000). Applying heat treatment at 45 °C

for 6 h to fruits before storage diminished CI symptoms of Sunrise papaya stored at 5 °C. It is possible to use a moderate treatment at non-lethal temperatures causing both a reversible delay of ripening and a decrease of fungal decay without significant changes in fruits quality (Lurie, 1998a). The goal of higher export rate with desirable quality and minimized CI has not been achieved yet. Thus, it is critical to evaluate the papaya physico-chemical characteristics after hot water treatment and during storage. Heat treatments will provide more commercial benefits for local and leader producers, even to comply with quarantine protocols from importer countries. The objective of this study was to investigate the effect of heat treatment and storage temperatures on postharvest quality characteristics of papaya during three weeks storage.

3.2 Materials and methods

3.2.1 Sample preparation and heat treatment

Papaya (*Carica papaya* cv. Frangi) fruits at maturity stage 2, with less than 10% yellow skin were harvested from a commercial farm in Lanchang, Pahang, Malaysia. Fruits were transported to the Postharvest Laboratory, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia within 3 h of harvest. Fruits of uniform size (450-550 g) and appearance and free from blemishes and defects were selected. Double-dip hot water treatment was applied on selected fruits by first immersing fruits in hot water at 42 °C for 30 minutes, and then followed immediately at 49 °C for 20 minutes (Alvarez and Nishijima, 1987). Hot water dip was performed in two water baths and the required temperature was kept constant with a thermostat. During the treatment, the

internal fruit's temperature was determined by inserting a thermocouple thermometer into the pulp of a few fruit samples. Hot water dip and control (without hot water dip) fruits were dipped in Folicur® (Tebuconazole fungicide, Bayer Advanced™) for less than 60 seconds, and then cooled for 20 minutes at room temperature (23-25 °C). Control and treated fruits were stored at 6 and 12 °C with 98% RH for 3 weeks. Changes in physical and chemical quality characteristics, CI incidence and enzyme activity of fruits were measured weekly. The experiment was repeated a total of three times, once each in June, July, August 2011. For each treatment, including the control, three replications of fruits were assessed.

3.2.2 Physical quality characteristics measurement

3.2.2.1 Firmness

Fruits firmness was measured using an Instron Universal Testing Machine (5543, Instron Corp. Minneapolis, USA) fitted with a 6 mm diameter cylindrical probe and a 5kg load cell. The Instron was used simultaneously with an Instron Merlin Software version M12-13664-EN. Each fruit was cut into three disks of 2cm thickness at the equatorial region. The probe was driven to a depth of 3 mm at a crosshead speed of 20 mm/min into each fruit disk. Firmness was measured at three equidistant points on each fruit disk, and the force was recorded in Newtons (N).

3.2.2.2 Color evaluation

Peel and pulp color changes were quantified in the CIE 1976 (L*, a*, b*) color space using a chroma meter (CR-300, Minolta Corp. Japan) equipped with an 8 mm diameter measuring head and calibrated with a standard white tile. Peel colour measurements were made at six random points on each fruits. Pulp colour was measured on six random points of each of the three fruits disks used for firmness measurement. The lightness coefficient, referred to as L*, ranged from black = 0 to white = 100 (McGuire, 1992). Hue angle (h° = arc tangent b*/a*) is defined as an angle on a colour wheel, 0° for red-purple color, 90° for yellow, 180° bluish-green and 270° for blue region. Chroma ($C^* = (a^{*2}+b^{*2})^{1/2}$) is the indicator of color purity with the variety from rich and vivid colors in circle circumference to the dull and grayish colors in the center.

3.2.2.3 Weight loss

Each fruit was weighed with a digital balance (B303, Mettler Toledo, Japan) before storage and then weekly for three weeks. Fruits percentage of weight loss was calculated according to the following formula:

$$\%\ \text{Weight loss} = \frac{\text{Fruit weight at } W_i - \text{fruit weight } W_{i-1}}{\text{Fruit initial weight}} \text{ x } 100$$

W_i = week = 1, 2 and 3.

3.2.3 Chemical quality characteristics measurement

3.2.3.1 Soluble solids concentration (SSC)

Treated fruits were peeled and diced. Then, a 20 g pulp sample was added to 80 ml distilled water and homogenized in a blender (MX-799S, Panasonic, Malaysia). The homogenate was filtered to get a clear juice through cotton wool. Two drops of the clear juice were dropped on the prism of a digital Refractometer (N-1E, Atago, Japan), and the reading was recorded. The SSC (%) was calculated with the following formula (Ranganna, 1997):

$$\text{SSC (\%)} = (\text{refractometry reading x dilution factor}) + 0.28$$

$$\text{Dilution factor} = \frac{1 + [\text{water volume (80 mL)}]}{[\text{Fruit sample weight (20 g)}]}$$

The readings were corrected to a standard temperature of 20 ºC by adding 0.28% to attain SSC percentage.

3.2.3.2 pH

Measurement of pH for extracted juice was with a pH meter (Delta 320, Mettler Toledo, Shanghai, China). The glass electrode was calibrated to pH 4 and 7 with the use of standard buffers before measurement. The electrodes of pH meter were put into each of the remaining filtrated sample of clear juice from the SSC measurement, and pH was

recorded when the reading became stable. Before and after measurement of each sample, the electrodes were washed with distilled water and wiped dry with a paper tissue, ready for the next sample.

3.2.3.3 Titratable acidity

The titration method was used to determine the samples' TA (Ranganna, 1997). Reagents were prepared as follows:

1. Phenolphthalein indicator (1%) was prepared by dissolving 0.5 g phenolphthalein in 50 mL ethanol solution. Then, the solution was kept in the refrigerator before use.
2. Sodium hydroxide (0.1 N NaOH) was prepared by dissolving 4 g of NaOH in 1 L distilled water. The solution was poured into a flask then the flask was sealed and kept in a cool place until use.

A 10 g sample of diced pulp from the SSC determination was homogenized with 40 ml distilled water in a blender (MX-799S, Panasonic, Malaysia). The homogenate was filtered through cotton wool. Five mL of the filtrate was poured into a conical flask, and two drops of phenolphthalein (1%) were added as an indicator. Then, the juice was titrated with 0.1 N NaOH until the color of the solution turned pink to an end point of pH 8.2. The volume of titration was recorded. The results were expressed as citric acid (Hernández et al., 2009), with the equivalent weight of 64 g.

$$\% \text{ Citric acid } = \frac{\text{mLNaOH x 0.1N NaOH x product vol. (100 mL) x citric acid g eq. weight (64)x 100}}{\text{Sample volume for titration (5 mL)x sample weight (10 g)x 1000}}$$

3.2.3.4 Ascorbic acid determination

Ascorbic acid content was determined quantitatively by using the dye method. Buffer solutions were prepared in advance as follows:

1. Metaphosphoric acid (3% HPO_3) was prepared by dissolving 3 g HPO_3 in 100 mL distilled water, and the solution was kept in a refrigerator.
2. Standard ascorbic acid solution was prepared by adding 0.1 g ascorbic acid to 3% HPO_3 until 100 mL volume was obtained. Then, 10 mL of the solution was diluted with 3% HPO_3 until 100 mL of ascorbic acid standard solution was obtained.
3. Dye solution was prepared by dissolving 21 mg sodium bicarbonate in 75 mL distilled water, and the mixture was boiled. Then, 25 mg sodium 2, 6-dichlorophenol-indophenol was added into the boiled solution.

   Dye factor calculation: 5 mL standard ascorbic acid was added into 5 mL of 3% HPO_3. The buffer was titrated with the dye until a distinct pink color remained for 15 seconds.

$$\text{Dye factor} = \frac{0.5}{\text{Titration volume (mL)}}$$

Twenty g of diced pulp from the SSC determination were homogenized with 80 mL of 3% HPO_3 in a blender (MX-799S, Panasonic, Malaysia) until a mixed homogenous juice was obtained. The sample was filtered through cotton wool. Five mL filtrate from each sample was taken and titrated with the former standardized dye solution until the juice color turned to pink. The volume of dye that was used for the titration was recorded each time, and ascorbic acid content was calculated according to the following formula (Ranganna, 1997):

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{mL used dye x dye factor x product vol. (100 mL) x 100}}{\text{Papaya sample weight (20 g) x sample vol. for titration (5 mL)}}$$

3.2.3.5 Ethylene and respiration rate

Fruits ethylene production and respiration rates were measured by using the static method, each week during the 6 and 12 °C storage duration. Three replications from control and treated fruits were chosen randomly and allowed to stabilize at room temperature. Measurements started 4 h after removal from storage. Each papaya was incubated for 3 h in a hermetically airtight container (1900 mL volume) fitted with a rubber septum. Ethylene and CO_2 production rates were measured simultaneously by withdrawing 1 mL of accumulated headspace gas with a syringe through the rubber septum. Then, the gas sample was injected into a gas chromatograph (Clarus-500, Perkin Elmer, USA) which was equipped with a flame ionization detector (FID) and thermal

conductivity detector (TCD), and fitted with a stainless steel porapak Q column (3m x 1/8 in; 50/80 mesh). Nitrogen with a flow rate of 30 mL.min^{-1}was used as the carrier gas while flow rates of hydrogen and air were 45 and 400 mL.min^{-1}, respectively. The injector, FID and TCD temperatures were maintained at 200 °C, and the oven temperature was 100 °C. Results for the ethylene production rate were expressed as µl C_2H_4/Kg FW.h and CO_2 production rate was expressed as mL CO_2/Kg FW.h. The gas chromatograph was calibrated by the standard gas injection (Appendix 4) and the ethylene and CO_2 production calculated.

$$\text{mL } CO_2/\text{Kg FW. h} = \%CO_2 \text{ x} \frac{\text{Container vol. (mL)} - \text{ fruit vol. (mL)}}{\text{Fruit weight (kg) x time (h) x 100}}$$

$$\text{Where } \%CO2 = \frac{\text{Area of the sample}}{\text{Area of the standard}} \text{x concentration of the gas standard (\%)}$$

$$\text{µl } C_2H_4/\text{Kg FW. h} = \text{ppm } C_2H_4 \text{ x} \frac{\text{Container vol. (mL)} - \text{ fruit vol. (mL)}}{\text{Fruit weight (kg) x time (h) x 1000}}$$

$$\text{Where } ppm\ C_2H_4 = \frac{\text{Area of the sample}}{\text{Area of the standard}} \text{ x concentration of the gas standard (ppm)}$$

3.2.4 Experimental design and data analysis

The experiments were conducted using a randomized complete block design (RCBD), in a factorial arrangement of treatments (2 heat treatment levels x 2 storage temperatures x 4 storage duration), with three replications of three fruits per replication. The data were subjected to analysis of variance (ANOVA), and means were separated by least significant difference (LSD) tests at P≤0.05 by Statistical Analysis System (SAS),

version 9.13 (SAS, Institute Inc., Cary, NC, USA). Regression analysis was carried out when the interaction effects between two factors were significant.

3.4 Results and discussion

3.4.1 Fruit color values (L*, C* and h°)

Papaya fruits peel and pulp were monitored each week for three weeks of storage to determine color changes during postharvest storage following heat treatment (double dip hot water treatment). There were no significant interaction effects between heat treatment, storage temperature and storage duration on peel L* (Table 3.1). Peel L* was also not significantly affected by the main effect of heat treatment and storage duration. However, peel L* was reduced significantly when the fruits were stored at 6 compared to 12 °C.

There were no significant interaction effects between heat treatment, storage temperature and storage duration on fruits peel C* (Table 3.1). Results showed no significant difference between peel C* of control and hot water dip fruits. Storage temperature at 6 °C significantly reduced fruits peel C* compared to storage at 12 °C. The significant positive correlation (r = 0.69) between peel L* and peel C* showed that an increase in peel L* value was followed by an increase in peel C* value (Table 3.2). The interaction effect between storage duration x storage temperature on peel h° of fruits was significant ($P \leq 0.05$). The heat treatment x storage temperature and heat treatment x storage duration had no interaction effects on peel h° (Table 3.1). There was a significant quadratic relationship ($R^2 = 0.73$) between peel h° and storage duration of fruits stored at 12 °C and 73% of the variability in peel h° was due to the storage duration (Figure 3.1).

Table 3.1 Main and interaction effects of heat treatment, storage temperature and storage duration on peel and pulp color (L*, C*, h°) of 'Frangi' papaya.

Factor	Peel			Pulp		
	L*	C*	h°	L*	C*	h°
Heat treatment (HT)						
Non hot water dip (control)	45.97 a[z]	25.00 a	115.41 a	50.29 a	32.09 a	48.49 a
Hot water dip	45.65 a	25.04 a	114.74 a	49.75 a	32.49 a	49.24 a
Storage temperature (ST) (°C)						
6	44.11 b	23.37 b	119.14 a	50.68 a	31.70 b	50.05 a
12	47.52 a	26.67 a	111.01 b	49.36 a	32.88 a	47.68 b
Storage duration (SD) (Weeks)						
0	46.17 a	21.89 b	119.98 a	51.29 ab	30.02 c	51.74 a
1	45.89 a	23.12 b	119.01 a	52.01 a	31.62 b	50.56 a
2	45.01 a	27.18 a	112.65 b	48.75 ab	31.91 b	47.34 b
3	46.18 a	27.88 a	108.65 c	48.02 b	35.61 a	45.83 b
HT x ST	ns	ns	ns	ns	ns	ns
HT x SD	ns	ns	ns	ns	ns	ns
SD x ST	ns	ns	*	ns	ns	ns
HT x ST x SD	ns	ns	ns	ns	ns	ns

[z] Means within a column and factor followed by the same letter are not significantly different by LSD test at P≤0.05.
ns = Non significant and * = Significant at P≤0.05.

During storage at 12 °C, peel h° was reduced dramatically by 17.5% between weeks 0 to 3 of storage, indicating that fruits were undergoing ripening. However, there was no significant relationship between peel h° and storage duration of fruits stored at 6 °C. The significant negative correlation (r = -0.32) between peel h° and peel L* indicated the increase in peel h° followed by a decrease in peel L* of fruits. Moreover, there was a significant negative correlation (r = -0.68) between peel h° and peel C*. It showed that the increase in peel h° corresponded with the decrease in peel C* (Table 3.2).

Table 3.2 Significant correlation coefficients (r) between 'Frangi' papaya peel and pulp (L*,C*,h°), firmness (Firm), soluble solids concentration (SSC), titratable acidity (TA), ascorbic acid (AA), pH, ethylene (C_2H_4), carbon dioxide (CO_2), weight loss (WL), peel electrolyte leakage (PEL), chilling injury (CI) peel and pulp, catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD).

	Peel L	Peel C	Peel h	Pulp L	Pulp C	Pulp h	Firmness	SSC	TA	AA	pH	C_2H_4	CO_2	WL	PEL	CI peel	CI pulp	CAT	APX	SOD
Peel L	--	0.69*	-0.32*	0.47*	------	-----	-----	-----	------	------	------	-----	-----	-----	------	------	-----	-----	-----	-----
Peel C		--	-0.68*	---	0.28*	-0.56*	0.64*	0.40*	-----	-----	-0.28*	-----	0.31*	0.54*	0.35*	-----	-----	0.34*	0.44*	----
Peel h			--	0.37*	-0.39*	0.59*	0.74*	-0.53*	-----	-0.30*	------	-----	-0.41*	-0.53*	------	-0.32*	-0.36*	-0.33*	-0.45*	----
Pulp L				--	-0.36*	0.39*	0.31*	-----	-----	-0.31*	------	-----	-----	-----	------	------	-0.37*	-----	-----	-----
Pulp C					--	----	-----	-----	----	-----	------	----	0.48*	0.60*	0.39*	0.44*	-----	-----	-----	-----
Pulp h						--	0.66*	----	-----	-----	-----	-----	-0.33*	-0.64*	-0.59*	-0.33*	-0.29*	-0.43*	-0.36*	-----
Firm							--	-0.38*	-----	-----	0.30*	-0.42*	-0.42*	-0.75*	-----	-0.33*	-0.32*	-0.39*	-0.56*	-----
SSC								--	----	0.42*	-----	-----	------	-----	-----	------	-----	0.30*	-----	-----
TA									--	-----	-----	------	------	-----	------	0.29*	0.44*	-----	-----	-----
AA										--	-----	-----	------	------	------	0.32*	0.42*	-----	-----	-----
pH											--	------	------	-0.29*	-----	-----	-----	-----	-----	-----
C_2H_4												--	-----	-----	-----	-----	-----	-----	-----	-----
CO_2													--	0.56*	-----	-----	-----	-----	0.45*	----
WL														--	-0.66*	0.39*	----	----	0.52*	0.30*
PEL															--	0.30*	-----	0.32*	-----	-----
CI peel																--	0.64*	-----	-----	------
CI pulp																	--	-----	-----	------
CAT																		--	0.34*	------
APX																			--	------
SOD																				--

Table 3.1 showed that there were no significant interaction effects between heat treatment, storage temperature and storage duration of papaya pulp L*. The pulp L* of control fruits was not significantly different from hot water dip fruits. Similarly, storage temperatures (at 6 and 12 °C) had no significant effect on pulp L*. Storage duration showed significant effects on pulp L*, with a significant reduction in L* from weeks 1 until 3 of storage. The correlation between peel and pulp L* showed a significant positive correlation (r = 0.47). Thus, the increase in peel L* could be followed by the increase in pulp L*. There was also a significant positive correlation (r = 0.37) between pulp L* and peel h° (Table 3.2).

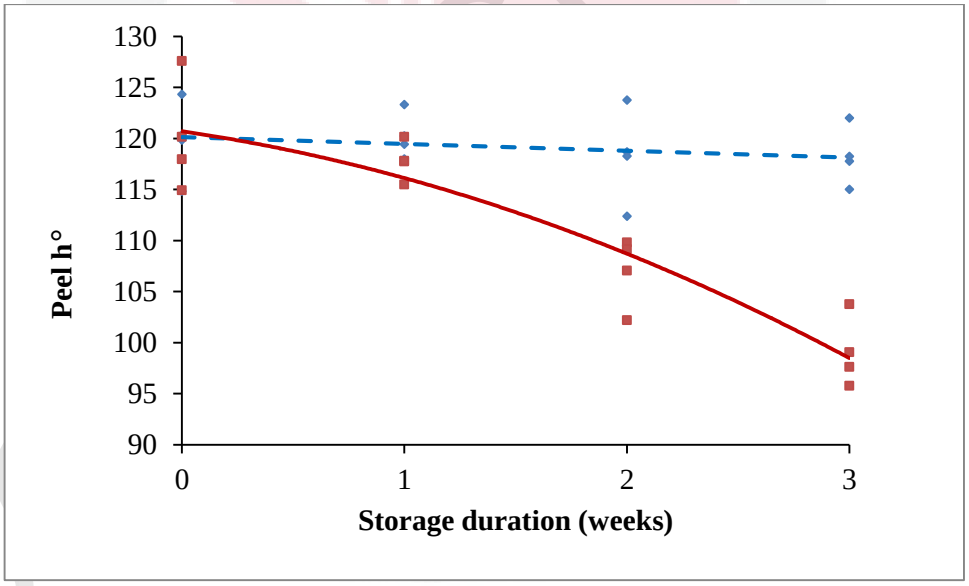

Figure 3.1 Relationship between peel hue (h°) and storage duration of 'Frangi' papaya stored at 6 °C (♦) and 12 °C (■).
y (12 °C) = 120.7-3.16x-1.41x^2 (R^2 = 0.73). Solid line indicates a significant quadratic relationship.

There were no significant interaction effects of heat treatment, storage temperature and storage duration on pulp C*. Pulp C* significantly affected by the storage temperature and fruits stored at 12 °C indicated a higher value compared with 6 °C storage. Pulp C* was also significantly affected by the storage duration and increased almost by 18/5% (Table 3.1). A significant positive correlation existed between pulp C* and pulp L* ($r = -0.36$). Also, there were significant positive correlations between peel C* and pulp C* ($r = 0.28$) (Table 3.2).

There were no significant interaction effects of heat treatment, storage temperature and storage duration on pulp h°. Storage temperature significantly reduced pulp h° by 5% when fruits were stored at 12 °C compared with stored fruits at 6 °C. Fruits h° significantly reduced from weeks 1 to 2 during the storage (Table 3.1).

In this study, heat treatment did not have significant effects on papaya peel and pulp color values. Heat treatment prevented the reduction of peel L* and C* values resulting in less harmful effects on fruits colour compared to fruits in the control treatment. However, different results were reported on banana. In control banana, peel L* increased sharply, but in hot water treated banana it increased slightly (Ummarat et al., 2011). The results obtained in this experiment were in agreement with Paull and Chen (1990). They found that exposure of fruits at 42 °C for 30 min, and then at 49 °C for up to 70 min, allowed papaya to develop normal yellow skin during ripening. Normal L* and C* in treated fruits could be the sign of delayed CI onset in treated fruits (Chen et al., 2008). The peel color progression from dark green in immature fruits to orange red in fully-ripe

fruits and, usually in climacteric fruits is due to the degradation of chlorophyll structure. The main factor for this degradation is pH changes (almost because of organic acid leakage from vacuoles), oxidative systems and chlorophyllase enzymes (Yang et al., 2009b). Color loss depends on one or all of these agents acting respectively to damage the chlorophyll structure. Normally, the failure to ripen in chilling temperature could be the consequence of interruption in the conversion of chloroplasts to chromoplasts due to the destruction of plastids (Yang et al., 2009b). In this study, peel L* and C* were lower at 6 °C (chilling temperature) compared to stored fruits at 12 °C, the same results were obtained by Chen et al. (2008) ,whereby, it could be an indicator of peel browning at the cooler storage temperature. The banana peel values decreased at 8 °C storage during 12 days. In addition, changes in the skin color values coincided with visible peel browning in banana.

The papaya fruits stored at 6 °C retained similar colour of grayish-green during the whole three weeks of storage period as evidenced by the non-significant linear relationship between peel h° and storage duration (Figure 3.1). During storage of carambola (star fruit) fruits at 5 °C, color progression was suppressed, and the fruits failed to develop their color (Zainon et al., 2004). They usually appeared dull, and sometimes some of the carambola fruits could not reach the fully ripened color stage. Papaya fruits color was affected by chilling temperature and did not change after being transferred from cold storage to 25 °C. The fruits remained with a dull greenish color and did not ripen normally (Lam, 1990). The mentioned results were the same as for this experiment as shown in Figure 3.1. Fruits stored at a lower temperature failed to ripen

normally, because of the prior progression of decay and tissue collapse (Wills and Widjanarko, 1997). Ripening inhibition occurred indirectly in HWRB of treated melons and tomatoes by prohibiting color development (Fallik et al., 2000). In the present study, all color parameters in the peel and pulp were not affected by heat treatment, and there were no significant changes between hot water dip and control fruits (Table 3.1). Similar findings were reported in tomato whereby heat treatment effect was not observed due to insufficient length of the treatment period (Lu et al., 2010).

During the first few days of storage, fruits colors as indicated by peel h° in the two different storage temperatures were relatively similar (Figure 3.1). As time passed, color differentiation became greater until the third week when h° was reduced at 12 °C. Thus, fruits looked more mature with respect to their yellow color. It seemed that fruits color development was sensitive to temperature changes. At higher storage temperatures, the color change from green to yellow or orange became faster compared to storage at lower temperatures (An and Paull, 1990). Papaya color began to change from green to greenish orange-yellow after 6-8 days at 10 °C, which means that fruits were capable of ripening at this temperature, but colour changes were not uniform (Proulx et al., 2005). In this study, papaya fruits stored at ambient temperature (12 °C) resulted in a normal color development from light green to fully yellowish-orange (Figure3.1). Earlier reports mentioned the evidences of changes in papaya peel C* and h°, when green spots among the yellowish-orange color existed when the fruits were moved to ambient temperatures (Paull and Chen, 1983). Furthermore, the papaya's L* values did not change at 0, 5 °C during storage in comparison with stored papaya at 15 and 20 °C.

It could be the result of chilling storage temperature and that fruits were not able to ripen normally (Paull and Chen, 1983). Pulp L* had no significant changes with storage temperature and heat treatment (Table 3.1), but other reports mentioned that heated papaya fruits at 49 °C for 70 min caused slower rate of internal carotenoid color development (Paull and Chen, 1990). The Japanese plum (*P. salicina Lindell* cv. 'Amber Jewel') had a lower flesh chromaticity L* value which could be attributed to CI due to flesh browning or translucency (Singh et al., 2009). The finding is similar to that in this experiment. Storage duration reduced the pulp L* during first and third weeks of storage, indicating a pulp color darkening (Table 3.1). Results on mango fruits indicated that the yellow-orange color of the mesocarp became more intense with an increase in carotenoid content as the storage time passed.

The increase in intensity of yellow-orange color of the epidermis and mesocarp of mango fruit was due to an increase in the C*, and a reduction in the L* and h°. The C* and L* were reported during the ripening of 'Nam Dokmai', 'Dashehari' and 'Kaew' mangoes (Mahayothee et al., 2004, Saranwong et al., 2004, Jha et al., 2006). The results on the L*, C* and h° values were obtained on Frangi papaya pulp color were similar (Table 3.1). In the present study, storage temperature at 12 °C had a lower pulp h° value of papaya fruits than those stored at 6 °C. Fruits at higher storage temperatures showed more yellow to orange-reddish flesh color than the fruits stored at lower storage temperatures. According to (Saltveit, 1999), the reduction of h° in higher storage temperature could be related to an increase in carotenoid synthesis as the fruits ripened normally.

In climacteric fruit such as mango, respiration rate increased in the higher storage temperature and consequently the carotenoid synthesis would also increase (Saltveit, 1999). In this study, fruit storage at 6 °C could decrease pulp carotenoid expression as the storage of 'Satsuma' mandarins at 5 °C slowly decreased carotenoid content in the flavedo (Matsumoto et al., 2009). The carotenoid content reduced in the pulp and consequently decreased the expression of key carotenoid biosynthetic genes in both tissues (Matsumoto et al., 2009). Storage of 'Navelina' orange fruits at 12 °C stimulated the expression of carotenoid biosynthesis genes and enhanced carotenoid contents, hence increased coloration in both pulp and flavedo of fruits. However, at 2 °C storage these parameters were almost unaffected (Carmona et al., 2012).

3.4.2 Chemical quality characteristics

There were no significant interaction effects of heat treatment, storage duration and storage temperature on SSC (Table 3.3). The SSC was not affected by heat treatment, but storage temperature had a significant effect on SSC. SSC of papaya fruits stored at 6 °C was reduced significantly by 7% compared to stored fruits at 12 °C. SSC remained consistent during storage except on week 1 when SSC of fruits was reduced significantly to almost 7 and 9% compared to SSC on weeks 2 and 3, respectively (Table 3.3). There was a significant positive correlation between SSC and peel C* ($r = 0.40$), moreover there was a significant negative correlation between SSC and peel h° ($r = -0.53$). SSC showed a significant negative correlation ($r = -0.38$) with firmness (Table 3.2).

Table 3.3 Main and interaction effects of heat treatment, storage temperature and storage duration on soluble solids concentration, citric and ascorbic acid and pH of 'Frangi' papaya.

Factor	Soluble solids concentration (%)	Titratable acidity (%)	Ascorbic acid (mg/100g)	pH
Heat treatment (HT)				
Non hot water dip (control)	11.64 a[z]	0.12 a	33.16 b	5.58 a
Hot water dip	11.21 a	0.12 a	36.33 a	5.57 a
Storage temperature (ST) (°C)				
6	11.04 b	0.12 a	34.66 a	5.58 a
12	11.81 a	0.12 a	34.83 a	5.56 a
Storage duration (SD) (Weeks)				
0	11.46 ab	0.11 a	35.00 ab	5.56 b
1	10.82 b	0.12 a	32.33 b	5.71 a
2	11.58 a	0.11 a	34.33 ab	5.50 b
3	11.84 a	0.14 a	37.33 a	5.52 b
HT x ST	ns	ns	ns	ns
HT x SD	ns	ns	ns	ns
SD x ST	ns	ns	ns	ns
HT x ST x SD	ns	ns	ns	ns

[z] Means within a column and factor followed by the same letter are not significantly different by LSD at P≤0.05.
ns = Non significant at P≤0.05.

The TA was determined and expressed as citric acid content since it was the predominant acid in the papaya fruits (Hernández et al., 2009). As the results showed, there were no significant main and interaction effects of heat treatment, storage duration and storage temperature on TA (Table 3.3). There were no significant interactions effects of heat treatment, storage duration and storage temperature on ascorbic acid content, but storage duration had variable effects on ascorbic acid content (Table 3.3).

The ascorbic acid content was reduced during week 1 compared to week 3 of storage. There was significant positive correlation between ascorbic acid content and SSC ($r = 0.42$). The significant negative correlation existed between ascorbic acid and peel h° ($r = -0.30$) (Table 3.2). There were no significant interaction effects of heat treatment, storage duration and storage temperature on pH. The values of pH were not significantly affected by the main effects of heat treatment and storage temperature. However, pH was affected significantly by the storage duration and was increased during week 1 then, decreased at week 2 and remained relatively constant afterwards (Table 3.3).

The results on SSC and TA obtained in this study were similar to the study on control and hot water treated Satsuma mandarins, where through the postharvest periods, limited changes occurred in fruits quality indices. However, the low metabolic activity during prolonged storage could lead to internal metabolite alterations (Ghasemnezhad et al., 2008). In this study, the increasing SSC trend during papaya ripening and softening was similar with correlation results and it was mentioned earlier by (Paull and Chen, 2000) (Table 3.3). The SSC varied from 6-19% depending on fruit cultivars. The minimum SSC of papaya should be 11.5% in order to be at the market level (papayas, 2005). Mango fruits had a significantly higher SSC during storage irrespective whether the fruits were given hot water treatment or not (Tovar et al., 2000). In present study, it could be concluded that fruits stored at 6 °C did not reach the same stage of ripeness as those stored at 12 °C. In papaya fruits, SSC values were maintained approximately constant during the storage (Argañosa et al., 2008). Proulx et al., (2005) showed that the fruits SSC were reduced during the storage. After 6 days storage at 20 °C fruits SSC

were reduced by 42% and 30%, respectively, compared to the preliminary values. Paull and Chen (2000) reported that there were no significant effects of heat treatment on SSC of grapefruit, tomato, mango, and orange.

In this study, TA was not significantly affected by heat treatment, storage temperature and storage duration (Table 3.3). However, Lazan et al., (1989) reported that TA increased during papaya fruit ripening (to 75% yellow skin color) and it was reduced thereafter. Reports on 'Golden' and 'Pococi' ('Sunrise' or 'Solo-type') papayas (Bron and Jacomino, 2006, Schweiggert et al., 2011) were similar with Lazan et al., (1989). 'Pluk Mai Lie' papaya had similar results, whereby the TA did not change significantly during ripening (Fuggate et al., 2010). Results of Proulx et al. (2005) and Maharaj and Sankat (1990) were in agreement with this experiment, there was no significant effect of the storage temperature or duration on the papaya's TA.

The ripeness alteration of papaya such as yellowing did not have an effect on the fruits acidity. Papayas contain small amounts of organic acids, and the most common acids are citric and malic acids (Lancashire, 2007). The acid concentrations are known to be reduced during the ripening process (Medlicott et al., 1986). Hernández et al., (2006) indicated TA values for a ripe papaya were approximately 72 ± 8 mg citric acid per 100 g fruit. The papaya in their study were harvested at mature green stage and ripened at 18 °C with a SSC of 12.1±1.1 °Brix. However, other studies indicated a TA value of 15±1 mg citric acid per 100 g fruit (Fernandes et al., 2006). The TA in chitosan coated 'Eksotika II' papaya ranged from 0.22% on the first day of storage at 12 °C and was

reduced to 0.18% during 4 weeks of storage (Ali et al., 2011). The ascorbic acid content changed during the storage as the concentration increased significantly by week 3 compared to week 1 (Table 3.3). The reduction in ascorbic acid concentration occurred in mango as the ripening improved (Tovar et al., 2001). Another hypothesis mentioned that the ascorbic acid increased on a fresh weight basis during storage and it might be related to water loss through the storage rather than the actual increase in ascorbic acid concentration (Nunes et al., 1998). Dea et al. (2010) mentioned that there was no significant difference in ascorbic acid amount of hot water treated and control 'Kent' mango. In another experiment, heat-treated strawberry fruits showed higher levels of ascorbic acid than control fruits (Vicente et al., 2006). The earlier reports mentioned that ascorbic acid content of papaya increased during ripening (Lee and Kader, 2000). In this experiment, storage duration affected ascorbic acid content and it fluctuated during the three weeks of storage. Hawaiian papayas (1/8 ripe stage) contained 45-55 mg L-ascorbic acid per 100 g fresh weight (Wall, 2006).

In present study, the pH value was not significantly affected by the storage duration and temperature and heat treatment (Table 3.3). As mentioned earlier, changes during the storage were so small, and it could not have produced considerable differences on fruits taste evaluation. No significant trend for pH was established which was related to the hot water treated fruits (Dea et al., 2010). It has been studied previously that there were no significant pH changes during the storage of mango fruits (Paull and Chen, 2000, Gil et al., 2006, Gonzalez-Aguilar et al., 2008a). The papaya pH value decreased at 15 or 20 °C storage (Proulx et al., 2005). Likewise, the pH value of cherry fruits stored at lower

temperatures remained quite stable through the storage (Yaman and Bayoindirli, 2002). Organic acids in papaya are mostly citric, and malic acids and these acids are the substrates for enzymatic reactions during respiration. Thus, an increase in pH value, and a decrease in acidity is expected during storage (Yaman and Bayoindirli, 2002). Solo papaya commercialized in four establishments of Brasilia (Distrito Federal) showed a range of pH values from 5.20 to 5.71 (Fagundes and Yamanishi, 2001).

3.4.3 Weight loss

There was a significant ($P \leq 0.05$) interaction effect of heat treatment x storage duration on weight loss of papaya fruits (Table 3.4). There was a significant linear and quadratic relationship between weight loss and storage duration of control and hot water dip papaya, respectively. The $R^2 = 0.83$ for control and $R^2 = 0.69$ for treated fruits indicated that 83% and 69%, respectively, of the variability in weight loss was due to the storage duration (Figure 3.2). In control fruits, the weight loss increased drastically until the end of the storage. The increasing trend was observed in treated samples, but after week 2 of storage, the weight loss increased with the lower percentage in treated fruits compared to the control fruits. The effects of hot water dip could be observed after week 2 of storage (Figure. 3.2). There was a significant ($P \leq 0.05$) interaction effect of storage duration x storage temperature on weight loss. However, there were no other interaction effects on weight loss of papaya (Table 3.4).

Table 3.4 Main and interaction effects of heat treatment, storage temperature and storage duration on weight loss and firmness of 'Frangi' papaya.

Factor	Weight Loss (%)	Firmness (N)
Heat treatment (HT)		
Non hot water dip (control)	2.07 a[z]	40.44 a
Hot water dip	1.93 a	35.24 b
Storage temperature (ST) (°C)		
6	1.74 b	47.16 a
12	2.26 a	28.52 b
Storage duration (SD) (Weeks)		
0	0 d	62.64 a
1	1.71 c	47.10 b
2	2.55 b	19.48 c
3	3.76 a	22.14 c
HT x ST	ns	ns
HT x SD	*	*
SD x ST	*	*
HT x ST x SD	ns	*

[z] Means within a column and factor followed by the same letter are not significantly different by LSD at P≤0.05.
ns = Non significant and *= Significant at P≤0.05.

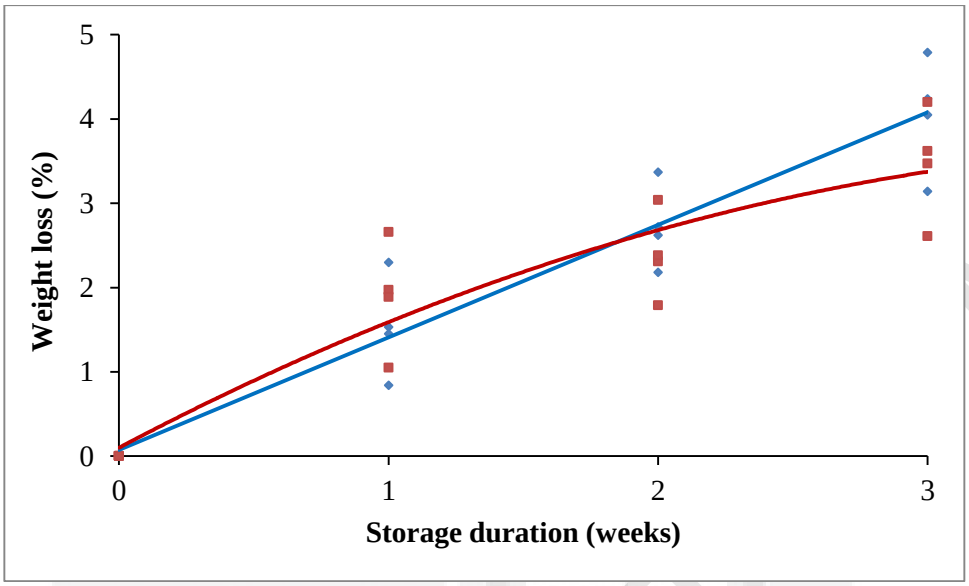

Figure 3.2 Relationship between weight loss (%) and storage duration of 'Frangi' papaya in control (♦) and hot water dip (■) fruits.
y (control) = 0.073+1.33x (R^2 = 0.83), and y (hot water dip) = 0.1+1.69x-0.2x^2 (R^2 = 0.69). Solid line indicates a significant quadratic relationship.

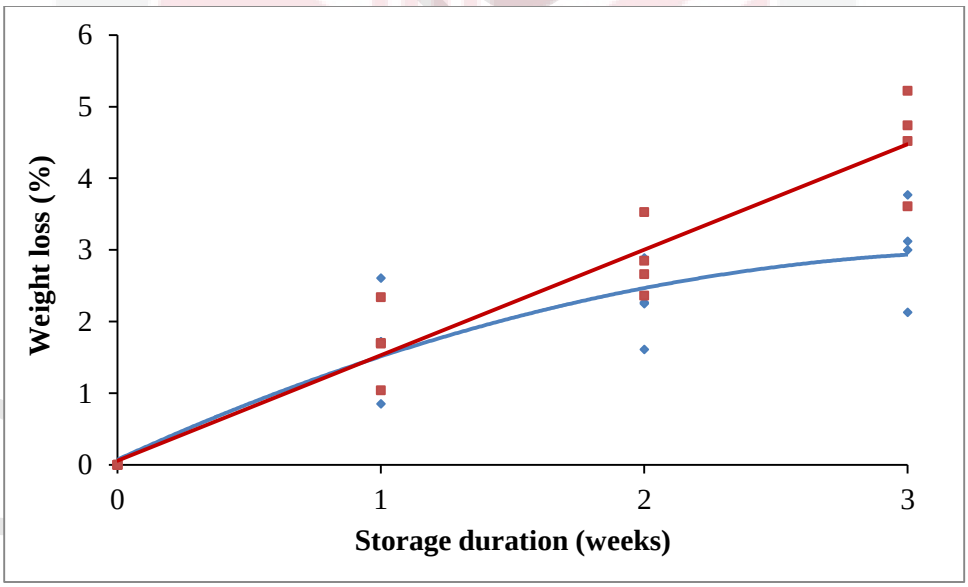

Figure 3.3 Relationship between weight loss (%) and storage duration of 'Frangi' papaya stored at 6 °C (♦) and 12 °C (■).
y (6 °C) = 0.072+1.68x-0.24x^2 (R^2 = 0.71) and y (12 °C) = 0.058+1.47x (R^2 = 0.90). Solid line indicates a significant quadratic relationship.

The regression analysis showed that there were significant linear and quadratic relationships between weight loss and storage duration at 6 and 12 °C storage. The R^2 was 0.71 and 0.90, respectively, indicating that 71% and 90% of the variability in weight loss was due to the storage duration (Figure 3.3). There were similar increases in weight loss up to week 1 of storage for both storage temperatures. However, from weeks 1 to 3, there was a sharp increase in fruits weight loss stored at 12 °C compared to the fruits stored at 6 ºC. Data mean in week 3 showed that, stored fruit at 12 °C had 1.52% higher weight loss than the fruit stored at 6 °C (Figure. 3.3). There was a significant negative correlation (r = -0.75) between weight loss and firmness (Table 3.2). This indicates that increasing fruits water loss resulted in reduction of fruits firmness. There was a significant positive correlation (r = 0.55) between weight loss and CO_2 production. Weight loss increase was accompanied by an increase in CO_2 production. Weight loss was correlated significantly with peel C* and h° (r = 0.54) and (r = -0.53), respectively (Table 3.2).

The correlation results indicated that as the fruits ripening progressed (increase in C* and decrease in h°) the fruits weight loss increases and it is similar with the obtained results on 'Flordaking' peach fruits during the storage (Tareen et al., 2012). The phenomenon could be due to the color saturation which resulted in higher transpiration from the surface of fruits, and it is evident from the weight loss percentage (Tareen et al., 2012). Reduction of fruits firmness was correlated significantly with the increase in fruits weight loss. The beginning of softening is related to the alteration of water-insoluble protopectin into water-soluble pectin, which finally caused cell wall

deterioration and increase in water loss (Fishman et al., 1993). Heat treatment is capable of increasing or decreasing fruits water loss, and it depends on treatment and the commodity (Hong et al., 2007). In this study, heat treatment induced weight loss reduction after week 2 of storage (Figure 3.2). The photomicrography of the papaya's epidermis after 1 min heat treatment at 45, 55 and 65 °C showed many crystalloids on the fruit's surface (Kechinski et al., 2012). Crystalloid formation is typically a characteristic of the outer wax layer in plant tissues and fruits induced by heat treatments (Roy et al., 1994, Montero et al., 2010). Montero et al. (2010) pointed out that wax formation was observed in apple fruits subjected to heat treatment. It occurred, after two weeks of storage, to fill in the cracks on the cuticle. Wax formation could be related to the inhibition of fruits dehydration. It can be concluded that the same phenomena occurred in this experiment and weight loss was reduced after week 2 of the storage (Figure 3.2).

Crystalloids do not appear naturally on papaya fruits. They appear as a result of heat treatment. As is shown in Figure 3.2, it is probable that crystalloids did not form in the control samples, and this is similar with results obtained by Kechinski et al. (2012) on application of HWRB method to clean and disinfect fresh harvested papaya at temperatures between 45, 55 and 65 °C for 60 seconds. The dominant form of crystalloid distinguished on 'Golden' papaya consisted of tubules that, in some cases, took shape after harvest. It has been reported that temperature has a direct effect on crystalloid formation. As the temperature increases, the crystalloid quantity will increase (Barthlott et al., 1998). The scanning electron microscope (SEM) images of the papaya's

cuticle revealed that heat treatment modified the natural wax layer of the papaya, and produce a more uniform covering with fewer cracks (Montero, 2007). In addition, hot water treatment could cover stomata and cracks with a wax layer. These cracks are potentially an essential entrance sites for pathogens into the fruits (Montero, 2007). It is vital how a postharvest treatment can have effects on the morphology of epidermal characteristics (Montero, 2007). The higher the temperature, the smoother the epidermal wax will cover the fruit surface. The combination of temperature and time applied in the treatment should be chosen carefully so as not to damage the papaya's epicarp. The heat treatment temperature of 65 °C causes heat damage (Kechinski et al., 2012). The application of heat treatments causes the increase in fruit resistance to molds. Hot water treatment will cause melting of the epicuticular waxes that cover the cracks. Consequently, the pathogen entry is inhibited (Schirra et al., 2000). Hot water treatment increases weight loss in 'Navel' oranges during storage (Birla et al., 2005), but reduces weight loss in 'Valencia' oranges, grapefruits (Mohammed and Brecht, 2002) and 'Star ruby' grapefruit (Porat et al., 2000c).

Weight loss of 'Satsuma' mandarin does not significantly change after hot water treatment. The heat treatment effect on weight loss could be due to the different fruits reactions to heat treatment (Hong et al., 2007). These responses to heat treatment resulted from a combination of factors such as storage temperature, host, time and temperature of exposure. The treatment methods and commodity physiological age also were prominent factors (Lydakis and Aked, 2003). In this study, the weight loss percentage increased significantly during 3 weeks of storage at 12 °C (Figure 3.3).

It might be related to the fruits ripening stage and progression. The results in this experiment (Figure 3.3) were similar with Proulx et al. (2005) who reported that fruits stored at higher storage temperatures lost more weight compared with fruits stored at a lower temperature. The loss of approximately 8% of the primary weight from Sunrise and Sunset mature-green papayas caused rubbery texture, slight to moderate skin shrivel, low-gloss, and unmarketable fruits (Paull and Chen, 1989). In this study, papaya weight loss never reached the 8% limit. Weight loss percentages in stored papaya at 12 °C were more than 4%, but stored fruits at 6 °C showed 3% weight loss (Figure 3.3).

The amount of water loss from fruits surface could be related to the fruits epidermis characteristics. Storage of carambola fruits at 10 °C considerably reduced water loss, but at 5 °C, water loss was increased relative to the storage at 10 °C, particularly after day 10 (Zainon et al., 2004). The results on carambola fruits were in contrary with the obtained results in this experiment. Stored papaya at 6 °C had reduced amount of water loss after 14 days, and from days 0-7, weight loss increased steadily in both storage temperatures (Figure 3.3).

In apple cultivars, changes in the appearance of cracks on the fruit surface occurred as a consequence of fruit expansion during ripening and development (Roy et al., 1994). In addition, the crack's appearance was related to humidity losses through the apple's cuticle (Veraverbeke et al., 2006). In this study, after the first week of storage at 6 °C water loss increased gradually as it could be the result of the disappearance of cracks, together with lenticels during cold storage (Veraverbeke et al., 2006).

3.4.4 Firmness

Firmness in papaya (*Carica papaya* L.) cultivars demonstrates a wide variation in fruits softening rates, a parameter that indicates fruits quality and shelf life, and also it is the result of cell wall degradation (Thumdee et al., 2010). In the present study, there was a significant ($P \leq 0.05$) interaction effect of heat treatment x storage duration on the firmness of the papaya fruits (Table 3.4). Regression analysis indicated that there was a significant linear and quadratic relationship between firmness and storage duration on control and treated samples. As illustrated in Figure 3.4, firmness declined in both control and treated fruits, but from weeks 0 to 2, control fruits remained firmer than the treated ones.

Firmness difference between control and treated samples was highest during the first week of the storage, and this was the greatest difference occurred during the whole storage. After the second week of storage treated samples reserved their firmness and the graph shows a constant trend until the end of storage. Firmness in control fruits was reduced continuously until the third week of storage. In the control samples $R^2 = 0.59$ and in the treated samples $R^2 = 0.53$ which indicates that the 59% and 53%, respectively, of the variability in firmness was due to the storage duration. Table 3.2 showed the significant positive correlation between peel h° and pulp L* with firmness ($r = 0.74$) and ($r = 0.31$), respectively. There was a significant negative correlation ($r = -0.64$) between firmness and peel C*. There was a significant ($P \leq 0.05$) interaction effect of storage duration x storage temperature on firmness (Table 3.4).

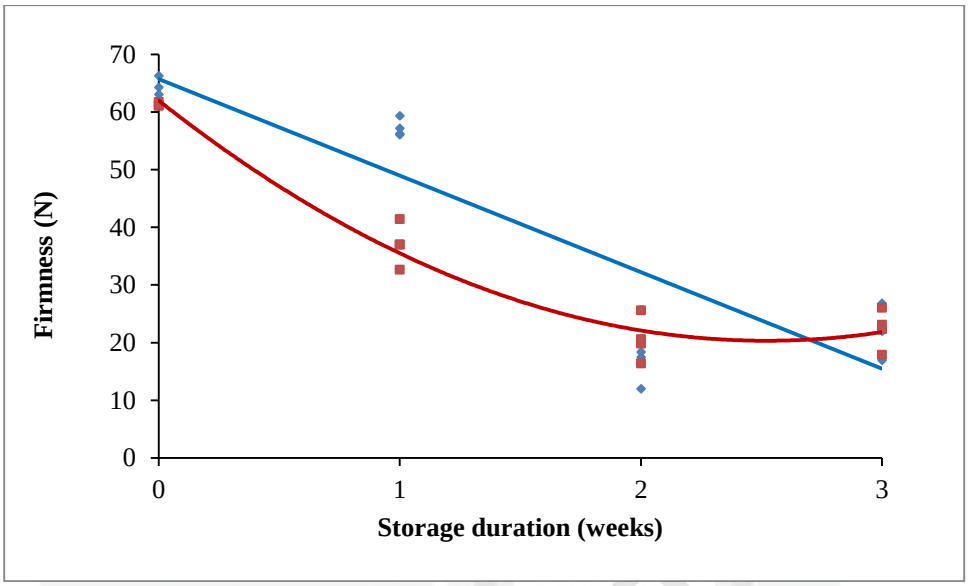

Figure 3.4 Relationship between firmness (N) and storage duration of 'Frangi' papaya in control (♦) and hot water dip (■) fruits.
y (control) = 65.29-16.56x (R^2 = 0.59) and y (hot water dip) = 61.57-32.58x+6.44x^2 (R^2 = 0.53). Solid line indicates a significant quadratic relationship.

However, there was no interaction effect of heat treatment x storage temperature on firmness. The results from the regression analysis show that there were significant linear and quadratic relationships between firmness and storage duration at 6 and 12 °C (Figure 3.5). Stored fruits at 6 °C indicated higher firmness than stored fruits at 12 °C. There was no significant difference on the firmness among the stored papaya at 6 and 12 °C during the first three days of storage. Fruits firmness at 12 °C storage reduced sharply from day 3 until day 21. The R^2 at 6 and 12 °C storage was 0.59 and 0.80, and it showed that 59% and 80%, respectively, of the variability in firmness was due to the storage duration. After week 1, stored fruits at 6 and 12 °C indicated a considerable difference in firmness reduction. The greatest firmness difference between 6 and 12 °C occurred at week 3.

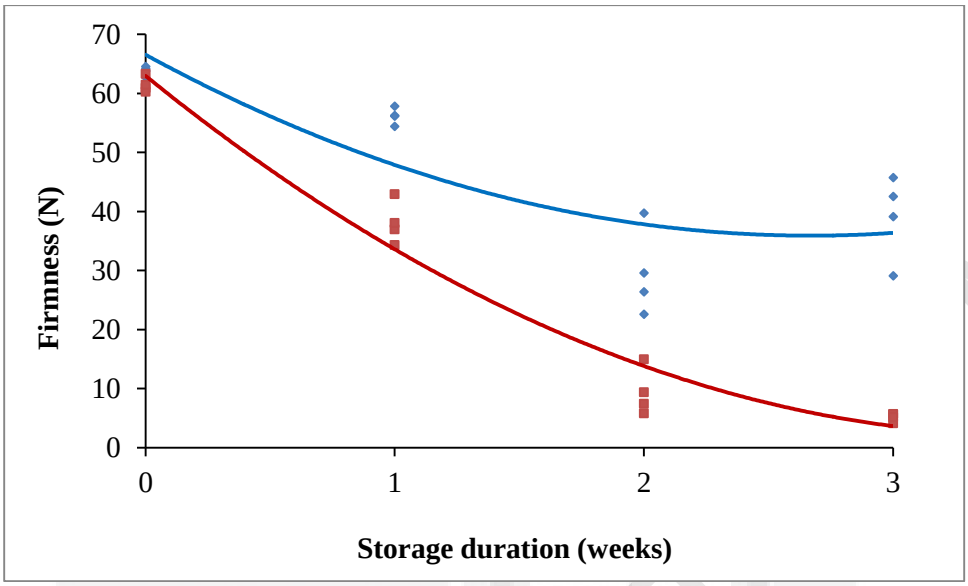

Figure 3.5 Relationship between firmness (N) and storage duration of 'Frangi' papaya stored at 6 °C (♦) and 12 °C (■).
y (6 °C) = 66.55-22.95x+4.30x^2 (R^2 = 0.59) and y (12 °C) = 62.97-34.16x+4.79x^2 (R^2 = 0.80). Solid line indicates a significant quadratic relationship.

Fruits firmness was reduced by 38.5% at 6 °C and 91% at 12 °C during 3 weeks of the storage. Stored fruits at 6 °C remained firmer than 12 °C and after week 2 at 6 °C firmness remained relatively constant (Figure 3.5). The degradation of hemicellulose, as well as pectin, may have a significant role in softening of papaya fruits (Paull and Chen, 1990). Figure 3.4 showed that firmness reduction in control fruits followed a decreasing trend from the first until the last day of storage. Meanwhile, softening occurrence in treated fruits was more than untreated samples, but after the second week of storage the graph showed a steady state until the end of the storage. The reduced softening in hot water dip fruits was related to the melting or re-crystallization of the wax layer after 2 weeks of storage (Fallik et al., 1999, Fallik et al., 2000). A wax layer covered obvious cracks in the cuticle that water could escape through. This covering of cracks or normal openings significantly decreased the weight loss and consequently maintained the fruits

firmness after long storage. Lurie (1998a) indicated that fruit softening reduction of heat treated fruits could be related to the pectin hydrolysis inhibition and a decreasing level of cell wall degrading enzyme activity. Consequently, fruit softening will cause inhibition of ethylene production due to a reduction in ethylene-forming enzyme (EFE) activity. In this experiment, fruits firmness reduction in treated fruits was inhibited after week 2 (Figure 3.4) and consequently ethylene production of treated fruit was reduced after week 1 of storage until week 2 (Figure 3.6). The obtained results were also similar with Paull and Chen (1990) who reported that softening in hot water treated papaya fruits was delayed as showed by higher deformation force 10 days after treatment. Some postharvest softening occurred as indicated by a reduction in deformation force in papaya fruits that were exposed for more than 40 min at 49 °C.

Softening disruption was not improved even after returning the fruits to the ambient temperature. However, softening due to more than 40 min at 49 °C took place in both the outer shell and inner mesocarp area near the seed cavity (Paull and Chen, 1990). The inner area of the mesocarp of papaya fruit is found to be commonly and severely affected with hard lumps in response to hot water treated induced failure of softening (Paull and Chen, 1990). Moreover, heat-injured mango fruits can also soften quickly or indicate abnormal softening and some flesh regions remain hard while others soften (Lurie, 1998a, Jacobi et al., 2001). In this study, until the second week of storage, there was no hot water dip effect on fruit firmness reduction (Figure 3.4). This result is in contrast with the findings of Jacobi and Giles (1997), who indicated that control mango fruits were firmer than vapor heat treated or hot water treated mangoes, both at the eat-

ripe phase and 10 days after storage. Heated fruits (with 25% or 50% of yellow skin) contained lower amounts of PG than fruits at the color-break phase after 65 min of exposure at 46 °C (Chan et al., 1981). This finding could be the result of denaturation or disruption of PG synthesis. PG showed first-order heat denaturation kinetics and was denatured by 60% in 20 min at 70 °C (Chan and Tam, 1982). It is expected that the papaya fruits exposure to 49 °C induced minimal denaturation. The failure to soften could be attributed to the mRNA suppression for wall softening enzymes as found out in tomatoes (Picton and Grierson, 1988). The recovery failure in papaya softening could be related to the limited production of mRNAs for cell wall. The mRNAs for cell wall degrading enzymes is produced only for a short period during specific ripening stage (Paull and Chen, 1990).

The papaya inner mesocarp showed considerable carotenoid progression with a less than 10% yellow skin while the outer mesocarp did not show carotenoids (Paull and Chen, 1983). It has been suggested that the inner mesocarp was susceptible to heat stress at the advanced ripening stage, even more than the less mature outer region. The inner mesocarp received an injurious temperature treatment that eventually disrupted softening. In comparison, the outer mesocarp even reached a higher temperature and was not damaged, because it was not at a susceptible stage (Paull and Chen, 1990). The production of papaya cell wall PG and xylanase occurred briefly during ripening (Grierson and Kader, 1986). It was in contrast with continual production of PG in tomatoes and cellulase in avocado (Grierson and Kader, 1986). In papaya, PG and xylanase have peak activity when the fruit is 40% to 60% yellow (Paull and Chen,

1983). The amount of protection of softening disruption is related with season, fruits ripeness stage during exposure, pretreatment time and temperature (Paull and Chen, 1990). In this study, Figure 3.5 shows that the flesh firmness was reduced during storage especially at 12 °C and after 2 weeks. Storage temperature has significant effects on firmness and firmness reduction progressed with storage time (Paull, 1999). Fruits firmness stored at 12 °C was no longer acceptable while the fruits firmness stored at 6 °C was acceptable for 2 weeks (Figure 3.5). Earlier studies mentioned storage at low temperature slowed down the fruits ripening. Stored pawpaw fruits at 4 °C for 4 weeks, exhibited minimal loss in quality and also softening continued slowly (Archbold et al., 2003, Galli et al., 2008).

Proulx et al., (2005) noted that fruits stored below 10 °C never reached the acceptable rating limit after storage for 14 days; these results were similar to the stored papaya at 6 °C, which never reach the acquired level of softening and ripening (Figure 3.5). In pawpaw fruits, firmness of all cultivars were reduced during cold storage (4 °C), but due to this reduction during cold storage fruits firmness already was lower than higher storage temperatures and did not reduce further during storage (Archbold et al., 2003; Galli et al., 2008). The same trend is shown in Figure 3.5 as reduction occurred sharply at 12 °C; also fruit firmness declined at 6 °C, but not as sharply as at 12 °C during the 3 weeks of storage. Storage of carambola fruits (*Averrhoa carambola* L. cv. B10), mature green (stage 1), at 5 °C for 21 days maintained fruit firmness, inhibited the solubilization and depolymerization of chelator-soluble wall polyuronides and considerably decreased the elevation in pectinesterase and β-galactosidase activities. At 10 °C, carambola fruits

demonstrated lower rates of the firmness loss and it is related with polyuronide solubilization and depolymerization. In addition, the enhancement in pectin esterase, β-galactosidase, PG and β-(1,4)-glucanase activities were delayed significantly in comparison with the fruits kept at ambient temperature (Zainon et al., 2004).

3.4.5 Ethylene production

There was no interaction effect of heat treatment x storage temperature on ethylene production rate of the papaya (Table 3.5). However, ethylene production rates were significantly ($P \leq 0.05$) affected by both interactions between heat treatment x storage duration and storage duration x storage temperature. There were significant linear and cubic relationships between ethylene production and storage duration of control and hot water dip fruits (Figure 3.6). Ethylene production of hot water dip papaya increased from week 0 until week 1 and then followed by sharp reduction until week 2 (Figure 3.6). From weeks 2 to 3 there was a slight increase in ethylene production. Control fruits showed a constant increase of ethylene production from weeks 0 until 3. The $R^2 = 0.41$ in control and $R^2 = 0.40$ in hot water dip fruit showed that 41 and 40% of the variability in ethylene production rate was due to the storage duration. There was a significant cubic relationship between ethylene production and storage duration of fruits stored at 12 °C (Figure 3.7). There was a significant linear relationship between ethylene production and storage duration of fruits stored at 6 °C. The ethylene production fluctuated during storage at 12 °C. Ethylene production increased sharply from weeks 0 to 1 and followed by a reduction until week 2.

Table 3.5 Main and interaction effects of heat treatment, storage temperature and storage duration on ethylene and carbon dioxide production of 'Frangi' papaya.

Factor	Ethylene (µl/ kg FW. h)	Carbon dioxide (ml/ kg FW. h)
Heat treatment (HT)		
Non hot water dip (control)	2.1 b[z]	6.43 a
Hot water dip	3.6 a	5.69 a
Storage temperature (ST) (ºC)		
6	2.6 a	4.84 b
12	3.1 a	7.28 a
Storage duration (SD) (Weeks)		
0	0.7 b	5.08 b
1	4.0 a	3.70 b
2	3.1 a	5.01 b
3	3.7 a	10.45 a
HT x ST	ns	ns
HT x SD	*	ns
SD x ST	*	*
HT x ST x SD	ns	ns

[z] Means within a column and factor followed by the same letter are not significantly different by LSD at P≤0.05.
ns = Non significant and * = Significant at P≤0.05.

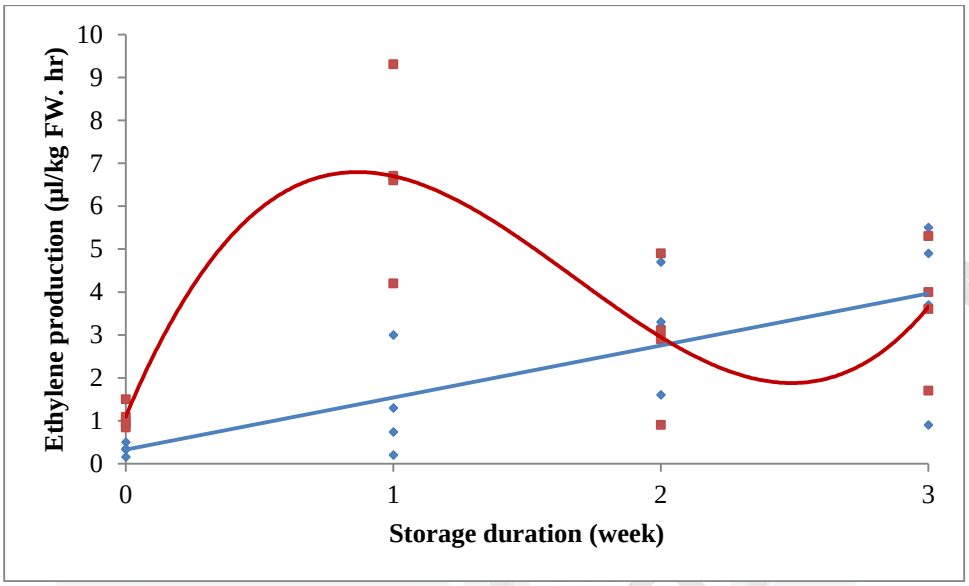

Figure 3.6 Relationships between ethylene production (µl/kg FW.hr) and storage duration of 'Frangi' papaya in control (♦) and hot water dip (■) fruits.
y (control) = 0.12+0.035x (R^2 = 0.41), and y (hot water dip) = 0.11+1.48x-1.15x^2+0.23x^3 (R^2 = 0.40). Solid line indicates a significant linear and cubic relationship.

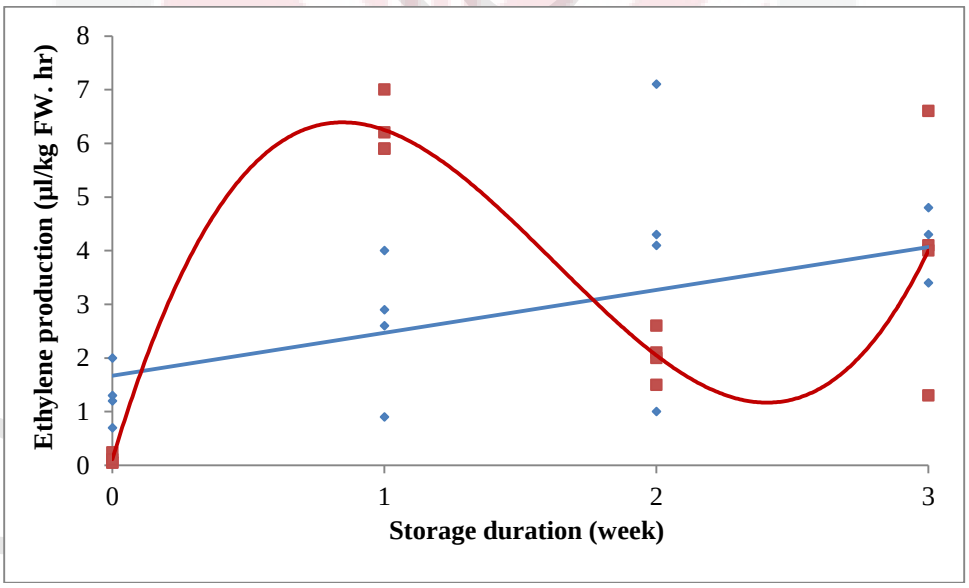

Figure 3.7 Relationships between ethylene production (µl/kg FW.hr) and storage duration of 'Frangi' papaya stored at 6 °C (♦) and 12 °C (■).
y (6 °C) = 0.13+0.088x (R^2 = 0.35), and y (12 °C) = 0.011+1.69x-1.34x^2+0.276x^3 (R^2 = 0.42). Solid line indicates a significant linear and cubic relationship.

The increasing trend of ethylene production at the end of storage at 12 °C was observed. Ethylene production at 6 °C showed an increasing trend from weeks 0 until 3 of the storage. Table 3.2 indicated that there was significant negative correlation (r = -0.42) between ethylene production rate and firmness. The correlation results in Table 3.2 indicated that the ripening progression is associated with increase in ethylene production rate and conversely decrease in fruit firmness. The ethylene production rate has been taken into account as a major physiological marker of the climacteric stage (Saltveit, 1999). Mango fruit softening was due to the increased of cell wall hydrolysis enzymes activities during ripening and is activated by ethylene (Ali et al., 2004). Ethylene production of heat-treated Satsuma mandarin was affected considerably by storage duration, and showed 50% reduction between weeks 4 and 8 of the storage (Ghasemnezhad et al., 2008). Satsuma mandarin dipped in 50 °C hot water for 2 min and stored in cold storage for 8 weeks had considerably suppressed ethylene increase as well as lower respiration rate (Ghasemnezhad et al., 2008).

In this experiment, ethylene reduction (55%) occurred after week 1 in hot water dip fruits. It can be concluded that ethylene production was accelerated by hot water dip up to week 1 of storage, and then followed by a reduction until after the second week of storage (Figure 3.6). Exposure to temperatures above 36 °C led to accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) content in apple tissue and induced a reduction in ethylene production (Yu et al., 1980). A rapid loss of 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) activity occurred in many fruits when exposed for a short period to temperatures of 38 °C or above (Dunlap et al., 1990; Klein and Lurie,

1990). The heat inactivation of ACO in papaya and cucumber is biphasic, and part of the ACO activity is heat resistant (Chan, 1986a, 1986b). The results obtained in this experiment were almost similar with Atta-Aly (1992) who mentioned that high temperature could also induce a reduction in ACC accumulation indicating vulnerability of the 1-aminocyclopropane-1-carboxylic acid synthase (ACS) to heat. However, subjecting fruit to periods of high temperature does not cause heat damage, and the fruit quickly recover its ethylene synthesis ability (Dunlap et al., 1990). Complete recovery of ACO activity took place within 3 days of heat removal in papaya (Paull and Chen, 1990), apples (Klein et al., 1990), and mango (Ketsa et al., 1999). Moreover, the ACS activity also was recovered in tomatoes after a high temperature treatment (Biggs et al., 1988). It seemed that ethylene production in hot water dip fruits was not inhibited until the first week of storage (Figure 3.6). Ketsa et al. (1999) showed an ethylene peak in heated fruits after 6 days. Whereas, heat treated apples produced more ethylene than the control fruits, when returned to 20 °C (Klein and Lurie, 1990).

The steady increase of ethylene production in control fruits could be the reason for elevated content of both ACC and ACS activity corresponding with ethylene production, but after 4 days the production was reduced (Ketsa et al., 1999). The results in present study were similar with the increasing ethylene production in untreated papaya fruits, but the ethylene production increased until the third week of storage. The results obtained were in contrast with heat treated (38 °C for 3 days) mangos stored at 5 °C, heated 'Keitt' mango produced less ethylene than un-heated fruits. Heat treated Keitt mango after 1 or 2 days at 38 °C followed by storage at 5 °C produced less ethylene than

unheated fruits (McCollum et al., 1993). Heat treatments can cause heat injury and also induce wound ethylene production 1-2 days after treatment with a subsequent advanced ethylene climacteric peak four days later (Paull and Chen, 1990). There are more numerous cells on the fruit skins which mean higher metabolic activity in fruits on a basis of volume or weight, and this could include ethylene production (Ketsa et al., 1999). The possibility of crystalloid formation in heat treated fruits after two weeks of storage support the findings in this experiment (Figure 3.6). Crystalloids could cover the numerous cells and will cause ethylene reduction with consequent effects on firmness and weight loss (Kechinski et al., 2012).

Ethylene production and respiration rate of different heat-treated fruits were affected significantly by storage duration. After 4 weeks of storage at 2 °C, ethylene evolution and respiration rate were correlated with CI development in Satsuma mandarin peel (Ghasemnezhad et al., 2008). Ethylene production of pawpaw fruits stored at 2 °C exhibited an increase through 4 weeks of cold storage (Galli et al., 2009). Similarly in the present study, ethylene production of papaya fruits stored at 6 °C indicated an increasing trend (Figure 3.7). The elevated ethylene production in pawpaw after removal from 2 °C storage during 4 weeks indicated that no irreparable injury happened to the ethylene biosynthetic pathway (Galli et al., 2009). In present study, papaya fruits stored at 6 °C exhibited a loss of firmness (Figure 3.5) and lower respiration and ethylene production rates during cold storage (Figure 3.7). Tomato is a climacteric fruit with a marked increase in ethylene synthesis at the beginning of the ripening. ‘Cedrico’ tomatoes have grown in New Zealand did not produce any significant amount of

ethylene when stored at either 2.5 or 6 °C. Fruits stored at 6 °C during 3 weeks did not show the ethylene peak as the stored fruits at 12 °C (Figure 3.7). With reference to the storage duration, ethylene response coincided with papaya color progression from week 0 to 3 (Table 3.1). In this experiment, the uneven coloration and production of stress ethylene could occur in heat treated or chilled papaya, but it is not easy to separate ripening and stress-related ethylene production (Watkins, 2002). The increase in ethylene production by Frangi papaya stored at 6 °C could be a stress reaction rather than being related with ripening (no fruit developed orange color). In addition, the higher ethylene production for fruits stored at 6 °C could be the integrated function of stress and decay as ethylene induction by infected fruits were previously reported (Barkai-Golan and Kopeliovitch, 1983). Tomatoes stored at 12.5 °C reached their ethylene climacteric peak, but the peak was delayed in comparison with fruits stored at 20 °C. However, tomato fruits stored at 6 °C showed a slight increase in ethylene production after 27 d of the storage (Biswas et al., 2012). The same results occurred in this experiment, as papaya fruits stored at 12 °C reached the ethylene peak in week 1.

3.4.6 Carbon dioxide production

CO_2 production rate was affected significantly ($P \leq 0.05$) by the interaction between storage duration x storage temperature (Table 3.5).Consequently, there were no other interaction effects on carbon dioxide production. The CO_2 production rate was not significantly influenced by heat treatment. Figure 3.8 showed that there was a significant quadratic relationship between CO_2 production and storage duration of fruits stored at

12 °C. However, there was no significant relationship between carbon dioxide production and storage duration of fruits stored at 6 °C. For fruit stored at 12 °C, the CO_2 production rate increased as the storage duration increased, but from weeks 0 to week 1, CO_2 production rate remained constant and an increasing trend followed from weeks 1 until 3. Fruits stored at 6 °C showed a reduction of CO_2 production rate, but the increasing trend was observed from weeks 2 to 3 of storage. From weeks 1 to 3, there was a 65% increase in CO_2 production of fruits stored at 12 °C while CO_2 production rate of fruits stored at 6 °C increased only by 25%. The reduction in CO_2 production of fruits stored at 6 °C was observed until week 2 of the storage duration (Figure 3.8). The $R^2 = 0.51$ in 12 °C fruits indicated that 51% of the variability in CO_2 production was due to the storage duration. There was a negative and significant correlation ($r = -0.42$) between carbon dioxide production and fruits firmness (Table 3.2).

The fruits firmness and respiratory intensity are indications of fruits senescence. In this experiment, reduction in firmness was followed by the enhancement of carbon dioxide production (Figure 3.5). The pawpaw fruits demonstrated an increase in respiration during 2 weeks of storage at 2 °C and 4 weeks of storage at -2 °C while fruits stored at 6 °C showed a respiration reduction (Galli et al., 2008). However, beyond 4 weeks, fruits from all storage temperatures (-2, 2, 4 and 6 °C) had a decline in respiration rate (Galli et al., 2008). In this study, the same reduction took place during the first and second week of storage. During cold storage (6 °C), fruits had lower CO_2 production compared with 12 °C (Figure 3.8). There was a delay of CO_2 production rate compared with ethylene production rate.

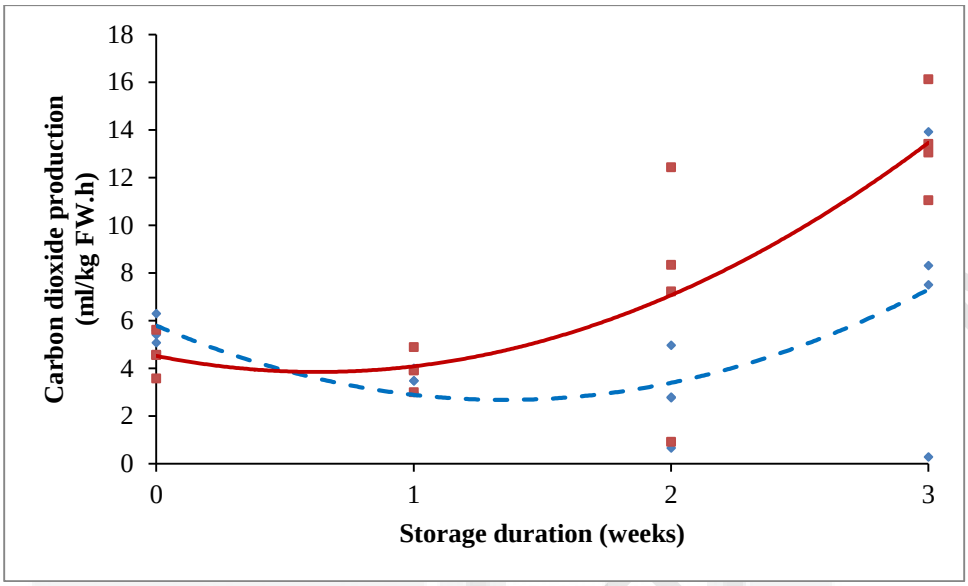

Figure 3.8 Relationship between carbon dioxide production (ml/kg FW.hr) and storage duration of 'Frangi' papaya stored at 6 °C (♦) and 12 °C (■).
y (12 °C) = $1.70x^2$-2.14x+4.52 (R^2 = 0.51). Solid line indicates a significant quadratic relationship.

This result is similar with the results obtained on pomegranate; reducing storage temperature from 15 to 5 °C reduced the CO_2 production during the storage (Caleb et al., 2012). In present study, the CO_2 production increased after the second week of storage at 6 °C, and it could be due to the onset of CI (Hernández et al., 2009). It could also be attributed to the climacteric nature of the fruits. The increased metabolic activities and enhanced respiration rate occurred at the transition from the breaker stage of the fruits to its senescence phase (Oms-Oliu et al., 2011). Cold storage is one of the most extensively applied technologies to reduce respiration rate and other metabolic procedures in order to enhance postharvest life of horticultural crops (Wang, 1992). Peach fruits stored at 20 °C showed a respiratory peak during 8 days of storage followed by a sharp decline. The stored fruits at 5 °C did not show changes in respiratory rate and followed a constant

trend (Kan et al., 2011). Storage of banana fruits at temperatures of 10, 15, 20, 25 and 30 °C indicated that respiration progressed at a faster rate with increasing temperature (Bhande et al., 2008). Similar findings were obtained in this experiment whereby the respiration rate was higher in the fruits stored at 12 °C than in the fruits stored at 6 °C (Figure 3.8).

3.5 Conclusion

Heat treatment affects many aspects of fruits ripening (chemical and physical quality characteristics), and induced changes at cellular and molecular levels. After double dip hot water treatment, fruits quality characteristics, like SSC, TA, ascorbic acid and pH, did not change significantly and hot water dip had no injurious effects on these quality characteristics.

Treated papayas had a better general appearance, and lower weight loss, but a lower firmness compared to the control samples. There were significant increases of weight loss during the storage of fruits both in control and hot water dip. The treated samples stored at 6 °C had lower weight loss after the second week of storage compared with control samples stored at 12 °C. There was significant firmness reduction during the three weeks of storage. Stored papaya at 6 °C remained significantly firmer than the fruits stored at 12 °C.

The higher storage temperature (12 °C) caused more ethylene fluctuation than papaya stored at a lower temperature (6 °C). The metabolism of 'Frangi' papaya was delayed by the application of cold storage temperature (6 °C), which delayed fruits ripening and color progression. Hot water dipped papayas considerably produced more ethylene than the control fruits, but reduction occurred after the second week of storage. In contrast, control papaya followed an increasing trend of ethylene production during the whole

storage period. With reference to the CO_2 production rate, the significant increase of CO_2 at 12 °C storage compared with 6 °C and it could be due to the higher metabolic activity occurred at higher storage temperatures. The non-damaging heat treatments could be able to reduce the fruits firmness and ethylene production. Interestingly considerable differences in weight loss and color progression occurred between heat treated and control papaya after the second week of storage. The fruits did not show significant differences immediately after hot water treatment. As a conclusion, it seems that hot water dip triggers many metabolic pathways after the second week of storage. The reduction of weight loss and firmness after week 2 in treated papaya makes hot water dip a proper choice for extending storage duration.

CHAPTER 4

CHILLING INJURY, ELECTROLYTE LEAKAGE AND ANTIOXIDANT ENZYME ACTIVITY OF HEAT TREATED *CARICA PAPAYA* var. 'FRANGI' DURING THREE WEEKS OF STORAGE

4.1 Introduction

Low temperature storage has been applied extensively in order to extend the postharvest life of horticultural products. Storage temperature reduction can significantly reduce the rate of many metabolic processes which result in fruits senescence, deterioration and loss of crop quality (Saltveit and Morris, 1990). Reducing the storage temperature of horticultural crops is a way of maintaining their quality during the storage after harvest. However, some tropical and sub-tropical fruits are sensitive to low temperature storage.

Low storage temperature will result in non-uniform or failure of ripening, appearance of skin pitting and other physiological disorders (McGlasson et al., 1979). All of these indications are symptoms of chilling injury (CI) (McGlasson et al., 1979). Changes in fruits membrane and structure are the first response of CI at the molecular level and affect the membrane permeability. A secondary reaction would appear with symptoms like electrolyte leakage, reduction of metabolic energy and cell lysis (Raison and Orr, 1990). Physiological demonstrations of CI often happen along with the manifestation of visible symptoms (Purvis, 2002). The CI severity is dependent on function of temperature and exposure duration. High temperature treatments have been applied to

control the disease and insects incidence as well as maintaining the fruits quality characteristics during storage (Mulas and Schirra, 2007). Heat treatment or its combination with other postharvest technologies has been applied to control insects and diseases. Heat treatment prior to cold storage could reduce or delay CI occurrence (heat induced chilling tolerance). hot water dip could also reduce disease incidence by stimulating specific responses in fruits (Sapitnitskaya et al., 2006).

Oxidative damage is considered to be a response of susceptible tissues to CI. Fruits subjected to abiotic stress, such as low or high temperatures (Sairam et al., 2000, Sala and Lafuente, 2004) produced elevated levels of the reactive oxygen species (ROS) that caused oxidative damage (Shigeoka et al., 2002). When plants are exposed to moderate heat treatments, weak oxidative stresses will be induced. This stress modulates antioxidant levels and causes a tolerance to the consequent severe stress (Li, 2003). Plants have an efficient antioxidant defense mechanism which is capable of preventing the ROS accumulation and repair oxidative injury. The activity of antioxidant enzymes has been related with tolerance to low temperatures and CI prevention (Imahori et al., 2008). The mechanism involves enzymes such as catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD). The function of SOD is to protect cells from damage by potentially toxic superoxide radical reaction products. The products are reduced to water by enzymes such as CAT and APX (Kochhar et al., 2003). Presently, there are no reports concerning the antioxidant defense mechanism involved in heat-induced chilling tolerance of 'Frangi' papaya. The objective of this experiment was to investigate the effects of heat treatment, storage at chilling and non-chilling

temperatures (6 and 12 °C) and storage duration on CI and peel electrolyte leakage (PEL) of Frangi papaya. In addition, antioxidant enzymes activity (CAT, APX, SOD) were monitored weekly during three weeks of storage.

4.2 Materials and methods

4.2.1 Sample preparation and treatment

In this experiment, the papaya samples used were heat treated. Control and hot water dip fruits stored at 6 and 12 °C storage temperature were obtained as the same method described in section 3.2.1. Fruits were divided into 2 sub-groups. Fruits in the first sub-group were used to assess CI, PEL and weight loss. Fruits from the second sub-group were used to measure postharvest quality characteristics and antioxidant enzyme activity. All the parameters were evaluated each week during three weeks of storage. Samples were assessed on day 0 of storage and it was considered as week 0.

4.2.2 Internal and external chilling injury assessment

Fruits from subgroup 1 were removed from storage on week 3 and allowed to incubate at ambient temperature (25±2 °C) for 2 days. Papaya fruits were assessed visually for external injuries such as the occurrence of skin pitting, scalds (from dark olive to light brown spots), shriveling and external water soaking. Pulp water soaking, hard lumps (around fruits seed cavity or throughout pulp) and internal mesocarp regions that failed to soften were evaluated as internal injuries. The areas, which failed to ripen, showed

slightly lighter orange color than normal tissue. The assessment was according to the following hedonic score: 1=no abnormality, 2=trace symptoms, small pits (1-15%), 3=moderate symptoms, small to medium pits, blotchy appearance (16-30%), 4=moderate to severe symptoms (31-50%), 5=severe symptoms (more than 50% affected) (Proulx et al., 2005). The CI incidence was calculated by logarithmic transformation of the data.

4.2.3 Peel electrolyte leakage determination

Papaya peel electrolyte leakage percentage was measured according to the method of (Woolf and Lay-Yee, 1997) with some modifications. The papaya peel was removed with a blade. The exposed pulp was cut into 10 disks of 2 g each using a 10-mm cork borer. Ten disks from each treatment and storage temperature were floated in separate conical flasks containing 10 ml of 0.4 M mannitol and then incubated for 3 h in a water bath (903, Protech, Malaysia) at 30 °C under continuous shaking. The initial conductivity was measured by a conductivity meter (Accumet AB30, Fisher Scientific, Singapore). To measure the total conductivity, conical flasks with floated peel disks were autoclaved at 120 °C for 20 min and cooled at ambient temperature. The electrolyte leakage percentage was calculated according to the following formula:

$$\%\text{Peel electrolyte leakage (PEL)} = \frac{\text{Initial conductivity}}{\text{Total conductivity}} \text{x } 100$$

4.2.4 Catalase extraction and assay

For the enzyme extraction, samples of fruits removed from storage on week 3 were kept immediately at -70 °C until measurements were made. One gram of papaya pulp (2 cm cubes from the fruit's equatorial region) was taken and grounded in liquid nitrogen with a mortar and pestle. The CAT activity was measured according to the method of (Blume and McClure., 1980), with some modifications. The extraction buffer contained 5 mL of 50 mM sodium phosphate buffer (pH 7.0). The reaction mixture (1 mL) contained 500 µL of 100 mM sodium phosphate buffer (pH 7.8), 150 µL of enzyme extract, 250 µL distilled water and 100 µL of 50 mM H_2O_2.The homogenate was centrifuged at 10,000 g for 15 min at 4 °C. The reaction was begun by adding 100 µL of 50 mM H_2O_2. The absorbance was measured at 240 nm using a spectrophotometer (UV-Visible Double Beam, U-2800, Hitachi, Japan). One unit of enzyme activity was defined as micromoles of hydrogen peroxide, oxidized per milliliter per minute at 25 °C. The results were expressed as enzyme unit per gram fresh weight (U/g FW). The extinction coefficient of 39.4 mM. cm^{-1} was used to calculate the CAT activity.

4.2.5 Ascorbate peroxidase extraction and assay

Remaining samples from subsection 4.2.4 were extracted with 5 mL extraction buffer. The extraction buffer contained: 250 µL of 50 mM sodium phosphate buffer (pH 7.0), 50 µL of 1mM L-ascorbic acid, 5 µL of 0.1 m methylenediaminetetraacetic acid (EDTA) and 1% polyvinylpyrrolidone (PVP).

The homogenate was centrifuged at 10,000 g for 15 min at 4 °C. APX activity was assayed according to the methods of (Nakano and Asada, 1981), with some modifications. The reaction mixture contained 500 µL of 100 mM sodium phosphate buffer, 100 µL of 5 mM L-ascorbic acid, 250 µL of distilled water, 50 µL of enzyme extract and 100 µL of 1 mM H_2O_2. The final reaction volume was adjusted to 1 mL and APX activity was measured by the oxidation of ascorbate at 290 mn. The reaction was started by adding 1 mM H_2O_2. One unit of enzyme activity was defined as micromoles of ascorbate oxidized per milliliter per minute at 25 °C. The results were expressed as (U/g FW). The extinction coefficient of 2.8 mM. cm^{-1} was used to calculate the APX activity.

4.2.6 Superoxide dismutase extraction and assay

Remaining samples from subsection 4.2.4 were extracted with 5 mL extraction buffer. The extraction buffer contained 5 mL of 50 mM sodium phosphate buffer (pH 7.8) and the homogenate was centrifuged at 10,000 g for 15 min at 4 °C. The assay was run using the SOD kit (Cell Technology Inc., USA) by drawing the SOD standard curve. The absorbance was recorded at 450 nm using a microplate reader (Labomed, UVD-2950, Power Wave X 340, Biotek Instruments Inc., USA). One unit of SOD activity inhibits the rate of increase in the absorbance (inhibition in reduction of WST-1 to a water-soluble formazan dye) at 440 nm by 50% under the conditions of the assay. The percent inhibition of the test sample correlates with SOD activity using a SOD standard curve. The results were expressed as (U/g FW).

4.2.7 Experimental design and data analysis

The experiments were conducted using a randomized complete block design (RCBD), in a factorial arrangement of treatments (2 heat treatment levels x 2 storage temperatures x 4 storage duration), with three replications of three fruits per replication. The logarithmic transformation of data were applied to calculate CI part and then data were subjected to an analysis of variance (ANOVA), and means were separated by least significant difference (LSD) tests at $P \leq 0.05$ by Statistical Analysis System (SAS), version 9.13 (SAS, Institute Inc., Cary, NC, USA). Regression analysis was carried out when the interaction effects between two factors were significant. The experiment was repeated three times, in June, July and August 2011.

4.3 Results and discussion

4.3.1 Peel and pulp chilling injury incidence

Table 4.1 shows that there was a significant ($P \leq 0.05$) interaction effect of heat treatment x storage temperature on peel CI incidence. There was no significant difference in peel CI incidence between control and hot water dip fruits stored at 12 °C (Figure 4.1). However, control fruits stored at 6 °C showed significantly higher CI incidence than hot water dip fruits stored at 6 °C.

There was no significant difference between hot water dip fruits stored at 6 and 12 °C. However, the peel CI incidence of control fruits stored at 6 °C was significantly higher than control fruits stored at 12 °C (Figure 4.1). There was a significant and negative correlation between peel and pulp h° and peel CI ($r = -0.32$) and ($r = -0.33$). Pulp C* and peel CI showed significant and positive correlation ($r = 0.44$). Peel CI incidence showed significant positive correlation with weight loss and PEL ($r = 0.39$) and ($r = 0.30$), respectively (Table 3.2). There was a significant interaction effect of storage duration x heat treatment on peel CI incidence (Table 4.1). Regression analysis showed that there was a significant quadratic relationship between storage duration and peel CI incidence of treated fruits. There was significant cubic relationship between storage duration and peel CI incidence of control fruits (Figure 4.2).

Table 4.1 Main and interaction effects of heat treatment, storage temperature and storage duration on peel and pulp chilling injury incidence and peel electrolyte leakage of 'Frangi' papaya.

Factor	Peel chilling injury (log[y])	Pulp chilling injury (log)	Peel electrolyte leakage (%)
Heat treatment (HT)			
Non hot water dip (control)	0.26 a[z]	0.18 a	21.36 a
Hot water dip	0.22 a	0.21 a	16.04 b
Storage temperature (ST) (°C)			
6	0.26 a	0.20 a	19.34 a
12	0.22 b	0.18 a	18.06 a
Storage duration(SD) (Weeks)			
0	0 d	0 c	10.81 c
1	0.24 c	0.17 b	19.75 b
2	0.33 b	0.23 b	20.10 b
3	0.39 a	0.40 a	24.15 a
HT x ST	*	*	ns
HT x SD	*	ns	ns
SD x ST	*	ns	ns
HT x ST x SD	*	*	ns

[y] Data were Log transformed.
[z] Means within a column and factor followed by the same letter are not significantly different by LSD at P≤0.05.
ns = Non significant and * significant at P≤0.05.

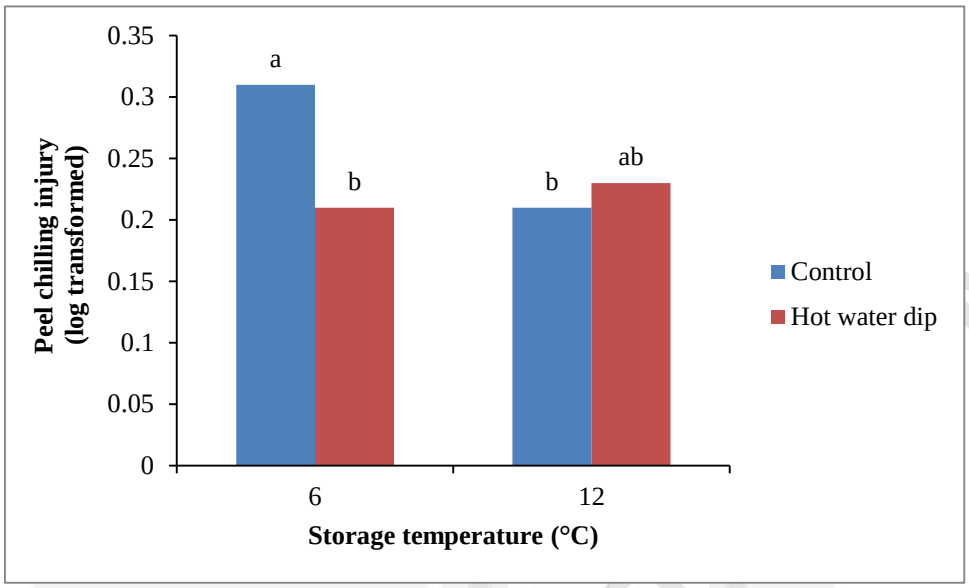

Figure 4.1 Effect of storage temperature on peel chilling injury of 'Frangi' papaya. Data shown are the means of three replications. Means followed by same letters are not significantly different by LSD test, P ≤0.05.

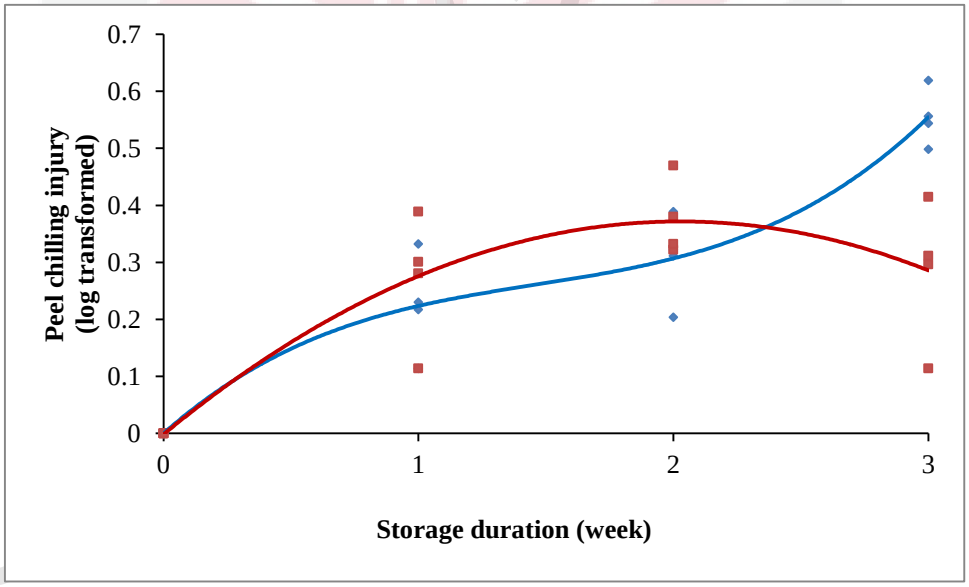

Figure 4.2 Relationship between peel chilling injury and storage duration of 'Frangi' papaya in control (♦) and hot water dip (■) fruits.
y (control)=0.395x-0.22x^2+3.05x^3 (R^2=0.76) and y (hot water dip)= -0.001+0.34x-0.09x^2 (R^2=0.56). Solid line indicates a significant quadratic and cubic relationship.

Peel CI incidences were observed on control fruits until week 3 of storage. As the storage period progressed to week 3, peel CI in control fruits showed a remarkable increase (Appendix1, 2, 3). During week 1 to 2 of the storage, control fruits showed relatively constant CI incidence. After week 2, control fruits revealed increasing decays and pitting areas on the fruits surface. Peel CI of treated fruits increased from week 0 until week 2 but reduced sharply thereafter (Figure 4.2). The R^2=0.76 in control fruits and R^2=0.56 in treated fruits indicated that 76% and 56% of the variability in peel CI incidence was due to the storage duration. Table 4.1 showed that there was a significant interaction effect of storage duration x storage temperature on peel CI incidence of Frangi papaya.

There was a significant, quadratic relationship between storage duration and peel CI of papaya stored at 6 and 12 °C storage temperature. The R^2=0.55 and 0.71 at 12 and 6 °C respectively indicated that 55% and 71% of the variability in peel CI incidence was due to the storage duration (Figure 4.3). Fruits stored at 6 °C revealed severe peel CI from week 0 until week 3. During the first 7 days of the storage, minor CI symptoms were observed. Fruits CI and pitting in two storage temperatures (6 and 12 °C) were detected from week 0 until 2 (Appendix 1, 2, 3). At the beginning of storage, fruits stored at 12 °C had low CI symptoms. Then between week 1 and 2, the CI manifestation coincided with 6 °C storage temperature (Appendix 1, 2, 3). The chilling symptoms did not progress after week 2 of storage, and it remained relatively constant during the last week of storage (Figure 4.3) (Appendix 1, 2, 3). There was a significant interaction effect between heat treatment x storage duration on pulp CI (Table 4.1).

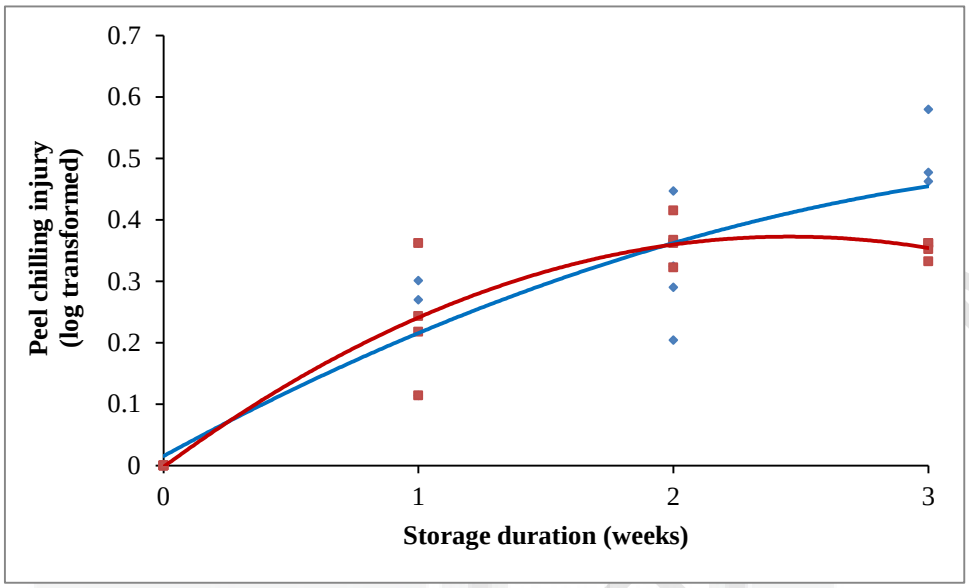

Figure 4.3 Relationships between peel chilling injury and storage duration of 'Frangi' papaya stored at 6 °C (♦) and 12 °C (■).
y (12 °C)=-0.001+0.29x-0.06x^2 (R^2=0.55) and y (6 °C)=0.01+0.22x-0.025x^2 (R^2=0.71). Solid line indicates a significant quadratic relationship.

Figure 4.4 indicates that there were significant differences between hot water dip and control fruits stored at 6 °C. Control fruits showed significantly higher pulp CI incidence at 6 °C than treated fruits. Hot water dip fruits showed significantly higher pulp CI incidence than control fruits when stored at 12 °C (Appendix 3). Hot water dip fruits stored at 6 °C indicated lower pulp CI incidence than heat treated fruits stored at 12 °C. In addition, control fruits stored at 6 °C had significantly higher CI incidence than control fruits stored at 12 °C (Appendix 3). There was a significant positive correlation (r = 0.64) between peel and pulp CI incidence. Also, there were significant negative correlations between peel and pulp h° with pulp CI, (r = -0.36) and (r = -0.29), respectively. There was a significant negative correlation between pulp L* and pulp CI incidence (r = -0.37) (Table 3.2).

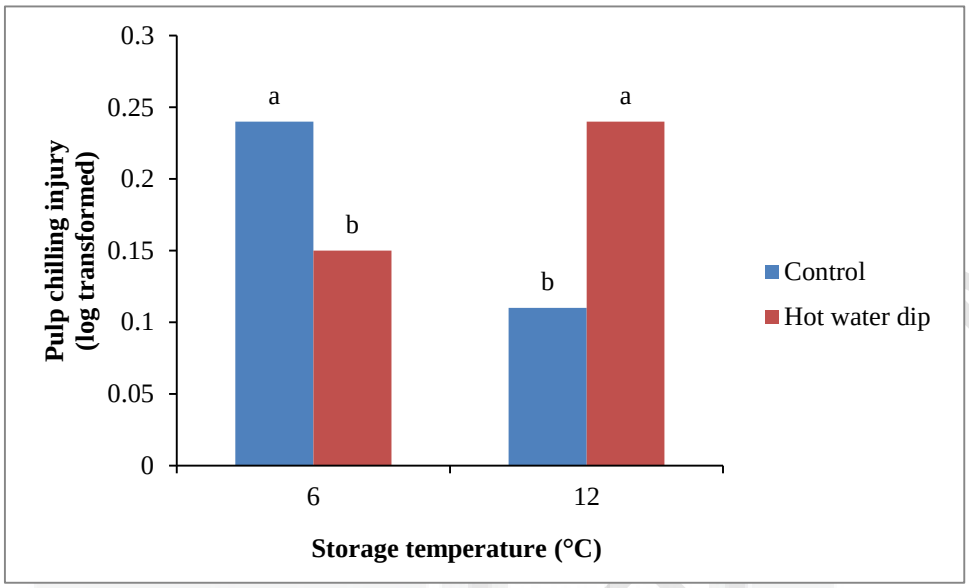

Figure 4.4 Effect of storage temperature on pulp chilling injury of 'Frangi' papaya. Data shown are the means of three replications. Means followed by same letters are not significantly different by LSD test, P ≤0.05.

The significant correlation between fruits color values and peel CI incidence could be due to the climacteric characteristics of the papaya fruits. Color development occurred within ripening progression and consequently CI increase with fruits ripening and senescence (Paull and Chen, 2000). Electrolyte leakage is a way to measure CI, and has been applied to determine the CI amount (Murata, 1989). Therefore, according to the correlation results, the increase in CI will lead to increase in PEL. Results in this experiment showed lower peel CI incidence of heat treated fruits stored at 6 °C compared with control fruits stored at the same temperature (Figure 4.1). Similarly, hot water treated bananas presented lower CI after 5 days of storage at 7 °C (Wang et al., 2012). Chen et al. (2008) reported that heat pretreatment of banana before storage at 8 °C delayed the induction of CI symptoms by 3 days. Application of hot water treatment in Satsuma mandarin delayed the onset of CI progression when fruits treated at

47.5 °C for 2 and 5 min and 50 °C for 2 min (Ghasemnezhad et al., 2008). Moreover, findings about Fortune mandarins were attributed to the protective effect of hot water treatment at 50-52 °C for 3 min against CI (Schirra and D'Hallewin, 1997). Cold conditioning along with hot water dip showed a decline in susceptibility of fruits to CI. The obtained results in this experiment (Figure 4.1) were similar to the reduction of CI in heat-treated grapefruit stored at 2 °C (Sapitnitskaya et al., 2006), heat treated pomegranate stored at 2 °C (Mirdehghan et al., 2007) and heat treated 'Flavorcrest' peach stored at 0 ± 0.5 °C (Murray et al., 2007).

In chilling-sensitive fruits, cold temperatures impair the energy state of cell and/or stimulate changes in membrane integrity. This may cause an increase of active oxygene species (AOS) proliferation. The increase of AOS will decrease the scavenging efficiency through factors like chilling-related inactivation of antioxidants and/or blocked antioxidant turnover (Hodges et al., 2004). Chilling temperatures change the equilibrium between AOS production and defense mechanisms such that oxidative induced CI is observed. As reported earlier, the stimulation of some detoxifying enzymes during storage might result from an induction of antioxidant enzymes during the production of superoxide radicals and hydrogen peroxide. This balance between the formation and detoxification of AOS is vital to cell survival through chilling storage (Hodges et al., 2004). These results demonstrated that SOD; CAT and APX actions may contribute, at least to some extent, to the CI alleviation of fruits stored at 6 °C. Application of hot water treatment as a pre-conditioning treatment induced chilling-tolerance by modulating antioxidant systems (Fallik, 2004).

This system could inhibit the AOS accumulation (Sala and Lafuente, 2000). In Navel oranges, by the end of 20 days quarantine storage at 1 °C (RH=85-90%), the highest CI incidence of fruits was found in untreated fruits (Bassal and El-Hamahmy, 2011). Hot water treatment considerably decreased the CI incidence (0-4%). 'Navel' and 'Valencia' oranges treated with hot water at 41 °C for 20 min presented significantly lower CI percentages. The number of pitted regions per fruit compared with the control fruits was lower (Bassal and El-Hamahmy, 2011). According to Figure 4.2, there were significant cubic and quadratic relationships between storage duration and peel CI of control and hot water dip fruits, respectively. In control samples, CI increased progressively as the storage duration increased. In Satsuma mandarins, chilling symptoms revealed after 4 weeks at 2 °C and the symptoms increased with storage duration (Ghasemnezhad et al., 2008).

Papaya fruits may demonstrate the decay occurrence after 8 days of storage at 15 °C (Aziz et al., 1975). At this study, the constant trend of peel CI incidence during week 1 and 2 (Figure 4.2) could be due to the increase of antioxidant enzymes activity in control fruits. It was reported that CAT might be a main antioxidant enzyme involved in the defense mechanisms of mandarin fruits against CI incidence (Sala and Lafuente, 2000). Other researches on ‘Qingnai’ plum and pomegranate fruits mentioned the increase of CI in control fruits during storage (Barman et al., 2011, Luo et al., 2011). Storage of control loquat fruits during 21 days at 1 °C induced higher internal browning index and severe flesh-leatheriness. Fruits developed the decay areas and pitting on the skin even before being sufficiently ripe to eat (Cao et al., 2009).

In Satsuma mandarins, there was a basic skin injury level which occurred in all heat treatments (nearly 20% of fruits exposed to 2 min dipping, and about 30% of fruits exposed to 5 min dipping). Because of this basic level of heat damage, 2 min treatments between 47.5 and 50 °C would be recommended as optimum under New Zealand conditions (Ghasemnezhad et al., 2008). It shows that, the basic heat treatment temperature of Frangi papaya as an optimum temperature might be reported under Malaysia conditions. Peel CI of hot water dip fruits increased until week 2 of the storage and reduced thereafter (Figure 4.2).The peel CI reduction of hot water dip papaya after week 2 attributed to alterations in surface gloss of the fruit. The possible modifications to the light reflectance characteristics could be induced by a loss of cuticular wax and/or changes of ultra-structure in the crystalline surface wax (Charles et al., 2008).

Any modification in the fruits skin could decrease the pathogen adhesion, and consequently colonization reduction. This wax formation was observed in apple fruits exposed to heat treatment, whereby, after two weeks of storage wax filled the cracks on the cuticle, to avoid fruits dehydration (Montero et al., 2010). Heat is known to have an effect on fruits surfaces, causing cuticular waxes to melt, and natural openings to close. Heat treatment obstructing the potential entrance sites for pathogens and will act as a physical obstacle against infection (Eckert and Eaks, 1988). Figure 4.3 showed the CI symptoms of Frangi papaya during storage, followed by an increasing trend at 6 °C from week 0 until end of the storage. The same results mentioned by Zainon et al. (2004) about the CI symptoms of carambolas and the symptoms began to appear in fruits stored at 5 °C during 10 days.

Reduction of the storage temperature to 5 °C intensified the CI progressions. Under 2 °C temperature regimes, CI symptoms were closely associated with increased browning potential as attributed with increasing polyphenol oxidase (PPO) and peroxidase (POD) activities (Pérez-Tello et al., 2001). The high incidence of CI during the chilling storage temperature has been reported earlier by (Chen and Ramaswamy, 2002), under chilling conditions, cell membrane lipids go through changes in physical state from liquid-crystalline to solid–gel state. These changes resulted in an increase in membrane permeability and leakage of ions. Moreover, adaptation or cold acclimation has been planned with an increase in proportion of unsaturation of membrane lipid, and this is considered as a key factor in maintenance of cellular integrity during chilling conditions (Galindo et al., 2004).

Oxygen free radicals have been mentioned as the mediator of membrane degradation in injuries related with low temperature storage (Scandalios, 2001). Therefore, the increased permeability to oxygen will result in increasing generation of AOS. The increase of AOS interrupt the antioxidant defense systems and leads to injury of low temperature stressed plant tissues (Scandalios, 2001). The results obtained about mume fruits during cold storage were the same as this experiment. Mume fruits showed severe CI on day 5, and the symptoms developed extremely fast at 6 °C. After 15 days of storage at 6 °C, numerous sunken areas appeared on the fruits surface, and pitting progressed over the whole fruits surface with CI (Imahori et al., 2008). In this study, peel CI incidence consistently increased during the storage at 6 °C storage. The most CI symptoms had been observed in week 3 of the storage (Figure 4.3).

Long storage duration resulted in external and internal damages of the fruits tissues such as pawpaw (Koslanund, 2003) and cherimoya (Alique et al., 1994). The chilling disorder might be related to the tissue deterioration or senescence, which resulted in membrane permeability changes. One of the reasons for CI induction was the decline in the degree of unsaturated fatty acids and membrane permeability. The physical characteristics of membranes are affected by low temperature and are related with low temperature-induced quality losses. The reduction in the ratio of unsaturated to saturated fatty acids may indicate an increase in lipid peroxidation during chilling and lipid peroxidation attributes to CI development (Wang, 1992). Some tropical or subtropical fruits such as banana or papaya are highly vulnerable to CI during low temperature (<13 °C) storage.

Low storage temperature can cause peel pitting, discoloration and abnormal ripening (Nguyen et al., 2003, Wang et al., 2007). Pawpaw fruits stored at cold temperature more than 4 weeks failed to ripen normally and augmented internal discoloration, tissue acidification, off-flavor and aroma (Archbold and Pomper, 2008). In this study, storage at 12 °C caused the CI reduction after week 2 of storage (Figure 4.3). The CI become manifested at temperatures above 0 °C, around 8 °C for subtropical plant species and around 12 °C for tropical ones (Lyons and Breidenbach, 1990). Mature-green 'Taiping' and 'Bentong' papayas from Malaysia manifested the CI progression after storage for 7 days at 15 °C (Nazeeb and Broughton, 1978). The obtained results were relatively similar to this experiment, when CI increased in Frangi papaya after 7 days of storage at 12 °C (Figure 4.3). The reduction of CI incidence after week 2 in fruits stored at 12 °C (Figure 4.3) could be due to the increased antioxidant enzymes activity (SOD), and it

will be discussed in the antioxidant enzyme section (subsection 4.3.3) later. Sufficient protection against destructive ROS reactions is supported by cellular antioxidant defense mechanisms, including SOD, CAT, and the ascorbate-glutathione cycle (Noctor and Foyer, 1998). The corresponding action of antioxidant enzymes help to decrease oxidative injuries during ripening and senescence (Noctor and Foyer, 1998).

The increase of peel CI incidence from week 0 to 2 at 12 °C (Figure 4.3) could be due to an early increase of ethylene production and respiration rate during the storage at 12 °C (Figure 3.7 and 3.8). Previous reports mentioned that, the early increase in ethylene production will cause the early increase in CI symptoms (Ren et al., 1999). Another hypothesis mentioned that the progression of CI during the optimum temperature storage (12 °C) could be related with preharvest factors. For example, in 'Delicious' apples, the decreased susceptibility to superficial scald, has been associated with relatively low preharvest temperatures, rainfall and sunshine; meanwhile high temperatures during the late season caused higher vulnerability to scald (Bramlage and Weis, 1995).

In this study, the susceptibility of Frangi papaya to the pulp CI during the optimum storage temperature at 12 °C might be the result of pre-harvest factors. Figure 4.4 showed that chilling injuries (hard lumps, abnormal ripening and flesh water soaking) of hot water dip fruits increased when stored at 12 °C. Temperatures of more than 47.5 and 50 °C for 2 and 5 min, respectively, caused fruits to become susceptible to heat injury in the form of rind browning (Ghasemnezhad et al., 2008). The increase of pulp CI incidence in present study in hot water dip fruits at 12 °C storage might be related to the

low persistence of antioxidant enzymes activity after hot water treatment as mentioned earlier about heat treated 'Fortune' mandarins by Sala and Lafuente (2000). They mentioned that CAT was related with the enhanced pulp CI in hot water treated mandarins. Pulp CI symptoms were revealed at relatively high temperatures (e.g. 12 °C), and the symptoms were exacerbated with reducing temperature (Chen et al., 2008). Many pulp CI symptoms may occur with fruits ripening progression and can be determined from senescence (Saltveit and Morris, 1990). The obtained results about heat treated banana fruit was in contrast with this experiment. Pulp firmness in banana during storage both at ambient temperature for 10 days and at low temperature (14 °C) for 8 days did not show any heat damage on treated fruits. Pretreatment at 38 °C, with 1 to 2 hours interval was required for maximum damage reduction (Ummarat et al., 2011). In this study, internal injury was lower in hot water dip fruits stored at 6 °C compared with control fruits stored at the same temperature (Figure 4.4).

Similarly, heat treated tomatoes (23 h) showed a stronger resistance to the pulp CI during chilling storage at 2.5 °C (Lu et al., 2010). The rate of pulp CI in pericarp tissue of mature-green tomato was significantly decreased by heating the fruit to 40 °C for 7 min prior to chilling at 2.5 °C for 14 days (Lu et al., 2010). In hot water treated mangoes stored at 7 °C in comparison with untreated fruits, there was an increase in carotenoids and polyphenols with a better antioxidant capability (Talcott et al., 2005). In addition stimulation of *HSP70* expression and the corresponding protein accumulation was related with the increase in the antioxidant enzymes activities, and this synergistic action between HSP70 and the antioxidant system caused an increase in membrane

integrity and resistance to CI (Aghdam et al., 2013). Treated fruits' response to lower temperature storage with lower pulp CI incidence could be related to the antioxidant defense mechanism or heat-shock treatment (Luengwilai et al., 2012). It has been recommended that HSPs act simultaneously with antioxidant enzymes or they could be associated with enzymes induction to reduce the pulp CI after hot water treatment (Aghdam et al., 2013). These antioxidant enzymes could be helpful to control oxidative responses that resulted from free radical species and ROS in living organism (Wahid et al., 2007).

4.3.2 Peel electrolyte leakage

From Table 4.1, it can be concluded that there was no significant interaction effects of heat treatment on storage duration and storage temperature on papaya peel electrolyte leakage. There was also no significant interaction effect of storage duration x storage temperature on Frangi papaya peel electrolyte leakage. Results showed that PEL was significantly influenced by heat treatment and heat treated fruits had lower (around 24%) peel electrolyte leakage. This factor was not significantly influenced by storage temperature. The PEL was also significantly affected by the storage duration, and followed a consistent increase (55%) until the end of the storage (Table 4.1). There were significant positive correlations between peel and pulp C* with PEL ($r = 0.35$) and ($r = 0.39$) respectively, however there was significant negative correlation between PEL and pulp L* ($r = 0.39$) (Table 3.2). The increase in chroma value was due to the increase in peel electrolyte leakage. The significant correlation between PEL and color values

could be due to the fruits ripening progression. Fruits ripening stage, exposure duration to heat treatment, storage temperature, and their interactions could have effects on electrolyte leakage levels (Nyanjage et al., 1999). In this experiment, the increase of ion leakage during storage (Table 4.1) could be due to the respiration rate and depends on fruits tissue integrity. The increase in respiration rate and ion leakage parameters are presumed to occur at the end of ripening or when the fruits is exposed to rigorous stress situations like processing, cutting and/or exposure to high or low temperatures (Nyanjage et al., 1999).

In present study, PEL of control papaya was higher by 21%, as compared to the heat treated fruits (Table 4.1). Bananas which were heat treated at 38 °C for 3 days showed lower PEL than control tissues during 12 days storage at 8 °C (Chen et al., 2008). At any storage time, PEL of heat-pretreated banana was markedly lower than the control tissues (Chen et al., 2008). The same useful effects of heat treatment have been reported earlier for other tropical and subtropical fruits, like pepper, tomato, cucumber and citrus (Lurie, 1998a, Sanchez-Ballesta et al., 2000, Saltveit, 2001). A protective effect of heat treatment was observed in tomato with less ion movement across the membranes and an enhanced resistance against chilling-induced damage (Saltveit, 2005). Relative conductivity of grape tissue was increased by 12.1% after 3 h chilling at -2 °C (Zhang et al., 2005). In grape berries, the increase of electrolyte leakage induced by chilling stress was suppressed by heat pretreatment and the constancy of cell membranes was preserved by the treatment (Zhang et al., 2005). Ultrastructural alterations related to CI have often appeared in membrane systems like chloroplasts, mitochondria, nuclei,

plasma membranes and tonoplast (Kratsch and Wise, 2000). Prolonged chilling stress induced changes of senescence characteristics in grap berries fruits tissues, particularly in cell walls (Zhang et al., 2005). The degradation related to the induced chilling stress was delayed by heat pretreatment. Results reported by Zhang et al. (2005) provided cytological proofs that chilling tolerance was induced by heat pretreatment. The study on strawberry potassium ion leakage and ethylene production showed that both lower electrolyte leakage and ethylene production observed in heat treated fruits during storage indicates a reduction in tissue damage during storage (Vicente et al., 2006). Therefore, the fruits integrity of membrane components such as plasmalemma and/or tonoplast was protected by heat treatment (Vicente et al., 2006).

4.3.3 Catalase, ascorbate peroxidase and superoxide dismutase activity

There was a significant ($P \leq 0.05$) interaction effect of heat treatment x storage duration on CAT activity (Table 4.2). There was no significant relationship between storage duration and CAT activity of hot water dip fruits (Figure 4.5). However, there was a significant cubic relationship between storage duration and CAT activity of control fruits. The CAT activity of control fruits reduced from week 0 to week 1 and increased consistently until week 2 and decreased drastically until week 3 (Figure 4.5). There were significant and negative correlations between peel and pulp h° with CAT activity, ($r = -0.33$) and ($r = -0.43$), respectively. In addition, there was a significant and positive correlation between peel C* and CAT activity ($r = 0.34$). There was a significant negative correlation between firmness and CAT activity ($r = -0.39$) (Table 3.2).

Table 4.2 also illustrates that there was a significant ($P \leq 0.05$) interaction effect of storage temperature x heat treatment on APX activity. There was a significant interaction effect of storage duration x storage temperature on APX activity. There was no significant interaction effect of heat treatment x storage duration on APX activity. For papaya fruits stored at 12 °C there was a significant cubic relationships between APX activity and storage duration (Figure 4.6). Enzyme activity increased from week 0 until week 2 of the storage and slightly decreased afterwards. The R^2=0.50 indicated that 50% of the variability in APX activity at 12 °C was due to the storage duration.

There was no significant relationship between APX activity and storage duration of papaya fruits at 6 °C. The correlation indicates that there was a significant and positive correlation between weight loss and APX activity (r = 0.52) (Table 3.2). There was a significant positive correlation between peel C* and APX activity (r = 0.44). Pulp h° and firmness showed a significant negative correlation with APX activity, (r = -0.36) and (r = -0.56), respectively. There was a significant difference in APX activity between control fruits stored at 6 and 12 °C (Figure 4.7). Control fruits stored at 12 °C had significantly higher APX activity than the control fruits stored at 6 °C. The APX activity of control and hot water dip papaya fruits stored at 6 °C were significantly different. However, there was no significant difference in APX activities between hot water dip and control fruits stored at 12 °C (Figure 4.7). Table 4.2 indicated no significant interaction effect of heat treatment x storage temperature, and heat treatment x storage duration on SOD activity. However, there was a significant ($P \leq 0.05$) interaction effect of storage duration x storage temperature on SOD enzyme activity of papaya.

Table 4.2 Main and interaction effects of heat treatment, storage temperature and storage duration on catalase, ascorbate peroxidase and superoxide dismutase activity of 'Frangi' papaya.

Factor	Catalase (U/g FW)	Ascorbate peroxidase (U/g FW)	Superoxide dismutase (U/g FW)
Heat treatment (HT)			
Non hot water dip (control)	2.22 a[z]	41.13 b	85.84 a
Hot water dip	1.77 b	48.07 a	84.40 a
Storage temperature (ST) (ºC)			
6	1.74 b	35.75 b	79.89 b
12	2.25 a	53.45 a	90.35 a
Storage duration (SD) (Weeks)			
0	1.84 b	34.59c	74.66 b
1	1.77 b	43.79 b	96.00 a
2	2.41 a	54.18 a	73.13 b
3	1.96 b	45.84 b	96.69 a
HT x ST	ns	*	ns
HT x SD	*	ns	ns
SD x ST	ns	*	*
HT x ST x SD	ns	*	*

[z] Means within a column followed by the same letter were not significantly different by LSD test at P≤0.05.
ns = Non significant and * = Significant at P≤0.05.

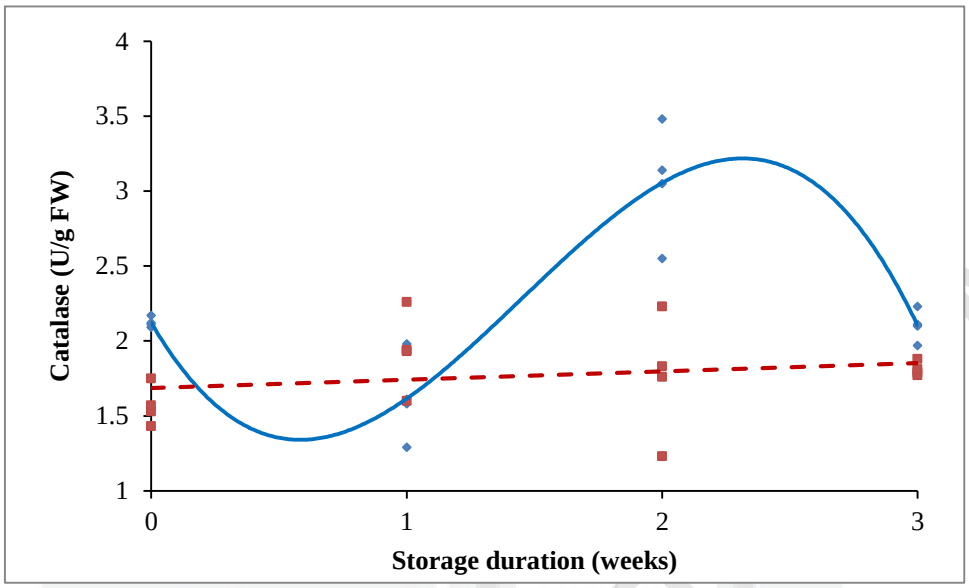

Figure 4.5 Relationship between storage duration and catalase activity (U/g FW) of 'Frangi' papaya in control (♦) and hot water dip (■) fruits.
y (control)=2.12-2.92x+3.14x^2-0.72x^3 (R^2=0.50). Solid line indicates a significant cubic relationship.

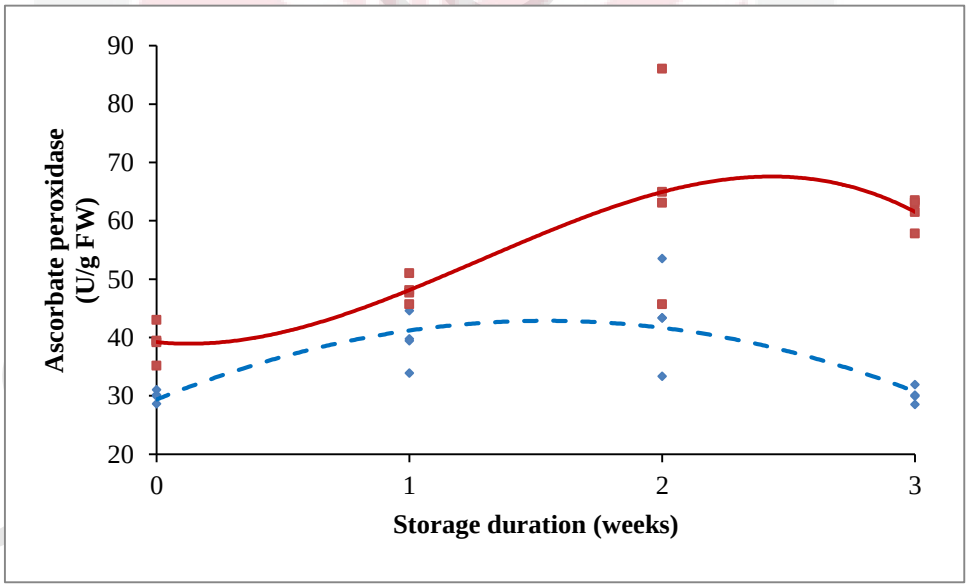

Figure 4.6 Relationship between ascorbate peroxidase activity (U/g FW) and storage duration of 'Frangi' papaya stored at 6 °C (♦) and 12 °C (■).
y (12 °C)=39.21-4.37x+17.98x^2-4.68x^3 (R^2=0.50). Solid line indicates a significant cubic relationship.

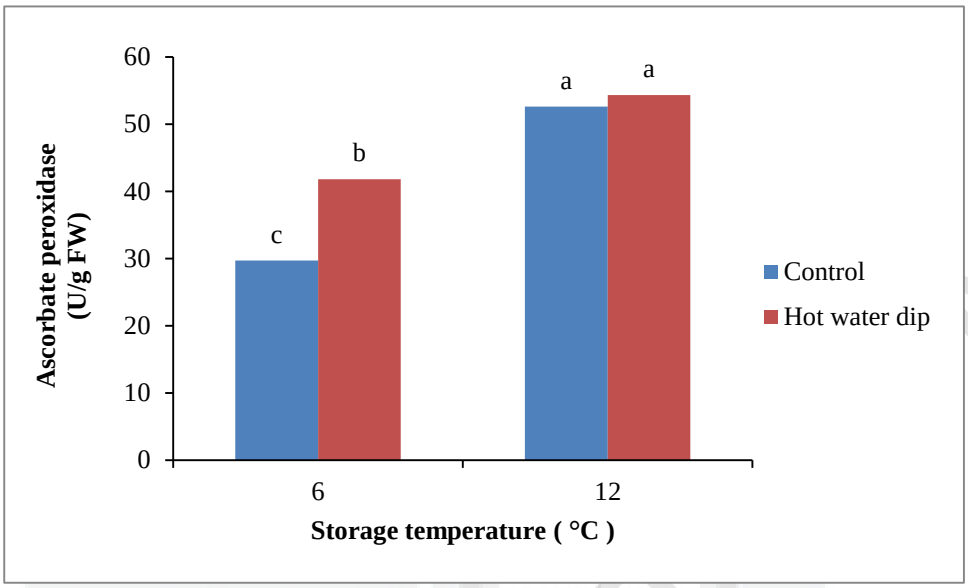

Figure 4.7 Effects of storage temperature and hot water dip on ascorbate peroxidase activity (U/g FW) of 'Frangi' papaya.
Data shown are the means of three replications. Means followed by the same letters were not significantly different by LSD test, P ≤0.05.

The result indicated that SOD enzyme activity had a significant positive correlation with weight loss (r = 30) (Table 3.2). There was a significant cubic relationship between storage duration and SOD activity of fruits stored at 12 °C (Figure 4.8). During the first week of the storage, SOD activity of fruits stored at 12 °C increased and then decreased markedly until week 2 of the storage. This was followed by an increase after week 2 until week 3 of the storage. There was no significant relationship between SOD activity and storage duration of papaya stored at 6 °C (Figure 4.8). The significant correlation between peel and pulp color values (C*, h°) and antioxidant enzyme activity, indicated the relationship between ripening and antioxidant enzymes activity.

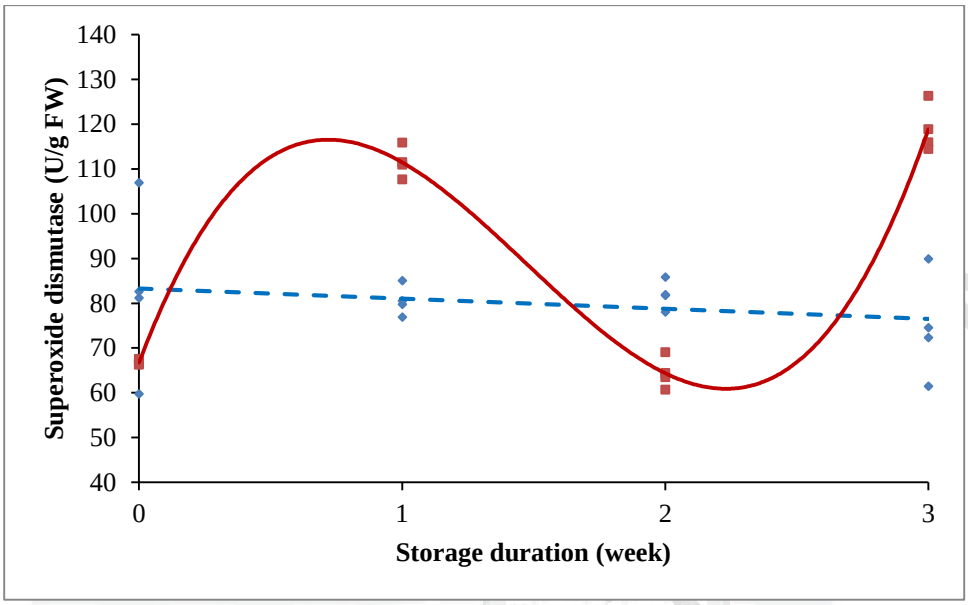

Figure 4.8 Relationship between storage duration and storage temperature on superoxide dismutase activity (U/g FW) of 'Frangi' papaya stored at 6 °C (♦) and 12 °C (■).
y (12 °C)=66.73+155x-142.5x^2-32.22x^3 (R^2=0.58). Solid line indicates a significant cubic relationship.

Fruits ripening process has been discussed as an oxidative phenomenon which needs a turnover of AOS, such as superoxide anion and H_2O_2 (Hamilton, 1974). It could be concluded that fruits ripening progression will result in an increase of antioxidant enzymes activity. However, correlation results of CAT activity in Sweet orange (*Citrus sinensis* (L.) Osbeck) showed considerable reduction as the ripening progressed (Huang et al., 2007). In this study, CAT activity was significantly affected by the storage temperature, as evidenced by the lower activity at 6 °C when compared with 12 °C (Table 4.2). It was similar with results on mume (*Prunusmume*) fruits stored at 6 °C, in which they indicated a lower CAT activity (Imahori et al., 2008). CAT is the enzyme that keeps cells against AOS; it catalyzes the decomposition of hydrogen peroxide to

oxygen and water (Ghasemnezhad et al., 2008). Therefore, the increase of CAT activity of fruits stored at 12 °C could be due to the decomposition of hydrogen peroxide which will cause the formation of hydroxyl radical and alleviation of chilling-induced peel injury (Table 4.1). Differences of CAT activity between storage temperatures reflect the physiological differences which are related to the function of the antioxidant system in fruits. The ROS accumulation is one of the mechanisms that contribute to the loss of membrane integrity and membrane-bound enzyme activities (Lacan and Baccou, 1998). The increase of H_2O_2 and lipid hydro peroxides was mentioned during fruits ripening and senescence (Lacan and Baccou, 1998). Lower CAT expression was found in hot water-treated bananas than in the control fruits during cold storage (Wang et al., 2012). The reported results were similar to those obtained in this experiment; treated fruits had lower CAT activity than the control fruits (Figure 4.5).

The fluctuating trend of CAT activity in control fruits during the storage could be the result of increasing H_2O_2 content during storage as it was mentioned by Huang et al. (2008) on 'Cara Cara' navel orange. They noted that, H_2O_2 content in control fruits increased, especially in the middle stage of fruits ripening (week 2 as shown on papaya). The findings on Navel and 'Valencia' oranges (Bassal and El-Hamahmy, 2011) were not in accordance with the results in this experiment. Hot water treatment significantly increased CAT activity in fruits peel and juice during the storage at 1 °C for 20 days. In addition, decrease in CI was paralleled with higher CAT activity (Bassal and El-Hamahmy, 2011). A hot water treatment at 53 °C for 3 min increased the CAT activity in Fortune mandarins.

After the fruits had been moved from high storage temperature to cold temperature storage, a quick reduction in CAT activity occurred, associated with enhanced CI. The different efficiencies of the heat-conditioning treatments in increasing the Fortune mandarin's chilling tolerance could be due to the induction of CAT activity during heat treatment. The CAT activity in grape berries was reduced to different levels after heat pretreatment, and also chilling stress caused a significant reduction in enzyme activity both in heat treated and control berries (Zhang et al., 2005). The reason might be attributed to the dominant production of H_2O_2 over the CAT scavenging activity (Huang et al., 2008).Their finding were similar with the result of this experiment and the constant trend of CAT activity observed in hot water dip fruits during three weeks of storage.

During storage of Satsuma mandarin at 2 °C for 8 weeks, a reduction in CAT activity was observed, while peroxidase activity increased. When temperature increased, CAT activity tended to increase, but there was a reduction at 55 °C after 8 weeks of storage (Ghasemnezhad et al., 2008). Antioxidant enzyme activity increased when the oxidant level increased with environmental stress (Ren et al., 1999). According to the results on papaya in this experiment, it did not indicate that higher CAT activity correlated with resistance to CI in hot water dip papaya. There was no significant relationship between APX activity and storage duration at 6 °C storage temperature (Figure 4.6). The results obtained in this study, were similar with those of Galli et al. (2009) who reported similar findings on pawpaw fruits stored at 4 °C. APX activity did not change significantly during fruit ripening in cold storage temperature.

Low APX activity could control dehydroascorbate (DHA) production, disable H_2O_2 scavenging system, and lead to H_2O_2 accumulation (Zhang and Kirkham, 1996). These cellular changes will result in oxidative stress injuries and increase postharvest senescence. The obtained results at 6 °C storage may indicate that ascorbate-glutathione cycle in papaya, did not provide the antioxidant protection during 3 weeks of cold storage (Barreiro et al., 2001, Mondal et al., 2006). In mume fruits, APX activity at 6 °C showed a quick reduction on the first day and slowly decreased during the entire storage period (Imahori et al., 2008). The first part of the results on mume fruits was in contrast with papaya stored at 6 °C, because during the first day of papaya storage, the APX showed increased activity until week 1.

The decreasing trend of the APX activity observed from week 1 to 3 in papaya fruits stored at 6 °C in this study was similar with mume fruits stored for 16 days at 6 °C (Imahori et al., 2008). In this experiment, the highest APX activity at 12 °C storage observed on week 3 (Figure 4.6). Similarly, the reduction of peel CI was observed on week 3 of storage (Figure 4.2). It seemed that in fruits stored at 12 °C, the APX isoforms were synthesized and caused the reduction of hydrogen peroxide concentration, and consequently papaya was protected from oxidative stress as previously mentioned by Ghasemnezhad et al. (2008). Figure 4.7 indicates that APX activity was significantly higher in hot water dip fruits stored at 6 °C. Optical density analysis of northern hybridization findings indicated that the hot water dip fruits showed higher MaAPX gene expression level than the control during cold storage. The higher expression rate of MaAPX gene probably is correlated to higher chilling resistance of the treated fruits

(Wang et al., 2012). The ROS content increased soon after hot water treatment of banana fruits (Wang et al., 2012). Hot water treatment developed a transient increase in APX (H_2O_2 and $O_2^{-\bullet}$) amount in banana in 0.5-1.5 h after the treatment. It seemed that as the ROS increased, consequently the APX activity also increased in treated fruits. APX activities in banana peel started to increase 0.5 h after hot water dip treatment, and reached a peak within 1.5-6 h, where significantly higher activities of APX were determined for the treated banana fruits compared to control fruits.

The increased activity of antioxidant enzyme (APX) induced by HT can protect the membrane damages produced by chilling stress, and consequently enhance chilling tolerance (Zhang et al., 2005). According to Table 3.2, treated samples showed higher ascorbic acid compared with control fruits, and at the same time, APX activity was higher in treated fruits. It could be explained that heat treatments mostly increased ascorbic acid content. Lower ascorbic acid amount of untreated fruits happened because of oxidation and lower amount of APX to reduce the ROS (Bassal and El-Hamahmy, 2011).

The APX enzyme, which is localized in mitochondria, chloroplasts, cytosol, and micro-bodies, is a key enzyme to decompose hydrogen peroxide in fruits and plants (Mittler, 2002). Because of the different affinity of APX and CAT for hydrogen peroxide, APX is probably responsible for the fine modulation of AOS for signaling while CAT might be a controlling factor to remove the excess AOS under stress (Mittler, 2002). The results of this experiment were similar with results for heat-treated strawberry fruits, in which

treated fruits had higher APX activity after 1 or 2 days at 0 °C (Vicente et al., 2006). APX activity was also increased or remained elevated during 7 days of refrigerated storage (Vicente et al., 2006). Control fruits stored at 12 °C showed significantly higher APX activity than control fruits stored at 6 °C (Figure 4.7). It is known that free radicals are produced during fruits ripening at optimum temperature and caused deteriorative changes related with senescence, but storage at chilling temperature retarded the normal ripening process and inactivated the production of free radicals (Huang et al., 2008). The induced high level of APX in cold storage may play a significant role in the alleviation of cold damage (Wang et al., 2012).

The results of other researchers were the same as Figure 4.1 which showed peel CI reduction of hot water dip fruits stored at 6 °C. Hot water treatment at 53 °C for 3 min produced a significant but temporary increase in APX enzymes activity directly after treatment in 'Persian' limes (*Citrus latifolia* T.), but this induction was not persistent during the storage period. These results suggested that the effectiveness of the conditioning treatment depends on fruits capacity to maintain the induction of antioxidant enzymes for the whole storage period (Rivera et al., 2004). Figure 4.8 showed the fluctuating trend of SOD activity during the storage at 12 °C and it could explain the role of SOD enzyme in removing superoxide radicals, and protecting cells from injury related to an excessive radical accumulation (Van Breusegem et al., 1999). During cold storage at 6 °C, SOD activity remained constant in comparison with the samples stored at 12 °C (Figure 4.8). The results obtained in this study, were the same as those obtained by Huang et al. (2008), there was a lower SOD activity in the pulp of

'Cara Cara' navel orange during storage at 6 °C than stored fruits at 20 °C. This phenomenon could be probably due to the lower production of ($O_2^{\bullet-}$) in cells at low temperature, or slow metabolism at the lower temperature. Changes in SOD activity are related with fruits cold-resistance and low temperature progressively activated SOD enzyme activity, but in contrast with this experiment low temperature did not cause the increase in SOD activity. The SOD activity in mangoes stored at 12 °C was higher than 6 °C storage (Kondo et al., 2005). These results suggest a link among SOD activity, and ($O_2^{\bullet-}$) scavenging activity at 12 °C (Kondo et al., 2005).

The role of SOD activity is to catalyze the dismutation of superoxide radical anions into H_2O_2 and molecular oxygen (Scandalios, 2001). Lowering the storage temperature (5 °C) caused the reduction of SOD activity in broccoli compared with 10 °C storage temperature. These results indicated that SOD plays a crucial role in retarding the senescence in broccoli but there are differences in the antioxidant mechanisms of broccoli stored below 10 °C (Zhang et al., 2009). The SOD activity is correlated conversely with the rate of superoxide radical production in chilling-sensitive fruits (Lukatkin, 2002). Peel CI occurrence in papaya fruits was not totally related with SOD production at 6 and 12 °C, but the constant trend of peel CI from week 2 to 3 in fruits stored at 12 °C (Figure 3.4) was similar with increase of SOD at 12 °C (Figure 4.8). The enhanced SOD activity after week 2 at 12 °C could contribute to inhibit the superoxide radical accumulation during storage and consequently reducing the tissue damage (Vicente et al., 2006). High SOD activity has been attributed to the stress tolerance in

plants. This enzyme neutralizes the reactivity of superoxide radical, which is more active under stress (Bowler et al., 1992). Dismutation of the superoxide radical by SOD may be the first defense step against the chilling temperature. The AOS causes cellular damage during extensive range of environmental stresses including chilling stress. The chilling effects on the SOD activity could be described by the different cold exposure duration, and its activity obviously depended on the temperature regime during chilling (Sala and Lafuente, 2004). The integrated action of SOD, CAT and APX, might efficiently remove the AOS. These enzymes removed the AOS through conversion of the potentially dangerous superoxide radicals and H_2O_2 to protect cellular components against the active hydroxyl radicals. The balance between SOD, CAT, and APX activities is essential to cell survival during cold storage (Sala and Lafuente, 2004). Plant injury caused by different biotic and abiotic stresses often involves an imbalance between the production and removal of AOS (Wise, 1995).

In grape berries, the CAT and SOD activities were steadily reduced under chilling stress (Zhang et al., 2005). The changes in enzyme activities were induced by chilling stress and decreased by heat pretreatment, then increased after 6 h chilling stress (Zhang et al., 2005). The increase of SOD activity could improve fruits ability to dismutate superoxide radicals. In addition, the increased CAT and APX activities could be related with the elimination of hydrogen peroxide. The injury of plant tissues has been related with AOS increase under stress situations such as exposure to chilling temperature. Oxidative damage is regarded to be an early reaction of the sensitive tissues to CI (Imahori et al.,

2008). Protection of the plant cell from oxidative damages during stress is the main mechanism of plants to resist under stress condition (Kraus and Fletcher, 1994).

4.4 Conclusion

The present experiment focused on the effects of heat treatment and two storage temperatures during 21 days of storage on CI incidence of papaya peel and pulp. Peel electrolyte leakage and antioxidant enzymes activity (CAT, APX and SOD) were measured during 3 weeks of the storage. Heat treatment was effective in reducing the fruits peel and pulp CI at 6 °C storage.

Hot water dip and 12 °C storage were capable of reducing the peel CI after week 2 of storage. Heat treatment could be an appropriate method to reduce the Frangi papaya peel and pulp CI incidence during the extended storage. The reduction of PEL percentage in heat treated fruits provided another reason for the application of heat treatment in Frangi papaya. The differences between the activities of the three antioxidant enzymes in papaya fruit from two storage temperatures indicated that they might have effects on peel CI symptom reduction at 12 °C. Among the three antioxidant enzymes, APX was found to be more active in hot water dip fruits stored at 6 °C than in control fruits. The reduction of peel CI symptoms at 12 °C after storage at week 2 could be attributed with the increase of SOD activity. The highest CAT activity in control fruits coincided with constant peel CI incidence of control fruits during the storage. In conclusion, the results of this experiment showed that the occurrence of peel CI symptoms in papaya fruits

developed faster and were more severe when susceptible fruits were stored at 6 °C compared to those stored at 12 °C. Heat treatment had significant effects on peel CI reduction after week 2 at 12 °C storage. Simultaneous action of SOD, CAT, and APX enzymes activity are important to reduce the peel and pulp CI incidence of heat treated fruits.

CHAPTER 5

SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

Results obtained in this experiment showed that there is a potential for hot water dip to reduce the peel and pulp CI incidence following storage at 6 and 12 °C for three weeks. The fruits quality characteristics were not significantly affected by hot water treatment. The peel color (h°) progression was suppressed by the chilling temperature. Heat treated fruits showed lower percentage of weight loss compared with control fruits. Firmness was reduced in treated fruits more than control fruits. Fruit stored at 6 °C caused a lower reduction in fruit firmness. Ethylene production rate fluctuate in heat treated fruits and there was no significant interaction effects between heat treatment and storage duration on carbon dioxide production rate. Heat treatment did not cause injurious effects on papaya quality characteristics and after week 2 of the storage, it was effective to inhibit the ethylene production and avoid the fruit firmness reduction. Hot water dip could be an option for fruits during the storage.

When control fruits were stored at 6 °C, peel and pulp CI symptoms increased but treated fruit stored at the same temperature caused the reduction in peel and pulp CI. Heat treatment reduced the pulp CI in week 1 of storage, but the value increased nearly until the end of the storage. Storage temperatures did not affect the peel electrolyte leakage, but hot water dip was effective to reduce the peel electrolyte leakage. The antioxidants enzymes are capable of reducing the pulp and peel CI in fruits and maintain the fruit nutritional values.

APX and SOD showed higher activities in treated fruits. Antioxidant enzymes are able to control the oxidative process in fruits during the storage. During week 2 of storage at 12 °C, higher APX and SOD activity was observed. The higher enzymes activity coincided with the reduction in peel CI incidence in week 2 of storage, and consequently was followed by ethylene production reduction and sustainable fruit firmness. Heat treatment triggered the antioxidant enzymes activity, and it was evident in APX activity. Different heat treatment temperatures and exposure could be applied to obtain the optimum potential of antioxidant enzymes activity in reducing the peel and pulp CI symptoms. In addition, to find the optimum storage temperature according to the antioxidant enzymes activity, it is necessary to store the papaya fruit in various temperatures.

By testing the narrow range of temperatures in different geographical regions and seasons, the optimum results will be obtained according to reduction of papaya peel and pulp CI. More research on tropical fruits, especially on Frangi papaya, are still required to observe their reactions on the overall effect of postharvest hot water dip on quality characteristics and nutritional value. It is necessary to investigate the relationship between the physiology and biochemistry of Frangi papaya after postharvest hot water treatments. Further research will lead to predict the Frangi papaya phenotypic and genotypic flexibility resulting in a stress tolerance. The genomics, proteomics, and transcriptomics approaches should be investigated for better perception of the Frangi papaya response to heat and chilling stresses and the involved mechanisms.

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APPENDICES

Appendix 1

Control 6 °C, week 0

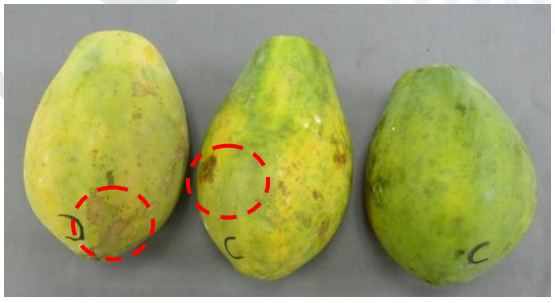
Treated 6 °C, week 0

Control 6 °C, week 1

Treated 6 °C, week 1

Control 6 °C, week 2

Treated 6 °C, week 2



Control 6 °C, week 3

Treated 6 °C, week 3

Dotted circles showed peel chilling injury progression of control and treated 'Frangi' papaya stored at 6 °C

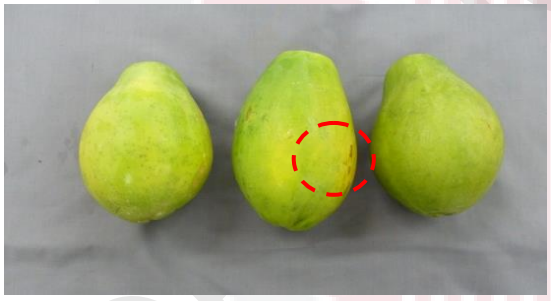
Appendix 2

Control 12 °C, week 0

Treated 12 °C, week 0

Control 12 °C, week 1

Treated 12 °C, week 1



Control 12 °C, week 2

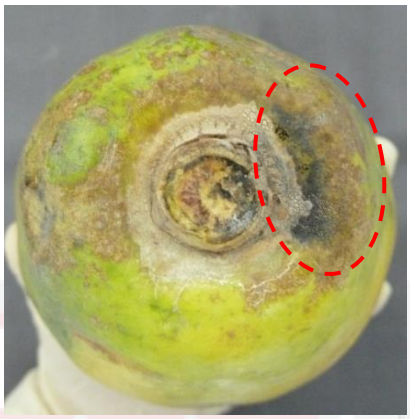
Treated 12 °C, week 2

Control 12 °C, week 3

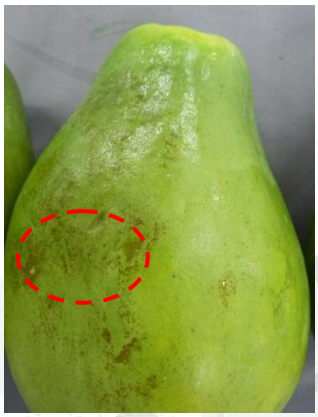
Treated 12 °C, week 3

Dotted circles showed peel chilling injury progression of control and treated 'Frangi' papaya stored at 12 °C

Appendix 3

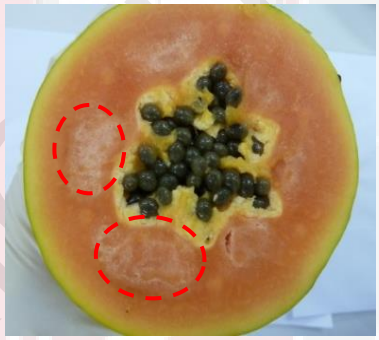


Control 6 °C, week 3

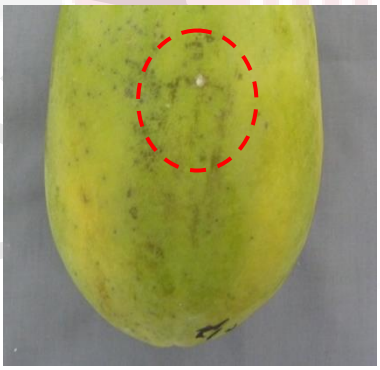


Treated 6 °C, week 3

Control 6 °C, week 3



Control 6 °C, week 3

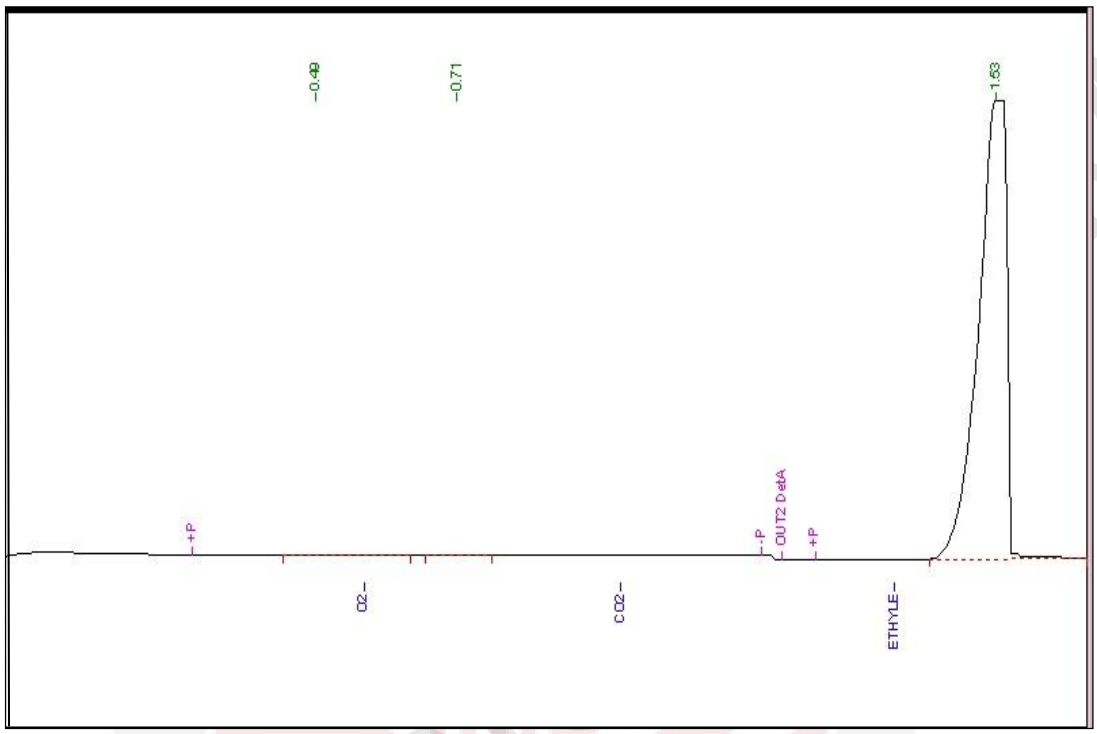


Control 12 °C, week 3

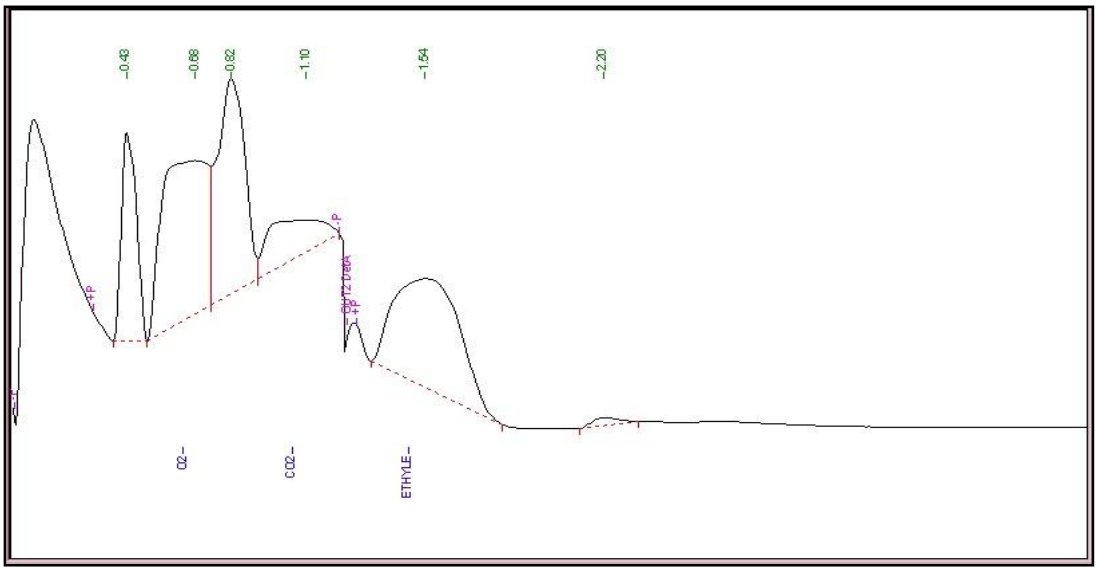
Treated 12 °C, week 3

Dotted circles showed progression of peel and pulp chilling injury in control and treated 'Frangi' papaya stored at 6 and 12 °C

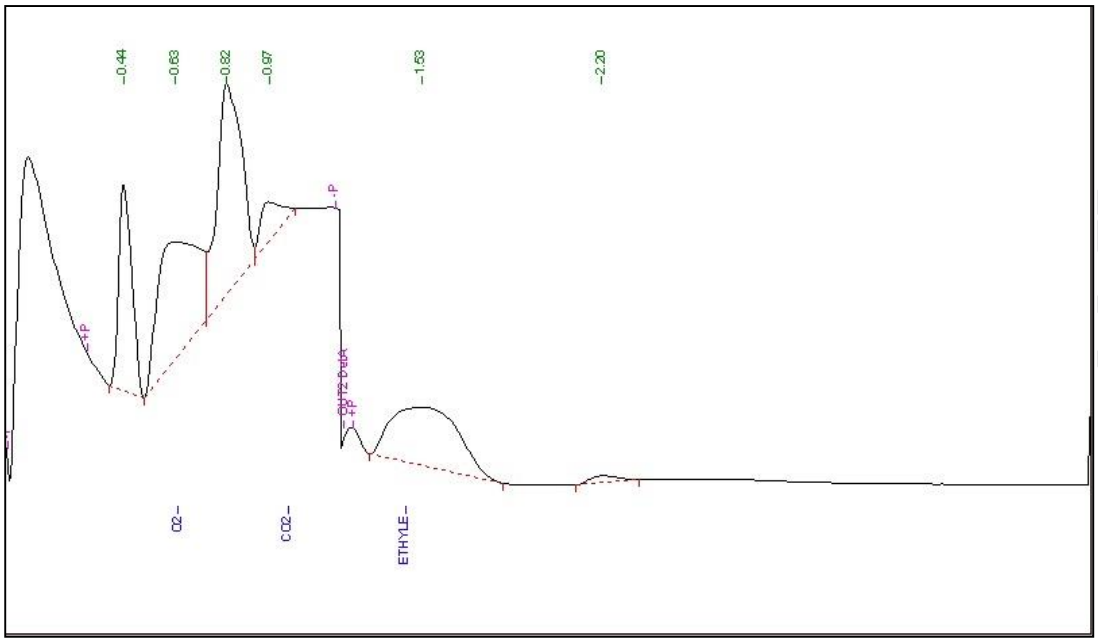
Appendix 4



A representative chromatograph of ethylene standard



A representative chromatograph of ethylene production rate in control samples



A representative chromatograph of ethylene production rate in treated samples

BIODATA OF STUDENT

Nasim Shadmani was born in Hamedan, Iran, on 9th December 1982. In 2001, she continued her bachelor study in Agriculture, Agronomy and Breeding in BU-Ali Sina University of Hamedan and graduated in 2005. She enrolled as a full-time master student in Universiti Putra Malaysia in December 2009 and completed her master program in the field of Agriculture, Postharvest in December 2012.

If you have any inquiries regarding this thesis, please feel free to contact her via email at: nasim.shadmani61@gmail.com.

LIST OF PUBLICATIONS

Shadmani, N., Ahmad, S. H., Phebe, D. & Saari, N.B. (21-23 November 2011). *Quality, Storage Life and Chilling Injury Incidence of Carica Papaya L.cv. Frangi after Postharvest Hot Water Treatment.* Paper presented at the 22nd Malaysian Society of Plant Physiology Conference: Approaches to Sustainable Plant Productivity and Safety in Challenging Environment, Grand BlueWave, Johor Bahru.

Shadmani, N., Ahmad, S. H., Phebe, D. & Saari, N.B. (25th to 29th June 2012). *Quality, Storage Life and Chilling Injury Incidence of Carica Papaya L.cv. Frangi after Postharvest Hot Water Treatment.* Paper presented at the 7nd International Postharvest Symposium: (IPS 2012), Putra World Trade Centre, Kuala Lumpur Malaysia.