UNIVERSITI PUTRA MALAYSIA

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

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By

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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

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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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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.

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' 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

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