### EFFECTS OF POSTHARVEST HOT WATER TREATMENT ON PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF EKSOTIKA II PAPAYA DURING RIPENING

**ARINA BINTI MHD ARSHAD** 

FACULTY OF SCIENCE UNIVERSITY OF MALAYA KUALA LUMPUR

2011

### EFFECTS OF POSTHARVEST HOT WATER TREATMENT ON PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF EKSOTIKA II PAPAYA DURING RIPENING

**ARINA BINTI MHD ARSHAD** 

### DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

### INSTITUTE OF BIOLOGICAL SCIENCES FACULTY OF SCIENCE UNIVERSITY OF MALAYA KUALA LUMPUR

2011

#### Abstract

The effects of postharvest hot water treatment (HWT) on the quality of 'Eksotika II' papaya fruit harvested at different harvesting maturities were investigated. HWT is a method for fruit fly disinfestation which is a quarantine requirement for the fresh papaya exportation industries in Malaysia. Fruit at maturity stages Index 1 (H1), Index 2 (H2) and Index 3 (H3) were harvested. From each maturity stage, one group of the papaya fruit batch was treated with hot water at 47±1°C fruit core temperature held for 10 minutes and another group was untreated. Fruit were left to ripen at ambient temperature  $(25^{\circ}C)$  and were taken into experiment at the subsequent ripening stages at Index 2, Index 3, Index 4 and Index 5. Physiological changes including skin colour (L\*a\*/b\*), weight loss, fruit firmness, total soluble solids (TSS), pH and chlorophyll fluorescence were determined at each different ripening stages. Biochemical changes including total sugar, total reducing sugar, total non-reducing sugar and cell wall degrading enzymes activities such as polygalacturonase (PG), pectin methylesterase (PME), pectate lyase (PL) and cellulase were also investigated. HWT did not give any significantly adverse effect to the physiology parameters evaluated, such as L\*a\*/b\* value, weight loss, TSS and pH. However, the treated Index 3 and Index 4 of H1 fruit was slightly firmer than the untreated fruit. The chlorophyll fluorescence parameters have shown that within one hour after treatment, the treated fruit experienced heat stress which was reflected in the lower chlorophyll fluorescence parameters values, Fv/Fm. The heat stress was found to be more severe by 14 - 25 % in the H3 fruit rather than in the H2 and H1 fruit. However, the Fv/Fm value in the H3 fruit recovered by 25% during the subsequent stages of ripening. Furthermore, the total sugar and the total reducing sugar content were not adversely affected by the HWT. The pattern of cell wall degrading enzymes activity showed that PME activity decreased during ripening, whilst PG, PL and cellulase activity increased progressively during ripening. It was also found

that the application of the HWT affected the cell wall degrading enzymes activity whereby it was found to be lower in the heat treated fruit. Nevertheless, pulp firmness was not severely affected since some of the enzyme activities managed to recover during the ripening period. For maximum fruit quality, particularly for export markets, it is recommended that Index 2 fruit are selected for HWT rather than H1 fruit since the H1 fruit will ripen poorly. The Index 3 Eksotika II papaya is only suitable for the local market because of its short shelf life. In summary, these observations indicated that the overall Eksotika II papaya fruit quality is still maintained following HWT when the fruit is allowed to ripen at ambient temperature. This suggests that postharvest HWT at 47 °C (fruit core temperature, held for 10 minutes) can maintain the postharvest quality of Eksotika II papaya fruit.

#### Abstrak

Kesan – kesan rawatan air panas lepas tuai terhadap kualiti buah betik 'Eksotika II' yang dituai pada pada tahap kematangan yang berbeza telah dikaji. Rawatan air panas adalah salah satu kaedah untuk membasmi lalat buah yang merupakan satu keperluan kuarantin yang perlu dipatuhi oleh industri pengeksportan betik di Malaysia. Buah dituai pada tahap kematangan Indeks 1 (H1), Indeks 2 (H2) dan Indeks 3 (H3). Satu kumpulan daripada buah yang dituai pada setiap tahap kematangan yang berbeza dirawat dengan air panas sehingga teras buah mencapai suhu  $47\pm1^{\circ}$ C dan suhu tersebut dikekalkan selama 10 minit. Manakala, satu lagi kumpulan buah tidak dirawat dan digunakan sebagai kawalan. Kemudian, kesemua buah tersebut dibiarkan melalui proses pemasakan pada suhu bilik (25°C) dan diambil untuk eksperimen pada setiap tahap pemasakan yang berlaku seterusnya iaitu pada tahap pemasakan Indeks 2, Index 3, Indeks 4 dan Indeks 5. Perubahan – perubahan fisiologi yang meliputi perubahan warna kulit buah (L\*a\*/b\*), kehilangan berat, tahap kekerasan buah, jumlah bahan larut (TSS), pH dan sinaran berpendarfluor klorofil telah dikenalpasti pada setiap tahap pemasakan. Perubahan – perubahan biokimia termasuklah jumlah keseluruhan gula, jumlah gula penurun, jumlah gula bukan penurun dan aktiviti enzim-enzim yang terlibat dalam penceraian struktur dinding sel seperti poligalakturonase (PG), pektin metilesterase (PME), pektate lyase (PL) dan cellulase juga telah dikaji. Rawatan air panas tidak memberikan kesan tidak baik yang signifikan terhadap faktor – faktor fisiologi yang dikaji seperti nilai L\*a\*/b\*, kehilangan berat buah, TSS dan pH. Walaubagaimanapun, buah H1 pada Indeks 3 dan 4 yang dirawat dengan air panas adalah lebih keras berbanding dengan buah pada Indeks yang sama yang tidak dirawat. Parameter sinaran berpendarfluor klorofil menunjukkan bahawa dalam masa satu jam selepas rawatan, buah yang dirawat mengalami tekanan yang disebabkan oleh suhu tinggi. Kesan ini boleh dilihat dari nilai Fv/Fm yang rendah. Tekanan yang disebabkan oleh suhu tinggi ini adalah lebih ketara (14 - 25%) pada buah yang lebih masak iaitu H3 berbanding buah yang kurang masak iaitu H2 dan H1. Walaubagaimanapun, nilai Fv/Fm ini pulih kembali sebanyak 25 % apabila melalui proses pemasakan yang seterusnya. Disamping itu, kandungan keseluruhan gula dan gula penurun tidak dipengaruhi oleh rawatan air panas yang dijalankan. Corak aktiviti enzim – enzim yang terlibat dalam proses penceraian struktur dinding sel menunjukkan bahawa aktiviti PME menurun semasa proses pemasakan, manakala aktiviti enzim – enzim PG, PL dan cellulase meningkat sepanjang proses pemasakan. Rawatan air panas memberi kesan terhadap aktiviti enzim – enzim ini dimana aktiviti enzim – enzim tersebut adalah lebih rendah didalam buah yang dirawat dengan air panas. Walaubagaimanapun, kekerasan buah tidak dipengaruhi oleh keadaan ini kerana ada diantara aktiviti enzim – enzim ini telah kembali pulih semasa proses pemasakan. Untuk kualiti buah yang maksimum, khasnya untuk pasaran eksport, adalah dicadangkan bahawa buah dituai pada tahap kematangan Indeks 2 untuk rawatan air panas, memandangkan buah yang dituai pada Indeks 1 (H1) tidak akan masak dengan sempurna. Manakala buah yang dituai pada Indeks 3 (H3) hanya sesuai untuk pasaran tempatan kerana jangka hayatnya yang pendek. Kesimpulannya, keputusan kajian ini menunjukkan bahawa kualiti buah betik 'Eksotika II' secara keseluruhannya dikekalkan walaupun selepas rawatan air panas apabila buah tersebut dibiarkan melalui proses pemasakan pada suhu bilik. Ini menunjukkan bahawa rawatan air panas lepas tuai pada suhu 47 °C (suhu teras buah yang dikekalkan selama 10 minit) dalam kajian ini dapat mengekalkan kualiti lepas tuai buah betik Eksotika II.

#### Acknowledgements

First and foremost, all praise to Allah, the Almighty, the most Gracious and the most Merciful, for His blessings and for giving me mental and physical strengths to complete this project.

I would like to express my deepest gratitude to both of my respected supervisors, Professor Dr. Amru Nasrulhaq Boyce and Dr. Chandran Somasundram for their invaluable guidance and advice throughout this study. Thanks are also expressed to Dr. Tung Heng Fong for her continuous assistance and guidance that helped me a lot to complete this study. I also would like to thank Prof. Helen Nair for her invaluable guidance and advice during the study period. I am also greatly indebted to Puan Zam Abdul Karim, for her assistance in finding the source for the papaya sample in this study. Many thanks to Encik Johari from DOA, Encik Harry Aziz from Exotic Star and Encik Rahim who provided papaya sample throughout my work. To all my friends of Postharvest Biotechnology Laboratory : Punitha, Wei Lim, Daniel, Wijenthiran, Zuliana, Kit Yew Sen, Loo Zhang Xin, Rebecca, Nadiah, Jasmine and Mr. Doraisamy, thank you very much for giving me courage and technical supports during this project.

A very sincere gratitude to my beloved father and mother, parents in law, brothers, sisters and all family members for their unconditional support and love in completing this project. Finally, a very special thanks goes to my beloved husband, Mohd Akhbar Ismail, for his love, care and support during the entire study period. Without the assistance of the people mentioned above, this study would have never reach completion.

## **Table of Contents**

	Page
Title	i
Original Literary Work Declaration	ii
Abstract	iii
Abstrak	v
Acknowledgements	vii
Table of Contents	viii
List of Figures	X
List of Tables	xiii
List of Abbreviations	xiv
List of Appendices	xvi
Chapter 1: Introduction	1
Chapter 2: Literature Review	4
2.1 Introduction	4
2.2 Papaya ( <i>Carica papaya</i> L.)	6
2.2.1 Botanical Description and Chemical Composition	6
2.2.2 Commercial Cultivation and Production	14
2.3 Fruit Ripening	17
2.3.1 Physiological and Biochemical Changes During Ripening	17
2.3.2 Cell Wall Degrading Enzymes Activity During Ripening	20
2.4 Postharvest Impediment: Fruit Fly Infestation	25
2.4.1 Common Postharvest Heat Treatment to Overcome Fruit Flies Infestation	26
2.4.2 Hot Water Treatment	27
2.5 Effects of Heat Treatment to Fresh Produce	29

Chapter 3: Physiological Properties of Hot Water Treated and Untreated Eksotika II Papaya	
3.1 Introduction	32
3.2 Materials and Methods	34
3.3 Results	40
3.4 Discussion	65
Chapter 4: Postharvest Changes in Sugar Accumulation of Hot Water Treated and Untreated Eksotika II Papaya	72
4.1 Introduction	72
4.2 Materials and Methods	74
4.3 Results	77
4.4 Discussion	86
Chapter 5: Cell Wall Degrading Enzymes Activity of Hot Water Treated and Untreated Eksotika II Papaya	90
5.1 Introduction	90
5.2 Materials and Methods	93
5.3 Results	103
5.4 Discussion	115
Chapter 6: Conclusion	122
Bibliography	125
Appendices	137

# List of Figures

## Page

Figure 2.1:	Tree of <i>Carica papaya</i> cv. Eksotika II	13
Figure 2.2:	Planted area and production of papaya in Malaysia (2006 – 2009)	15
Figure 2.3:	Mode of action of pectin degrading enzymes	21
Figure 3.1:	Colour indices of Carica papaya L. cv. Eksotika	35
Figure 3.2:	L*a*/b* values of (a) untreated and (b) treated Eksotika II papaya fruit harvested at three different maturity stages	41
Figure 3.3:	L*a*/b* values of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	42
Figure 3.4:	L*a*/b* values of treated and untreated Eksotika II papaya fruit at different ripening stages	43
Figure 3.5:	Relationship between the ripening index and the skin colour (L*a*/b*) values of Eksotika II papaya fruit during ripening	45
Figure 3.6:	Visual observation of representative hot water treated and untreated Eksotika II papaya fruit at each ripening stage. Fruit were harvested at maturity stage Index 1 (H1)	47
Figure 3.7:	Visual observation of representative hot water treated and untreated Eksotika II papaya fruit at each ripening stage. Fruit were harvested at maturity stage Index 2 (H2).	48
Figure 3.8:	Visual observation of representative hot water treated and untreated Eksotika II papaya fruit at each ripening stage. Fruit were harvested at maturity stage Index 3 (H3).	49
Figure 3.9:	Percentage of weight loss of (a) untreated and (b) treated Eksotika II papaya fruit harvested at three different maturity stages	51
Figure 3.10	Percentage of weight loss of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	52
Figure 3.11	Initial pulp firmness values of treated and untreated Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	53
Figure 3.12	Pulp firmness values of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	54
Figure 3.13	Initial pH of treated and untreated Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	55

Figure 3.14: The pH of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	56
<b>Figure 3.15:</b> Initial total soluble solids value of hot water treated and untreated Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	57
<b>Figure 3.16:</b> Total soluble solids values of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	58
<b>Figure 3.17:</b> Initial photosynthetic yield (Quantum efficiency of PSII, Fv / Fm) of treated and untreated Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	60
<b>Figure 3.18:</b> Photosynthetic yield (Quantum efficiency of PSII, Fv / Fm) of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	61
<b>Figure 3.19:</b> Chlorophyll fluorescence parameters (Fo, Fm and Fv) of hot water treated and untreated Eksotika II papaya fruit at different ripening stages. Fruit were harvested at maturity stage Index 1 (H1)	63
<b>Figure 3.20:</b> Chlorophyll fluorescence parameters (Fo, Fm and Fv) of hot water treated and untreated Eksotika II papaya fruit at different ripening stages. Fruit were harvested at maturity stage Index 2 (H2)	63
<b>Figure 3.21:</b> Chlorophyll fluorescence parameters (Fo, Fm and Fv) of hot water treated and untreated Eksotika II papaya fruit at different ripening stages. Fruit were harvested at maturity stage Index 3 (H3)	64
<b>Figure 4.1 :</b> Initial total sugar content in Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	78
Figure 4.2: Total sugar content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	79
Figure 4.3: Initial total reducing sugar content of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	81
Figure 4.4: Total reducing sugar content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	82
Figure 4.5: Initial total non-reducing sugar content of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	84
<b>Figure 4.6:</b> Total non-reducing sugar content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	85

Figure 5.1:	Initial protein content of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	104
Figure 5.2:	Protein content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	105
Figure 5.3:	Initial pectin methylesterase activity of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	106
Figure 5.4:	PME activity in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	107
Figure 5.5:	Initial polygalacturonase activity of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	109
Figure 5.6:	Polygalacturonase activity in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	110
Figure 5.7:	Initial pectate lyase activity of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	111
Figure 5.8:	Pectate lyase activity in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	112
Figure 5.9:	Initial cellulase activity of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3	113
Figure 5.10	Cellulase activity in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	114

## List of Tables

#### Page

Table 2.1: Top world papaya producing countries in 2008	5
Table 2.2: Top world papaya exporters in 2008	5
Table 2.3: Common papaya varieties in commerce and breeding	9
Table 2.4: Some chemical compounds occurring in Carica papaya L.	11
Table 2.5: Top Malaysian's papaya importers in 2007	15
<b>Table 3.1:</b> Proposed Eksotika II papaya fruit maturity indices value (L*a*/b*)range for the six maturity stages	44
Table 5.1: Preparation of standard BSA solution for protein estimation assay	94
Table 5.2: Preparation of standard galacturonic acid (GA) solution for PME assay	96
Table 5.3: Preparation of standard GA solution for PG assay	98
Table 5.4: Preparation of standard glucose solution for cellulase assay	101

## List of Abbreviations

ASEAN	Association of South-East Asian Nations
cm	Centimetre
СМС	Carboxymethylcellulase
ddH <sub>2</sub> O	Double distilled water
dH <sub>2</sub> O	Distilled water
DMRT	Duncan Multiple Range Test
DOA	Department of Agriculture, Malaysia
et al.	et alia (and others)
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
Fm	Maximum fluorescence yield
Fo	Minimum fluorescence yield
Fv	Variable fluorescence yield ( $Fv = Fm - Fo$ )
Fv/Fm	Optimum quantum yield of photosystem II
FW	Fresh weight
g	Gram
GA	Galacturonic acid
H1	Eksotika II papaya fruit harvested at Index 1
H2	Eksotika II papaya fruit harvested at Index 2
H3	Eksotika II papaya fruit harvested at Index 3
HSP	Heat shock proteins
HWT	Hot water treatment
Kgf	Kilogram force
m	Metre

М	Molar
MARDI	Malaysian Agricultural Research and Development Institute
mg	Milligram
μg	Microgram
μΜ	Micromolar
μmol	Micromoles
ml	Millilitre
mM	Milimolar
mm	Millimetre
PG	Polygalacturonase
PL	Pectate lyase
PME	Pectin methylesterase
PRSV	Papaya Ringspot Virus
RT	Room temperature
SPS	Sanitary and Phytosanitary
TSS	Total soluble solids
UDP	Uridine diphosphate
UK	United Kingdom
UTP	Uridine triphosphate
USA	United States of America

# List of Appendices

		Page
Appendix 1:	The certificate of patents – Fruit fly's Ridding System	137
Appendix 2:	Eksotika II papaya core temperature is being monitored using a thermometer	139
Appendix 3:	Immature fruit of Eksotika II papaya	139
Appendix 4:	L*a*/b* values against days after harvest of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	140
Appendix 5:	L*a*/b* value against ripening stages of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	140
Appendix 6:	Weight loss percentage of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	141
Appendix 7:	Pulp firmness of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	141
Appendix 8:	pH of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	142
Appendix 9:	TSS value of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	142
Appendix 10	: Fv/Fm values of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	143
Appendix 11	Fo, Fm and Fv values of hot water treated and untreated Eksotika II papaya fruit harvested at Index 1 (H1) during ripening	143
Appendix 12	Fo, Fm and Fv values of hot water treated and untreated Eksotika II papaya fruit harvested at Index 2 (H2) during ripening	144
Appendix 13	Fo, Fm and Fv values of hot water treated and untreated Eksotika II papaya fruit harvested at Index 3 (H3) during ripening	144
Appendix 14	: Total sugar, total reducing sugar and total non-reducing sugar content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	145
Appendix 15	: Total protein value of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening	146

Appendix 16: Pectin methylesterase activity of hot water treated and untreated	
Eksotika II papaya fruit harvested at different maturity stages	
during ripering	

- Appendix 17: Polygalcturonase activity of hot water treated and untreated 147 Eksotika II papaya fruit harvested at different maturity stages during ripening
- **Appendix 18:** Pectate lyase activity of hot water treated and untreated Eksotika II 147 papaya fruit harvested at different maturity stages during ripening
- Appendix 19: Cellulase activity of hot water treated and untreated Eksotika II 148 papaya fruit harvested at different maturity stages during ripening