

## **UNIVERSITI PUTRA MALAYSIA**

# PHYSICO-CHEMICAL, ANTIOXIDANTS AND STRUCTURAL CHANGES DURING DEVELOPMENT OF RED-FLESHED DRAGON FRUIT (Hylocereus polyrhizus)

**NUR ADILLA JAMALUDIN** 

FP 2010 13



# PHYSICO-CHEMICAL, ANTIOXIDANTS AND STRUCTURAL CHANGES DURING DEVELOPMENT OF RED-FLESHED DRAGON FRUIT

(Hylocereus polyrhizus)

# **NUR ADILLA JAMALUDIN**

# MASTER OF SCIENCE UNIVERSITI PUTRA MALAYSIA

2010



# PHYSICO-CHEMICAL, ANTIOXIDANTS AND STRUCTURAL CHANGES DURING DEVELOPMENT OF RED-FLESHED DRAGON FRUIT (Hylocereus polyrhizus)

# By NUR ADILLA JAMALUDIN

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

September 2010



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the

requirement for the degree of Master of Science

PHYSICO-CHEMICAL, ANTIOXIDANTS AND STRUCTURAL CHANGES

**DURING DEVELOPMENT OF** 

RED-FLESHED DRAGON FRUIT (Hylocereus polyrhizus)

By

**NUR ADILLA JAMALUDIN** 

September 2010

Chairman: Phebe Ding, PhD

**Faculty: Agriculture** 

A study was conducted on the changes in physico-chemical, antioxidant content and cellular

structures of red-fleshed dragon fruit at every 5 days interval starting from 5 until 35 day after

pollination (DAP). The experiment was conducted using randomized complete block design

with four replications. Three fruits per replicate were used. Data from measurements of

length, diameter, stomatal density, peel and pulp fresh and dry weight, moisture content,

ethylene and carbon dioxide rate, pulp firmness, soluble solids concentration (SSC), titratable

acidity, pH, peel and pulp betacyanin content and chlorophyll content were analyzed using

analysis of variance and differences between means were determined by Duncan's New

Multiple Range Test. Peel and pulp L\*, C\* and h° values were determined by regression.

Correlation carried out using Pearson's correlation coefficient. Fruit colour changes and

cellular structure were documented as photographs and micrographs, respectively. In this

study, fruit were non-climacteric with no respiration and ethylene peaks were observed. A decrease in stomatal density with closed stomata was found throughout fruit development and maturation. The moisture content in peel increased, while decreased in pulp was observed as DAP progressed. Colour changes of red-fleshed dragon fruit in pulp occur earlier than the peel. The manifestations of red-violet betacyanin only appear after seeds have matured, then followed by pulp colour changes and finally peel change from green to red-violet. The redviolet betacyanin manifested itself gradually in pulp at 25 DAP followed by peel at 30 DAP. The high retention of betacyanin content during development and maturation had caused it to correlate significantly with soluble solids concentration as in process of betacyanin synthesis, sugar derivatives was needed to form red-violet betacyanin. Titratable acidity and pH in fruit were not correlated with betacyanin content in peel but titratable acidity significantly correlate with betacyanin content in pulp. There were significant relationship between DAP with L\*, C\* and h° value of peel and pulp. Betacyanin determination using reversed-phase HPLCphotodiode array detection, was confirmed betanin and isobetanin in the fruit. Fruit has high content of antioxidant activity, yet relatively low levels of ascorbic acid and total phenolic contents. This suggest that major source of antioxidant activity of fruit may not contributed from ascorbic acid or phenolics but rather from betacyanin. The developmental processes of fruit were examined using scanning electron microscope (SEM). As DAP progressed, parenchymatous cell lose their integrity due to cell hydrolysis which leads to losing of firmness. The SEM micrographs had revealed that starch granules in pulp decreased in size and intensity as DAP progressed. The degradation of starch granules contribute to softening of pulp tissue. Various form and shape of crystals such as raphides and druses were observed



in scale tissues. It is clear, the physico-chemical and betacyanin accumulation of red-fleshed dragon fruit changed as fruit developed, matured and ripened which coincide with structural changes.



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

FISIKO-KIMIA, KANDUNGAN ANTIOKSIDA DAN PERUBAHAN STRUKTUR SEL SEPANJANG PERTUMBUHAN BUAH NAGA MERAH (Hylocereus polyrhizus)

Oleh

**NUR ADILLA JAMALUDIN** 

September 2010

Pengerusi: Phebe Ding, PhD

Fakulti: Pertanian

Kajian ini dijalankan untuk melihat perubahan fisiko-kimia, kandungan antioksida dan perubahan struktur sel buah naga merah. Kajian dijalankan pada setiap 5 hari bermula pada hari ke-5 sehingga hari ke-35 selepas pendebungaan (DAP). Eksperimen dijalankan menggunakan blok lengkap secara rawak dengan empat replikasi. Tiga sampel buah bagi setiap replikasi. Hasil dapatan data dari ukuran panjang, diameter, kepadatan stomata, berat kering dan berat basah kulit dan isi buah, kandungan air, kadar etilena dan karbon dioksida, kerapuhan isi, kandungan pepejal larut (SSC), keasidan boleh titrat, pH, kandungan pigmen merah-violet betacyanin dan kandungan klorofil dianalisis menggunakan analisis varians manakala perbezaan min dianalisis menggunakan Ujian Duncan New Multiple Range. Nilai L\*, C\* dan h° kulit dan isi buah naga merah dianalisis menggunkan regresi analisis. Korelasi menggunakan koefisien korelasi Pearson. Perubahan warna dan struktur sel buah naga merah masing-masing didokumentasikan dalam bentuk foto dan mikrofoto. Keputusan kajian

menunjukkan buah naga merah boleh diklasifikasikan sebagai buah tidak klimaterik apabila tiada puncak respirasi dan pengeluaran etilena didapati. Semasa perkembangan dan kematangan buah naga merah, liang stomata dalam keadaan tertutup. Manakala, kepadatan stomata di bahagian kulit buah naga merah didapati menurun sepanjang DAP berlaku. Semasa DAP berlaku, didapati kandungan lembapan pada kulit buah naga meningkat, sementara penurunan lembapan berlaku di bahagian isi buah. Perubahan warna buah naga merah di bahagian isi berlaku lebih awal berbanding perubahan warna pada kulitnya. Pengeluaran pigmen merah-ungu betasianin hanya berlaku selepas biji buah naga merah matang dan diikuti dengan perubahan warna pada isi buah pada hari ke-25 DAP dan perubahan warna pada kulit daripada warna hijau berubah menjadi warna merah pada hari ke-30 DAP. Pengeluaran pigmen merah-ungu betasianin sepanjang pengembangan dan pematangan buah menyebabkan korelasi yang signifikan dengan SSC. Ini disebabkan oleh gula derivatif diperlukan semasa proses penghasilan untuk membentuk pigmen merah-ungu betasianin. Keasidan boleh titrat dan pH kulit buah naga merah didapati tidak mempunyai korelasi signifikan dengan kandungan pigmen merah-ungu betasianin tetapi keputusan keasidan boleh titrat pada isi buah naga merah mempunyai korelasi signifikan dengan keasidan boleh titrat. Hubungan regresi diantara DAP dan nilai L\*, C\* dan h° kulit dan isi buah naga didapati signifikan. Untuk mengenalpasti kandungan pigmen merah-ungu betasianin dengan menggunakan HPLCfotodioda array pengesanan, di mana ia mengesahkan kandungan betanin dan isobetanin di dalam buah naga. Buah naga merah mempunyai kandungan aktiviti antioksida yang tinggi, tetapi kandungan askorbik asid dan kandungan fenol total (TPC) yang rendah. Ini menunjukkan kandungan aktiviti antioksida bukanlah daripada askorbik asid tetapi adalah



disebabkan oleh kandungan pigmen merah-ungu betasianin. Proses pengembangan buah diteliti dengan menggunakan Scanning Elektron Mikroskop (SEM). Sepanjang DAP berlaku, sel parenkima kehilangan integritinya disebabkan pemecahan sel yang mana menyebabkan buah naga merah hilang kekerasannya. Microfoto SEM menunjukkan granul gula di bahagian isi buah berkurang dari segi saiz dan kepadatan sepanjang DAP berlangsung. Penguraian granul gula didapati penyumbang kepada isi buah hilang kerapuhan. Juga didapati pelbagai bentuk dan corak hablur yang dikenali sebagai raphide dan druses dibahagian tisu sisik buah naga. Jelas sekali perubahan fisiko-kimia dan penghasilan pigmen merah-ungu betacyanin semasa pengembangan dan pematangan mempunyai hubungkait yang rapat dengan perubahan struktur sel buah naga merah.

#### **ACKNOWLEDGEMENT**

This thesis would not have been possible, and I would like to begin by giving praise to the Almighty God, the great I AM, Alhamdulillah. Without His grace and mercy, none of this would have been possible.

Next, I would like to show my gratitude to my supervisor, Dr. Phebe Ding for her enlightening suggestions and patient guidance from the preliminary to the end of this process. Without which I would not be able to make it to the end. I am also grateful to Prof. Dr. Azizah Abdul Hamid for being the committee.

I am deeply indebted to my dear family members as they had shown ceaseless support the whole time especially to my parents (Dr. Jamaludin Badusah and Mrs. Hashimah Hashim) and my brothers (Mr. Mohd Illuddin, Mr. Mohd Nasruddin and Mr. Mohd Latif). They had lent a helping hand whenever I faced any difficulties. I remain indebted to my husband, Mr. Mardian Matasan and my beautifully daughter Baby Marha Nabilah who stand beside me and had accepted my selfish time all the while, starting from first year right until this very day. It is because of their loving kindness which motivates me to fight hard and never give up until the end. Words cannot express my appreciation towards their time and effort.

To family members of Postharvest Laboratory, I would like to thank each and every one of you for your valuable advices and feedbacks. It is through your scrutinizing observations which made this system complete without error. To Miss Joanna Cho, I am grateful to the companion. The project can come to its completion was a result of our knowledge exchange and sharing. I owe my deepest gratitude to Miss Atiqah Sharif, Miss Nor Elliza Tajidin Miss Munirah Mohamad, and Mr. Humam Wan Mansor for all those constructive advices and formulae explanation, thank you very much.

Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

I would like to dedicate this thesis to my late grandfather (Mr. Badusah Abas) and late uncle (Mr. Bekkari Badusah) as both gave me moral support I required within two and half years of completion.

Nur Adilla Jamaludin



#### **APPROVAL 1**

I certify that an Examination Committee has met on (date of viva voce) to conduct the final examination of Nur Adilla Jamaludin on her Master of Science thesis entitled "Physicochemical, Antioxidant and Cellular Structure of Red-fleshed dragon fruit during Development and Maturation" in accordance with Universiti Pertanian Malaysia (High Degree) Act 1980 and Universiti Pertanian Malaysia (High Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

#### Siti Aishah Hassan, PhD (Chairperson)

(Associate Professor)
Faculty of Agriculture
Universiti Putra Malaysia

#### Yahya Awang, PhD (Internal Examiner)

(Associate Professor) Faculty of Agriculture Universiti Putra Malaysia

#### Mahmud Tengku Muda Mohamed, PhD (Internal Examiner)

(Associate Professor)
Faculty of Agriculture
Universiti Putra Malaysia

#### Ruzita Ahmad, PhD (External Examiner)

Head Cluster of Integrative Medicine Advanced Medical and Dental Institute Universiti Sains Malaysia Pulau Pinang, Malaysia

**BUJANG KIM HUAT, PhD** 

Professor and Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date:



#### **APPROVAL 2**

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

#### Phebe Ding, PhD

Lecturer Faculty of Agriculture Universiti Putra Malaysia (Chairman)

#### Azizah Abdul Hamid, PhD

(Associate Professors)
Faculty of Food Science
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, 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.

NUR ADILLA BT. JAMALUDIN

Date: 23 September 2010

## LIST OF TABLES

<b>Fable</b>		Page
3.1	Physicals characteristics of red-fleshed dragon fruit as day after pollination progressed from 5 to 35.	57
3.2	Correlation coefficient (r) for fruit weight (FW), length (FL), diameter (FD), stomatal density (SD), peel and pulp betacyanin contents (BCPe and BCPu, respectively) of red-fleshed dragon fruit.	64
3.3	Correlation coefficient (r) for red-fleshed dragon fruit peel and pulp colour (L*, C* and h°) and betacyanin content.	69
3.4	Physiological characteristics of red-fleshed dragon fruit as day after pollination progressed from 5 to 35.	72
3.5	Chemicals characteristics of red-fleshed dragon fruit as day after pollination progressed from 5 to 35.	74
3.6	Correlation coefficient (r) for pH, soluble solids concentration (SSC), titratable acidity (TA), peel and pulp betacyanin contents (BCPe and BCPu, respectively) of red-fleshed dragon fruit.	76
3.7	Retention times, area of peaks, concentrations and total betacyanin content for peel of red-fleshed dragon fruit during fruit development and maturation.	81
3.8	Retention times, area of peaks, concentrations and total betacyanin content for pulp of red-fleshed dragon fruit during fruit development and maturation.	82
4.1	Correlation coefficient (r) of ascorbic acid (AA), total phenolics (TPC), antioxidant activities (DPPH and FRAP) and betacyanin content (BC) in pulp of red-fleshed dragon fruit.	96



## LIST OF FIGURES

rigure		Page
2.1	Betalains are synthesized from the amino acid tyrosine through L-DOPA into the two subclasses, betacyanins (reds and purples) and betaxanthins (yellows).	31
3.1	Fruit fresh weight (a), fruit size (length and diameter) (b) and fruit shape (length to diameter ratio) (c) of red-fleshed dragon fruit as day after pollination progressed. Vertical bars denote $\pm$ SE for means of 12 measurements.	56
3.2	Stomata (in circle) of red-fleshed dragon fruit at 5, 10, 25 and 30 day after pollination (DAP) as obtained from nail polish imprint.	58
3.3	Changes stomata density of red-fleshed dragon fruit as day after pollination progressed. Values are means $\pm$ SE (n=12).	58
3.4	Change in peel and pulp colour of red-fleshed dragon fruit as fruit developed from 5 to 35 day after pollination (DAP).	66
3.5	The peel and pulp lightness (L*) with peel L* values=-0.02x2-1.557x+96.99, R²=0.92 and pulp L* values= $51.62x^2+0.54x-0.024$ , R²=0.97 (a), chroma (C*) peel C* values= $0.04x^2-0.87x+21.36$ , R²=0.66 and pulp C*= $0.034x^2-0.9.0x+32.48$ , R²=0.88 (b) and hue (h°) peel h°= $-0.12x^2+13.57x-14.64$ , R²=0.62 and pulp h°= $-0.24x^2+5.27x+99.26$ , R²=0.93 (c) of red-fleshed dragon fruit as day after pollination progressed. Values are means $\pm$ SE (12).	68
3.6	Change of chlorophyll content and betacyanin content in the peel and the pulp of red-fleshed dragon fruit as day after pollination progressed. Values are means $\pm$ SE (12).	78
4.1	Change in peel and pulp ascorbic acid of red-fleshed dragon fruit as fruit developed from 5 to 35 day after pollination. Data shown are the means of four replicates vertical bar represents $\pm$ standard error.	92
4.2	Change in peel and pulp total phenolic content of red-fleshed dragon fruit as fruit developed from 5 to 35 day after pollination. Data shown are the means of four replicates vertical bar represents +	94



#### standard error.

4.3	Change in peel and pulp antioxidant activity of red-fleshed dragon fruit as fruit developed from 5 to 35 day after pollination. Data shown are the means of four replicates vertical bar represents $\pm$ standard error.	98
4.4	Change of ferric-reducing antioxidant power (FRAP) in pulp extracts of red-fleshed dragon fruit as day after pollination (DAP) progressed. Data shown are the means of four replicates.	99
5.1	Picture shows the scales, peel, pulp, peel-pulp transition and seeds of red-fleshed dragon fruit.	106
5.2	Scanning electron micrographs shows cross section of red-fleshed dragon fruit peel, peel-pulp transition and pulp region as day after pollination (DAP) progressed. (a) Fruit at 5 DAP showing regular shapes and thin-walled cells. (b) Fruit at 15 DAP showing vascular bundle started to develop in transition region (circle). (c) Fruit at 20 DAP showing matured vascular bundle with sclerenchyma fibers in transition region. (d) Fruit at 30 DAP showing cell wall breakage and cell collapse (arrows). (e) Fruit at 35 DAP showing peel and pulp areas of further cell breakage resulting from ripening. SF, sclerenchyma fibers. $\times 100$ . Magnification bar = $10  \mu m$ .	108
5.3	Scanning electron micrograph shows a cross section of red-fleshed dragon fruit peel as day after pollination (DAP) progressed from 5 (a), 10 (b), 15 (c) and 20 (d) DAP. XY, xylem; PH, phloem; SF,,sclerenchyma fibers; CL, collenchymas. a, and b, $\times 150$ ; c, and d, $\times 250$ . Magnification bar = $100  \mu m$ .	109
5.4	Scanning electron micrographs of red-fleshed dragon fruit peel at 15 day after pollination. (a) Cross section of peel showing the organization of cells, with thickened cell walls and small intercellular spaces. (b) Close views of circle area from (a). (c) Close views of cell wall and intercellular spaces. CW, cell wall; IS, intercellular spaces. a, $\times 270$ ; b, $\times 1900$ ; c, $\times 1000$ . Magnification bar = a, $50~\mu m$ ; b,c, $10~\mu m$ .	110
5.5	Scanning electron micrographs shows cross section in red-fleshed	114

dragon fruit pulp containing funicle at 5 day after pollination. a,  $\times 270$ ; b,  $\times 300$ ; c,  $\times 250$ ; d,  $\times 600$ . Magnification bars = a, b, 50  $\mu$ m; c,

 $100~\mu m;$  d,  $20~\mu m.$ 



- 5.6 Scanning electron micrographs shows a cross section of red-fleshed dragon fruit pulp containing starch granule as day after pollination (DAP) progressed. (a) Pulp at 5 DAP shows low density of starch granules and starch granules were attach to cell wall, thin cell wall is also apparent. (b) Pulp at 20 DAP shows various size of starch granules. (c) Pulp at 25 DAP shows higher density of starch granules in the inner cells, cell wall become thicker. ST, starch granules. ×900. Magnification bar = 20 μm.
- 5.7 Scanning electron micrographs shows a size of starch granule as day after pollination (DAP) progressed. (a) Starch granules at 15 DAP.
  (b) Biggest size of starch granules at 20 DAP. (c) Smallest size of starch granules at 35 DAP. ×6,500; Magnification bar = 2 μm.
- 5.8 Scanning electron micrograph shows longitudinal section of redfleshed dragon fruit scale as day after pollination (DAP) progressed. (a) Scale at 5 DAP shows a crystal idioblast cell containing raphides (circle) and club-shaped cystolith, calcium carbonate (arrow). (b) Close views shows crystals from (a). (c) Scale at 15 DAP shows crystals appears within cells (d) Scale at 15 DAP shows block-like rhombohedral or prismatic crystals. (e) Scale at 20 DAP shows raphide crystals, a needle-shaped calcium oxalate crystals (arrow). (f) Scale at 25 DAP contains raphide crystals. (g) Scale at 30 DAP shows different shapes of crystal. a, ×1200; b, c, e, f, g, ×4300; d, ×3000. Magnification bar = a, 10 μm; b, c, d, e, f, g, 5 μm.
- 5.9 Scanning electron micrographs shows cross section of red-fleshed dragon fruit scale as day after pollination (DAP) progressed (a) 15 DAP. (b) 20 DAP. (c) 30 DAP (d) 35 DAP. MC, deposited mucilage; RD, raphides. ×1100. Magnification bar = 10 μm.



#### LIST OF ABBREVIATIONS

AA - Ascorbic acid

DAP - Day after pollination

DOPA - Dihydroxyphenylalanine

DPPH - 1,1-diphenyl-2-picrl-hydrazil

FRAP - Ferric-reducing antioxidant power

SEM - Scanning electron microscope

SSC - Soluble solids concentration

TA - Titratable acidity

TPC - Total phenolics content

## TABLE OF CONTENTS

		Page
ABS	ГКАСТ	ii
ABS	iv	
ACK	viii	
APPI	ROVAL	ix
DEC	LARATION	xi xii xiii
	OF TABLES	
	OF FIGURES	
LIST	OF ABBREVIATIONS	xvi
СНА	PTER	
1	INTRODUCTION	1
2	LITERATURE REVIEW	4
	Red-fleshed dragon fruit	4
	Botany of red-fleshed dragon fruit	4
	Economical value of red-fleshed dragon fruit	5
	Fruit development	6
	Growth stage	7
	Maturation stage	8
	Ripening stage	9
	Senescence stage	10
	Physical characteristics	11
	Physiological changes during fruit development	18
	Chemical changes during fruit development	21
	Structural changes during fruit development	26
	Betacyanin pigment	28
	Biosynthesis of betacyanin	29
	Economical value of betacyanin	30
	Antioxidants	32
	Antioxidant content	34
	Antioxidant activity	38



3	PHYSICO-CHEMICAL AND BETACYANIN CONTENT OF			
	DRAGON FRUIT DURING FRUIT DEVELOPMENT AND MA			
	Introduction	41		
	Materials and Methods	43		
	Plant material	43		
	Physical analyses	44		
	Physiological analyses	47		
	Chemical analyses	48		
	Results and Discussion	53		
	Physical changes	53		
	Physiological changes	71		
	Chemical changes	73		
	Conclusion	85		
4	PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF RED-FLESHED DRAGON FRUIT DURING FRUIT DEVELOPMENT AND MATURATION			
	Introduction	86		
	Materials and Methods	87		
	Betacyanin content determination	87		
	Ascorbic acids determination	88		
	Total phenolics content determination	89		
	DPPH determination	90		
	FRAP assay determination	90		
	Statistical analysis	91		
	Results and Discussion	91		
	Antioxidant content	91		
	Antioxidant content  Antioxidant activity	97		
	Conclusion	102		
	Conclusion	102		
5	CELLULAR STRUCTURE OF RED-FLESHED DRAGON I FRUIT DEVELOPMENT AND MATURATION	FRUIT DURING		
	Introduction	102		
	Materials and Methods	105		
	Tissue preparation for SEM studies	105		
	Results and Discussion	105		
	Cellular changes in peel region of red-fleshed dragon fruit	107		
	Cellular changes in Peel-pulp transition region of	107		
	red-fleshed dragon fruit	113		
	Cellular changes in Pulp region of red-fleshed dragon fruit	113		
	Cellular changes in Scale region of red-fleshed dragon fruit	120		
	Conclusion	125		



6	GENERAL CONCLUSION AND	127
	RECOMMENDATIONS FOR FUTURE RESEARCH	
R	EFERENCES	130
$\mathbf{A}$	PPENDICES	153
B	IODATA OF STUDENT	154
L	IST OF PUBLICATIONS	155

