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CHANGES IN PHYSICO-CHEMICAL, CELLULAR STRUCTURE AND ANTIOXIDANT ACTIVITIES OF *Carissa Congesta* L. FRUIT DURING GROWTH AND DEVELOPMENT

MARIANI BINTI MOHAMMAD

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By

MARIANI BINTI MOHAMMAD

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

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CHANGES IN PHYSICO-CHEMICAL, CELLULAR STRUCTURE AND ANTIOXIDANT ACTIVITIES OF *Carissa congesta* L. FRUIT DURING GROWTH AND DEVELOPMENT

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Chairman: Phebe Ding, PhD
Faculty: Agriculture

A study on the changes in physico-chemical, antioxidant activities and cellular structure changes of *Carissa congesta* fruit was conducted on weekly intervals from 1 until 13 weeks after anthesis (WAA) to establish its growth pattern. A series of the mentioned trait changes was carried out in order to establish the growth pattern and its compositional changes. For these purposes, fully bloomed flowers of *C. congesta* were tagged. In this study, the fruits were thinned to allow only five per cluster. The experiment was conducted by using a randomized complete block design with three replications. The morphological traits (fruit length, diameter, fresh weight and volume) were subjected to logistic regression analysis. Data from the measurements of colour, firmness, chemical (soluble solids concentration (SSC), pH, titratable acidity (TA) and moisture content), physiological (respiration rate and ethylene production), phytochemical (phenolic content, anthocyanin and antioxidant activities), and mineral nutrients were analyzed by using analysis of variance while means were separated by least significant difference. Growth of *C. congesta* fruit at cellular level was documented by using light microscopy (LM) and scanning electron microscopy (SEM). Results showed that the fruit took approximately 100 days from anthesis until ripening. The fruit exhibited a single sigmoid growth pattern where three physiological stages (S1, S2 and S3) were identified. Cells of *C. congesta* fruits at S1 underwent rapid division, followed by cell expansion at S2 before it reached physiological maturity at S3. These changes were in accordance with cellular structure as observed under LM and SEM. *C. congesta* fruits appeared whitish-pink in colour at early weeks of development before it changed to red and then dark purple at later weeks. The visual colour changes of *C. congesta* fruit were in line with L^* C^* and h^o values as fruit grew and developed. Fruit firmness and TA increased initially, then decreased during ripening while SSC and pH increased as WAA progressed. Respiration rate of *C. congesta* fruit was high at initial stage of growth, then reduced at

later stages. While no ethylene was detected throughout 13 weeks of observation. Fruits at 13 WAA exhibited the highest total antioxidant activities, phenolic and anthocyanin contents. Vitamin C content in *C. congesta* fruits was much higher than any other well-establish and known fruits at any stages of growth. *C. congesta* fruits demonstrated high levels of nitrogen and potassium and a wide diversity of other mineral constituents throughout fruit growth and development. As conclusions, it is clear that the quality of *C. congesta* fruit changed as it developed, matured and reached the stage of palatability. Since *C. congesta* fruits constitute a good vitamin and mineral source, high phenolic and antioxidant levels, it increases the value of this fruit to be commercialized as an economic alternative to the natural food colourant or pharmaceutical production.

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

PERUBAHAN CIRI FIZIKO-KIMIA, STRUKTUR SEL DAN AKTIVITI ANTIOKSIDA BUAH *Carissa congesta* L. SEMASA PERTUMBUHAN DAN KEMATANGAN

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Suatu kajian dijalankan ke atas perubahan fiziko-kimia, perubahan aktiviti antioksida dan struktur sel buah *Carissa congesta* pada setiap minggu bermula minggu pertama sehingga minggu ke-13 selepas antesis (WAA) bagi menentukan paten pertumbuhannya. Siri perubahan bagi ciri-ciri tersebut dijalankan bagi menentukan pola pertumbuhan dan perubahan komposisi buah tersebut. Untuk tujuan ini, bunga *C. congesta* ditanda. Selepas berputik, proses penjarangan dilakukan untuk memastikan hanya lima buah yang membesar di setiap kelompok. Eksperimen ini dijalankan dengan menggunakan reka bentuk blok rawak lengkap dengan tiga replikasi. Ciri-ciri morfologi buah (panjang, diameter, berat dan isipadu) tertakluk kepada analisis regresi logistik. Data untuk ukuran warna, kekerasan, kimia (kepekatan pepejal terlarut (SSC), pH, asid tertirat (TA) dan kandungan kelembapan), fisiologi (kadar respirasi dan pengeluaran etilena), fitokimia (kandungan fenolik, antosianin dan aktiviti antioksida) dan juga nutrien mineral dianalisa dengan menggunakan kaedah analisis varians manakala min dibezakan dengan menggunakan kaedah perbezaan terkurang bererti. Pertumbuhan buah *C. congesta* di peringkat selular direkod dengan menggunakan mikroskop cahaya (LM) dan mikroskop elektron pengimbas (SEM). Keputusan kajian menunjukkan bahawa buah *C. congesta* mengambil kira-kira 100 hari dari antesis sehingga ranum. Buah ini mempamerkan jenis pola pertumbuhan sigmoid di mana tiga peringkat fisiologi (S1, S2 dan S3) dikenalpasti. Pada mulanya, pertumbuhan secara perlahan diperhatikan pada peringkat S1 di mana pembahagian sel-sel giat berlaku. Kemudian, pengembangan sel pesat berlaku apabila buah memasuki peringkat S2 sebelum mencapai kematangan fisiologi di peringkat S3. Perubahan ini sejajar dengan perubahan struktur selular yang diperhati di bawah LM dan SEM. Buah *C. congesta* berwarna merah jambu pada peringkat pertumbuhan sebelum ia berubah menjadi merah-kemerahan pada peringkat matang dan ungu kehitaman pada

peringkat akhir pertumbuhan. Perubahan warna visual buah *C. congesta* di sepanjang pertumbuhan sejajar dengan nilai-nilai L^*, C^* dan h^o. Kekerasan buah dan TA meningkat pada awal penilaian, kemudian ia berkurangan di sepanjang proses matang dan ranum. SSC dan pH pula didapati meningkat sejajar dengan pertambahan minggu kajian. Kadar respirasi buah *C. congesta* tinggi di awal perkembangan kemudian ianya berkurangan di peringkat akhir pertumbuhan. Manakala tiada etilena dikesan di sepanjang 13 minggu pemerhatian. Aktiviti antioksida, kandungan fenolik dan antosianin buah dicatatkan paling tinggi pada 13 WAA. Vitamin C dalam buah *C. congesta* adalah paling tinggi berbanding dalam buah terkenal yang lain pada mana-mana peringkat pertumbuhan. *C. congesta* menunjukkan tahap nitrogen dan potassium yang tinggi dan juga kepelbagaian jenis mineral yang lain sepanjang pertumbuhan dan perkembangan buah. Kesimpulannya, kualiti buah *C. congesta* berubah-ubah sepanjang ia berkembang, matang dan mencapai tahap yang sesuai dimakan. Memandangkan buah *C. congesta* mengandungi sumber vitamin dan mineral yang baik, paras fenolik dan antioksidan yang tinggi, ini meningkatkan nilai komersial buah ini sebagai pewarna makanan semulajadi alternatif atau pengeluaran farmaseutikal.

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Mariani Mohammad

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 the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

$(C_2H_3)NaO_2.3H_2O$	Sodium acetate
1-MCP	1-methylcyclopropene
A	Absorbance
ABTS	2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
$ABTS^{\cdot+}$	ABTS radical cation
ACC	1- aminocyclopropane-1-carboxylic acid
ACO	ACC oxidase
ACS	ACC synthase
ANOVA	Analysis of variance
AVG	Aminoethoxyvinylglycine
C^*	Chroma
C_2H_4	Ethylene
$C_2H_4O_2$	Glacial acetic acid
Ca	Calcium
DPPH	Diphenyl-2-picrylhydrazyl
DRI	Dietary Reference Intakes
$FeCl_3.6H_2$	Ferric chloride solution
$FeSO_4.7H_2O$	Ferrous sulphate
FRAP	Ferric ion reducing antioxidant power
GAE	Gallic acid equivalent
H_2O_2	Hydrogen peroxide
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
h^o	Hue
HPO_3	Metaphosporic acid
K	Potassium
L^*	Lightness
LM	Light microscopy
LSD	Least significant difference
Mg	Magnesium
N	Newton
N	Nitrogen
NaOH	Sodium hydroxide
$O\bullet_2^-$	Superoxide anions
ORAC	Oxygen radical absorbance capacity
P	Phosphorus
PAL	Phenylalanine ammonia lyase
PG	Polygalacturonase
PL	Pectate lyase
PME	Pectin methylesterase
PPO	Polyphenol oxidases
RCBD	Randomized complete block design
ROS	Reactive oxygen species
SAM	S-adenosyl-L-methionine
SEM	Scanning electron microscopy

SSC	Soluble solids concentration
TA	Titratable acidity
TPC	Total phenolic content
TPTZ	2,4,6-tris (1-pyridyl)-5-triazine
USDA	United State Department of Agriculture
UV	Ultraviolet
WAA	Week after anthesis
β-GAL	β-galactosidase

CHAPTER 1

INTRODUCTION

Fruits are rich in nutrients such as sugars, organic acids, vitamins, minerals and polyphenolic compounds as well as other elements like fiber and secondary metabolites. Berry fruits in particular are precious in this regard. Most berries have been vastly cultivated and documented upon its commercial and nutritional importance. However, there are still quite a huge number of neglected crops which have been used for centuries in localized areas merely for the source as protective food, vitamins and mineral necessities. These species have been overlooked due to the lack of recognition of its highly nutritious and medicinal assets (Malik, 2010). This includes *Carissa congesta*, the underused berry which is still gaining its place to receive further attention from research, technology providers and consumers.

Fruit growth, generally, refers to a change in length, diameter, mass, or shape (Perkins-veazie, 1995). Fruit development comprises chronological stages which are growth, maturation, ripening and senescence (Opara, 2000; Kuang et al., 2011). Throughout development, they involve a sequence of physico-chemical and phytochemical changes at both macro and micro levels (Opara, 2000). The knowledge of these changes is important to determine harvesting time and maintain fruit quality. Fruits harvested immature tend to lack sufficient organoleptic properties and they may not ripen properly while harvesting overripe fruits may be unsuitable for the commercial use due to early deterioration and shorter storage life (Tadesse et al., 2002).

The morphological parameters of fruits like diameter, length, weight and volume are commonly used as indices of fruit growth (Jamaludin et al., 2011). By knowing the evolution of these physical traits, the fruit growth would be able to be presented in graphical analysis either single sigmoid curve or double sigmoid curve depending on species. At cellular level, as fruits grow, mature and ripen, it coincides with various degrees of cell division and expansion giving differences in number, size and intercellular air space size of cells (Opara, 2000; Valero and Serrano, 2010). Therefore, cellular characteristic can be also subscribed to further support the fruit growth and development.

Regarding consumer acceptability, consumers tend to choose a better quality of fruit such as fruit colour, taste and visual aspect (Iglesias et al., 2008; Fawole and Opara, 2013a). During growth, the fruits import and accumulate water, minerals, sugar, pH, organic acids and synthesize flavour and aroma compounds. These are strongly related with ripening process which could be also defined as physiological process comprising physical, chemical and biochemical changes of the fruits (Conde et al., 2007). Thus, the physico-chemical changes of fruit during growth and development in which colour and

firmness (physical quality); sugar, organic acids and pH (chemical quality); respiration rate and ethylene production (physiological quality) also play an important role in determining the suitable stage of fruit for consumer consumption (Fawole and Opara, 2013a; b). The group of phytochemicals that contribute to antioxidant capacity including phenolic compounds, flavonoids and vitamins such as vitamin C are also useful to be used as indices of growth because of the concentrations of these substances are commonly interrelated with fruit growth (Fawole and Opara, 2013b).

Several growth prediction models and physico-chemical profiles have been established for crops like arazá (*Eugenia stipitata*) (Hernández, 2007), red-fleshed dragon fruit (*Hylocereus polyrhizus*) (Jamaludin et al., 2011) and Rastali banana (*Musa* AAB) (Tee et al., 2012) but no such work has been done for *C. congesta* fruit. Many researchers only focused on chemical compositions and potential importance of *C. congesta* without clearly stating the stage of the fruit used in their work (Siddiqi et al., 2011; Patel and Rao, 2011). Thus, there is a need for a comprehensive study covering physico-chemical, cellular structure and phytochemical changes in fruits during growth and development. Understanding these changes will lead to appropriate identification of harvest time in order to maintain fruit quality and finally to improve their productivity and profitability. Hence, the objectives of this study were:

1. To characterize the growth pattern of *C. congesta* fruit with particular reference to its cellular structure changes.
2. To investigate physico-chemical and phytochemical changes from fruit set to ripening. How about antioxidant activities??

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BIODATA OF STUDENT

Mariani Binti Mohammad was born in Kuala Terengganu, Terengganu on September 25th 1983. She is the youngest child in a family with total of seven family members. She had her early education at Sekolah Rendah Kebangsaan Pagar Besi, Kuala Terengganu before continuing her secondary education at Sekolah Menengah Kompleks Mengabang Telipot, Kuala Terengganu. After completing her secondary school, she was offered to continue her studies at KPM Matriculation. She furthered her study in Universiti Putra Malaysia on April 2002 and graduated on August 2005 with a Bachelor of Science with Honours in Biology. She got married on the same year and became a full-time housewife. After seven years, she decided to further study. In 2012, she enrolled as a full-time Master candidate at Universiti Putra Malaysia and completed her Master of Science programme in August 2017.

LIST OF PUBLICATIONS

Journal papers published:

Mariani, M., Cahaya, N. M. and Ding, P. (2018). Physicochemical characteristics of *Carissa congesta* fruit during maturation. *Acta Horticulturae*, 1213:461-464.

Proceeding papers:

Mariani, M., Mashah, N. C. and Ding, P. (2016). Physico-chemical Characteristics of *Carissa carandas* Fruit During Maturation, in abstracts book of The 3rd Asia Pacific Symposium on Postharvest Research, Education and Extension, 9−11 December 2014, Victory Hotel, Ho Chi Minh City, Viet Nam, 2014.

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