UNIVERSITI PUTRA MALAYSIA

GROWTH AND MATURATION PROCESSES OF PURPLE PASSION FRUIT (Passiflora edulis Sims) THROUGH PHYSICAL AND METABOLOMICS APPROACHES

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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 Doctor of Philosophy

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The purple passion (*Passiflora edulis* SIMS) fruit has enormous potential to be cultivated on a mass scale in Malaysia since it gains high demand in the global market. This study is aimed to understand the growth and maturation process of passion fruit through physical and metabolomics approaches for producing high quality fruit for fresh fruit and varieties of industries. Growth and development of passion fruit were tracked starting from 7 days after anthesis (DAA) until the fruit detached from the plant. The first study was conducted to establish the fruit optimal harvesting stage. Logistic model showed all physical traits of passion fruit fitted well with single sigmoid curve with high regression coefficient (R^2) around 0.984-0.992. The anatomical study showed that passion fruit have three distinct growth phases; stage 1 (S1) (0-7 DAA), stage 2 (S2) (14-28 DAA) and stage 3 (S3) (35-63 DAA). Purple passion fruit obtained maturity at 49 DAA and ripened at 56 DAA. The S3 phase was investigated intensively in studies 2 and 3 because it is a vital period for maturation and ripening. In the second and third studies, metabolomics approaches have been implemented in studying the dispersion of metabolites during maturation and ripening process. In the study 2, juice and seed were subjected to ^{1}H NMR analysis as both compartments play significant role in fruit eating quality. Around 30 and 32 metabolites were found in juice and seed, respectively. Result showed the primary metabolites dominating both compartments consist of sugar (glucose, sucrose, fructose), organic acid (tartaric acid, citric acid, malic acid) and amino acid (lysine, threonine, methionine, leucine) while secondary metabolites present as minor compounds (chlorogenic acid, epicatechin and phenylacetic acid). Segregating the metabolites using principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) have outlined how the metabolites in both compartments changed dynamically throughout the maturation and ripening process. Variable importance in the projection (VIP) has sorted 13 and 18 metabolites as highly influential metabolites in juice and seed. Glycolysis, TCA cycle, shikimate pathway, ethylene and polyamine were detected as important biochemical

pathways that were responsible for the fruit's maturation and ripening. The third study analysed juice, seed and peel since all fruit compartments have equal benefits to be exploited as industrial products. Result showed that each fruit compartments have different secondary metabolites contents correlated with their antioxidant activity. During ripening, β-carotene and chlorophyll pigments degraded while anthocyanin accumulated, resulting peel in a deep purple. The peel was abundant with different types of phenolic acid (chlorogenic acid, caffeic acid and its derivatives) and flavonoid components (isorientin, rutinoside and vitexin-2"-O-rhamnoside) possessed the highest antioxidant activity determined by FRAP (732.91- 1089.61 µmol TE. 100 g^{-1} FW), ABTS (62.34-70.10 µmol TE. g^{-1} FW) and DPPH (52.362- 67.66 µmol TE. g-1 FW) as compared to seed and juice. All passion fruit compartments should be completely exploited for fresh consumption and product manufacturing. Hence, herein a comprehensive view of passion fruit growth and development may benefit Malaysia's agriculture and processing industries.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PROSES PERTUMBUHAN DAN KEMATANGAN BUAH MARKISA UNGU (*Passiflora edulis* Sims) MELALUI PENDEKATAN FIZIKAL DAN METABOLOMIK

Oleh

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Buah markisa ungu (*Passiflora edulis* SIMS) mempunyai potensi besar untuk diusahakan secara besar-besaran di Malaysia memandangkan ia mendapat permintaan tinggi di pasaran global. Kajian ini bertujuan untuk memahami proses pertumbuhan dan kematangan buah markisa melalui pendekatan fizikal dan metabolomik untuk menghasilkan buah berkualiti tinggi bagi industi buah segar dan pelbagai. Pertumbuhan dan perkembangan buah markisa dijejaki bermula dari 7 hari selepas antesis (DAA) sehingga buah tertanggal daripada pokok. Kajian pertama telah dijalankan untuk menentukan peringkat penuaian buah yang optimum. Model logistik menunjukkan semua ciri fizikal buah markisa dipasang sesuai dengan keluk sigmoid tunggal dengan pekali regresi tinggi (R^2) sekitar 0.984-0.992. Kajian anatomi menunjukkan bahawa buah markisa mempunyai tiga fasa pertumbuhan yang berbeza; peringkat 1 (S1) (0-7 DAA), peringkat 2 (S2) (14-28 DAA) dan peringkat 3 (S3) (35-63 DAA). Buah markisa ungu memperoleh kematangan pada 49 DAA dan masak pada 56 DAA. Fasa S3 telah disiasat secara intesif dalam kajian 2 dan 3 kerana ia merupakan tempoh penting untuk proses matang dan masak. Dalam kajian kedua dan ketiga, kaeadah metabolomik telah dilaksanakan bagi mengkaji penyebaran metabolit semasa proses pematangan dan masak. Dalam kajian 2, jus dan biji benih tertakluk kepada analisis ^{1}H NMR kerana kedua-dua bahagian memainkan peranan penting dalam penilaian kualiti buah. Sekitar 30 dan 32 metabolit dikenalpasti dalam jus dan biji, masing-masing. Keputusan menunjukkan metabolit primer yang mendominasi kedua-dua bahagian terdiri daripada gula (glukosa, sukrosa, fruktosa), asid organik (asid tartarik, asid sitrik, asid malik) dan asid amino (lisin, treonin, metionin, leucine) manakala metabolit sekunder hadir sebagai sebatian kecil. (asid klorogenik, epicatechin dan asid phenylacetik). Pengasingan metabolit menggunakan analisis komponen utama (PCA) dan analisis diskriminasi persegi terkecil separa (PLS-DA), telah mendapati metabolit dalam kedua-dua bahagian berubah secara dinamik sepanjang proses pematangan dan masak. Kepentingan berubah dalam unjuran (VIP) telah menyusun 13 dan 18 metabolit sebagai metabolit yang sangat berpengaruh dalam jus dan biji. Glikolisis, kitaran TCA, laluan shikimat, etilena dan poliamina telah dikesan sebagai jaluran biokimia penting yang bertanggungjawab untuk

kematangan dan masak buah. Kajian ketiga menganalisis jus, biji dan kulit kerana semua bahagian buah mempunyai manfaat yang sama untuk diproses sebagai produk industri. Keputusan menunjukkan setiap bahagian buah mempunyai kandungan metabolit sekunder yang berbeza yang berkaitan dengan aktiviti antioksidannya. Semasa masak, pigmen β-karotena dan klorofil terdegradasi manakala antosianin terkumpul, menghasilkan kulit berwarna ungu tua. Kulitnya kaya dengan pelbagai jenis asid fenolik (asid klorogenik, asid caffeic dan derivatifnya) dan komponen flavonoid (isorientin, rutinoside dan vitexin-2"-O-rhamnoside) mempunyai aktiviti antioksidan tertinggi yang ditentukan oleh FRAP (732.91- 1089.61 μmol TE. 100 g^{-1} FW), ABTS (62.34-70.10 μmol TE. g^{-1} FW) dan DPPH (52.362- 67.66 μmol TE. g^{-1} FW) berbanding dengan biji dan jus. Semua bahagian markisa harus dieksploitasi sepenuhnya untuk kegunaan segar dan pembuatan produk. Oleh itu, di sini pandangan menyeluruh tentang pertumbuhan dan pembangunan buah markisa yang boleh memberi manfaat kepada industri pertanian dan pemprosesan di Malaysia.

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^1H NMR	Proton nuclear magnetic resonance
1-MCP	1-methylcyclopropene
$ABTS^{\bullet+}$	2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	ACC oxidase
ACS	ACC synthase
AdoMet	S-adenosyl-l-methionine
ANOVA	Analysis of variance
ANT	Anthocyanin
BC	β-Carotene
CA	Chlorogenic acid
CE	Cyanidin-3-glucoside equivalent
CLA	Chlorophyll A
CLB	Chlorophyll B
CME	Cellulose mixed ether-membrane
CRD	Completely randomised design
D_2O	Deuterium oxide
d_4-CD_3OD	Deuterated methanol
DAA	Days after anthesis
DAD	Diode array detector
DPPH	1,1-Diphenyl-2-picrylhydrazyl
DW	Dry weight
ESI	Electrospray ionisation interface

$FeCl_3 . 6H_2O$	Ferric chloride hexahydrate
FRAP	Ferric reducing antioxidant power
FW	Fresh weight
GAE	Gallic acid equivalents
GC	Gas chromatography
HPLC	High performance liquid chromatography
HPO_3	Metaphosphoric acid
KEGG	Kyoto encyclopaedia of genes and genome
KH_2PO_4	Potassium dihydrogen phosphate
LSD	Least significant difference
M6PR	NADPH-mannose-6-phosphate reductase
MeOH	Methanol
MS	Mass spectrometry
MTA	5-Methylthioadenosine
MTBE	Methyl tert-butyl ether
Na_2CO3	Sodium carbonate
NaOH	Sodium hydroxide
NI	Neutral invertase
NPK	Nitrogen, phosphorous and potassium
PCA	Principal component analysis
PE	Pectinesterase
PG	Polygalacturonase
PL	Pectate lyase
PLS-DA	Partial least square discriminant analysis

ppm	Part per million
PRESAT	Pre-saturation
PRPP	Phosphoribosyl diphosphate
Q2	Cross-validate predictive ability
QE	Quercetin equivalents
R2	Total variance
RQ	Relative quantity
SAM	S-Adenosylmethionine
SPP	Sucrose-6-phosphate phosphatase
SPS	Sucrose-6-phosphate synthase
SSC	Soluble solids concentration
T6P	Trehalose-6-phosphatase
TA	Titratable acidity
TCA cycle	Tricarboxylic acid cycle
TE	Trolox equivalent
TFC	Total flavonoid content
TPC	Total phenolic content
TPTZ	2,4,6-Tri-(2-pyridyl)-s-triozine
Trolox	6-Hydroxy2,5,7,8-tetramethyl-chroman-2-carboxylic acid
TSP	3-(Trimethylsilyl) propionic-2, 2, 3, 3-d_4 acid sodium salt
USDA	United State Department of Agriculture
UV	Ultraviolet
V2R	Vitexin-2"-O-rhamnoside
VIP	Variable importance in the projection

vol	Volume
β-GAL	β-Galactosidase

CHAPTER 1

INTRODUCTION

1.1 Background of study

Fresh fruit is highly perishable, and the post-harvest loss occur naturally as soon after the fruit harvested from the parent plant. It is estimated about 18-28% of loss occur as soon after the harvesting stage (Md Nor et al. 2020). The loss continually develops during transportation, marketing, and trading levels, negatively impacts fruit farmers, consumer prices, nutritional quality produces, and income of the exporting countries (Md Nor et al. 2020). Due to this, Malaysia suffers around 17-35% of income loss, which mainly attributable to deteriorated fruit quality during local and global trading (Mahmud, 2017). The key strategy in producing high quality fruit is by determining the optimal harvesting. The Right harvesting stage is critical to make sure fruit achieves its optimal eating quality during harvesting and can maintain a longer post-harvest life (Mohammad and Ding, 2019; Tee et al. 2012).

Purple passion fruit (*Passiflora edulis* Sims.) is the focus of the current research. This fruit is classified as minor topical fruit which grabbed increasing demand globally. The enticing flavour makes the fruit suitable for dessert or processed into a variety of confectionary products such as fresh juice, cordial, jams, and concentrates (Pham et al., 2019; Natnicha et al., 2019). In recent years, passion fruit has gained popularity since its applications have expanded beyond the fresh fruit and processed food industries. The peel and seed of the fruit are processed for nutraceutical, pharmaceutical, food ingredients, cosmetics, and skincare (Ramli et al., 2020; Oliveira et al., 2016; López-Vargas et al., 2013). The increasing demand for passion fruit in the world market should be viewed as an opportunity to strengthen the country's revenue. The passion fruit has immense potentials to be cultivated commercially in Malaysia since the fruit can be processed to multifunction products (The Insight Partners, 2020). In preparing Malaysia for passion fruit commercial cultivation, it is essential to produce high-quality fruit, whether fresh or processed.

Fruit maturity has profound effects on the final fruit quality. Fruit such as banana (Tee et al., 2012), mango (Syed et al., 2021), pomelo (Gupta et al., 2021) and pitaya (Magalhães et al., 2019) have dynamic physicochemical characteristics that affect determination of optimal harvesting stage. Non-optimal harvesting could compromise the fruit eating quality and post-harvest life (Ghosh et al., 2017). Commercial producer countries such as Colombia, Brazil, and India have conducted studies on the optimal harvesting stage for passion fruit. Nevertheless, most of the studies were only focus on physical and psychochemical aspects. None of the studies have ever integrated the concrete analysis from wide aspects, for example by anatomical perspective of passion fruit. Integrating anatomical research with physical and physicochemical features will allow for precise maturation and ripening stage determination, while also contribute to fundamental knowledge in understanding the cellular process of growth and development.

Passion fruit has three main compartments: juice, seed and peel. In fresh fruit and food industries, the juice and seed are valuable compartments as they are graded by eating quality. Metabolomics is an efficient bioanalytical strategy that enables the visualisation of an organism's metabolites during a biological process by utilising comprehensive and high-throughput analysis (Lin et al., 2021). Metabolomics provides a holistic view of the physiological, structural, physical, and biochemical changes that occur in fruit during maturation and ripening. Primary metabolites such as sugars and organic acids are important determinants of fruit's eating quality (Tijero et al., 2021), whereas secondary metabolites such as phenolic and flavonoid compounds determining phytonutrients of a fruit (Del-Castillo-Alonso et al., 2021). Studying the fruit quality aspects analysing the juice and seed metabolome will understand the key metabolites that responsible for fruit maturation and ripening.

Numerous studies demonstrate that passion fruit have potential as useful source of natural antioxidants in neutralising or inhibiting free radicals activity that benefit to human health (He et al., 2020). Due to this, extensive research is being carried out to isolate and identify functional bioactive compounds that responsible for the antioxidant properties (Guimarães et al., 2020; Lin et al., 2021). All of the passion fruit compartment including peel may possess the antioxidant activity that can exploited beyond the fresh fruit and processing industries. Quantifying the primary and secondary metabolites will understand dynamic changes of antioxidant capacity during passion fruit growth and development. In addition, mechanism in pigment accumulation in passion fruit peel that responsible for colour remain unknown (Gioia et al., 2020). The study of the fruit metabolome and its correlation with antioxidant activity will help to understand the phenotypic development and functionality of each fruit compartment so that it can be fully exploited in industries.

1.2 Aims and objectives

The study aims to understand passion fruit's growth and maturation process through the physical and metabolomics approaches. Passion fruit is intended to be produced in optimal quality, with all fruit compartments being fully utilised in a variety of industries. The comprehensive study during growth and development will provide valuable knowledge in fruit's physiology as the fundamental knowledge in horticulture study.

The aims were achieved through the following set of objectives:

1. Characterise the growth and development and determine the optimal harvesting stage for passion fruit cultivated in Malaysia.
2. Identify and quantify metabolites that are responsible in fruit maturation and ripening.

3. Understand how targeting metabolites affect the fruit's phenotype and its relationship with antioxidant activity

1.3 Scope of thesis

This thesis is outlined in 6 chapters:
Chapter 1 provides an overview of the research background and aims of the study. The passion fruit quality, maturity of harvesting, and need for metabolomics study are briefly discussed.

Chapter 2 focuses on comprehensive review on literature related to research. The horticulture background about passion fruit, demand in global and fundamental knowledge in passion fruit development are highlighted. Study on metabolomics that discussing the differences between primary and secondary metabolites in plant metabolism are also incorporated.

Chapter 3 reports the optimal harvesting of passion fruit. The fruit's optimal harvesting stage was scrutinised from the data of physical, anatomical and physicochemical characteristics. Cellular study using scanning electron microscope was performed to distinguish the growth phases. Physically, fruit were rated for its firmness and colour, while physiology was rated according to respiration and ethylene production. The eating quality was rated according to biochemical analysis that commercially applied in post-harvest study. Fundamental knowledge on growth profile of passion fruit was established from this study, became the primary reference for the subsequent chapters.

Chapter 4 apply the nuclear magnetic resonance (NMR) spectroscopy and chemometric methods in achieving the metabolic fingerprint during passion fruit's maturation and ripening stage. Juice and seed were analysed as these compartments serve as major entities in determining the fruit eating quality. Through NMR analysis, majority of metabolites detected belong to primary metabolites while secondary metabolites detected as minor compound. The model of principle component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were established to understand the segregation of metabolites in juice and seed that response toward different harvesting stage. The highly influential metabolites that responsible for maturation and ripening in juice and seed were identified using variance importance projection (VIP) and further quantified using relative quantification. These metabolites were denoted as key metabolites that influencing the maturation and ripening of passion fruit.

Chapter 5 provides extension study to chapter 4. Peel compartment was included besides the juice and seed. It because the peel can be further exploited as industrial products even it does not contribute to fruit eating quality. Calorimetric analysis was conducted to preliminary quantify the secondary metabolites content and asses the antioxidant activity. The specific secondary metabolites were identified and quantified using the high-performance liquid chromatography (HPLC). Relationship between antioxidant content and its activity was established using Pearson's correlation. For seed and juice, a partial Least Square (PLS) regression analysis was performed to establish a correlation between antioxidant activity and the entire fruit metabolome, taking into account previous ^{1}H NMR data with secondary metabolites that were quantified by calorimetric and HPLC analysis.

Chapter 6 summarised the general conclusion obtain from the whole studies conducted in this thesis. Limitation of study and future perspective of result are embedded at the end of the conclusion.

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