UNIVERSITI PUTRA MALAYSIA

CAROTENOID PROFILES AND PRELIMINARY INVESTIGATION ON CAROTENOID BIOSYNTHESIS IN THE OIL PALM (*ELAEIS GUINEENSIS*) MESOCARP

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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 Universiti Putra Malaysia

March 2002

For my Dad & Mom.....

Here is the endeavour of my absences

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Associate Professor Ong King Kok, Ph.D.

Faculty: Medicine and Health Science

The oil palm fruit is one of the richest sources of carotenoids. However, little is known about the pathway for carotenoid synthesis in the oil palm. Therefore, the aim of this study is to provide some basic information on carotenoid synthesis in the oil palm mesocarp. In order to do so, we needed to firstly extract carotenoids from the oil palm mesocarp. A technique for extracting carotenoids from the oil palm mesocarp was optimised. Cold saponification was used to remove unwanted lipid materials from the crude carotenoid extract. Ultra violet-Visible (UV-Vis) spectrophotometry was utilized to compare the absorbance profiles of the carotenoid extracts of oil palm fruits at different stages of development i.e 15-20 weeks after anthesis (WAA). The results showed that in unripe oil palm fruit, carotenoids absorb strongly in the UV range while in ripe fruit the carotenoids absorb strongly in the visible range as more carotenoids are formed. Reverse phase high pressure liquid chromatography (RP-HPLC) coupled with a photo-diode array (PDA) detector was used in this study for characterizing the carotenoid profiles of the saponified carotenoid extracts of the oil palm mesocarp at different stages of development. The saponified carotenoid extracts were separated on both C18 and C30 columns and the results of the HPLC separations showed that separation on the C30 column was better as it yielded more peaks in all the saponified carotenoid extracts. α-Carotene, β-carotene and their isomers were identified in all the saponified extracts. Lycopene was not detected in any of the saponified carotenoid extracts. Thus, it can be concluded that lycopene is not present in oil palm fruit. In order to understand carotenoid biosynthesis in oil palm mesocarp, the incorporation of various ^{14}C-labelled substrates into carotenoids in the mesocarp slices of 20-WAA oil palm fruits was carried out. Incorporation studies showed that isopentenyl pyrophosphate (IPP) is the main intermediate for carotenoid synthesis in oil palm fruits. Thin layer chromatography (TLC) was carried out to study the flow of the various radiolabelled substrates into the carotenoid fractions. The results of this study suggested that carotenoid synthesis in oil palm fruits follows the acetate/mevalonate pathway.

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

PROFIL KAROTENOID DAN KAJIAN PERMULAAN TERHADAP BIOSINTESIS KAROTENOID DALAM MESOKARPA POKOK SAWIT (*ELAEIS GUINEENSIS*)

Oleh

JANE SONIA KAUR

Mac 2002

Pengerusi: **Profesor Madya Ong King Kok, Ph.D.**

Fakulti: **Perubatan dan Sains Kesihatan**

Buah sawit merupakan salah satu sumber yang kaya dengan karotenoid. Namun, tidak banyak yang diketahui tentang tapak jalan untuk sintesis karotenoid dalam buah sawit. Oleh itu, tujuan utama kajian ini adalah untuk memberikan maklumat asas berkenaan dengan sintesis karotenoid dalam mesokarpa pokok sawit. Untuk memenuhi tujuan ini, pengekstrakan karotenoid daripada mesokarpa buah sawit dijalankan terlebih dahulu. Satu teknik pengekstrakan karotenoid daripada mesokarpa buah sawit telah dioptimumkan. Saponifikasi sejuk telah dilakukan untuk menyingkir bahan lipid yang tidak dikehendaki daripada ekstrak mentah karotenoid. Spektrofotometri cahaya nampak-ultra lembayung (UV-Vis) telah digunakan untuk membandingkan profil penyerapan ekstrak karotenoid dalam buah sawit pada peringkat perkembangan buah yang berlainan iaitu 15 hingga 20 minggu selepas antesis (WAA). Hasil kajian menunjukan bahawa karotenoid dalam buah belum masak menyerap lebih dalam lingkungan cahaya ultra lembayung sementara karotenoid dalam buah masak menyerap lebih dalam lingkungan cahaya nampak

disebabkan oleh pembentukan lebih banyak karotenoid. Kromatografi cecair bertekanan tinggi-fasa berbalik (RP-HPLC) dengan gandingan pengesan 'array' foto-diod (PDA) telah digunakan dalam kajian ini untuk mencirikan profil karotenoid dalam ekstrak karotenoid pada peringkat perkembangan berlainan yang telah disaponifikasikan Pemisahan ekstrak karotenoid telah dijalankan dengan menggunakan kolum C18 dan C30. Hasil pemisahan menunjukan bahawa kolum C30 adalah lebih baik kerana ia dapat memperolehi lebih banyak puncak dalam kesemua ekstrak karotenoid. Karotenoid yang telah dikenalpasti dalam kesemua ekstrak karotenoid adalah α-karotena, β-karotena serta isomernya. 'Lycopene' tidak dapat dikesan dalam kesemua ekstrak karotenoid. Oleh it, dapat disimpulkan bahawa 'lycopene' tidak wujud dalam buah sawit. Bagi tujuan memahami biosintesis karotenoid dalam mesokarpa pokok sawit, pelbagai substrat yang dilabelkan dengan isotop ^{14}C telah dimasukkan ke dalam hirisan mesokarpa 20-WAA. Hasil kajian menunjukan bahawa isopentanil pirofosfat (IPP) merupakan bahan perantaraan utama bagi sintesis karotenoid dalam buah sawit. Teknik pemisahan kromatografi lapisan nipis (TLC) telah dilakukan ke atas kesemua ekstrak karotenoid 20-WAA yang telah dilabel dengan pelbagai substrat ^{14}C, untuk menentukan tapak jalan biosintesis karotenoid dalam buah sawit. Keputusan mencadangkan bahawa tapak jalan biosintesis karotenoid dalam buah sawit mengikut tapak jalan asetat/mevalonat.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A_{446nm}	absorbance at 446nm
ABA	abscisic acid
acetyl-CoA	acetyl coenzyme A
APCI	atmospheric pressure chemical ionization
CI	chemical ionization
DE	diethyl ether
DMAPP	dimethylallyl pyrophosphate
EI	electron impact
ESI	electrospray ionization
FAB	fast atom bombardment
FPP	farnesyl pyrophosphate
G3P	glyceraldehyde-3-phosphate
GGPP	geranylgeranyl pyrophosphate
GPP	geranyl pyrophosphate
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HPLC	high pressure/performance liquid chromatography
IPP	isopentenyl pyrophosphate
KOH	potassium hydroxide
λ_{max}	lambda maxima
LCMS	liquid chromatography mass spectrophotometry
LYC	lycopene cyclase
M	Molar
mCi	milliCurie
MES	2 - [N-Morpholino] ethanasulfonic acid

mmol	millimol
MVA	mevalonic acid
N_2	nitrogen
NMR	nuclear magnetic resonance
PDA	photo-diode array
PDS	phytoene desaturase
PE	petroleum ether
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
PP	polypropylene
PPPP	prephytoene pyrophosphate
PSY	phytoene synthase
Py	pyruvate
RE	retinol equivalent (a unit to define vitamin A activity of β-carotene as retinol)
RT	retention time
rpm	resolution per minute
TLC	thin layer chromatography
3D	three-dimensional
2D	two-dimensional
μCi	microCurie
vol.	volume
WAA	weeks after anthesis
ZDS	zeta-desaturase synthase

CHAPTER 1

INTRODUCTION

Background

Elaeis guineensis, which originated from West and Central Africa, is an agricultural crop that is thriving in Malaysia Today, palm oil derived from *Elaeis guineensis* is widely used for many commercial purposes It is used in the making of food (e g chocolates & margarine), non-food products (e g soaps & cosmetics) and in the near future, as fuel for motor vehicles

The use of palm oil can be traced back to Egypt during the reign of the Pharaohs In the mid-15th century, the people of West Africa used the oil extensively for cooking In South East Asia, oil palm was initially grown as an ornamental plant, but it was planted for commercial purposes when the palm oil export trade developed in the nineteenth century The seedlings from this humble plant first arrived in South East Asia from West Africa in 1848 The Dutch shipped the seeds from Nigeria to their experimental gardens in Bogor, Java, Indonesia From there, the oil palm was sent to the Botanical Gardens in Singapore in 1875, and subsequently brought to Malaya (as West Malaysia was then known) in 1878

Elaeis guineensis is a perennial tree that flourishes in the humid tropics It fruits all year long and is the highest oil-yielding crop It produces an average of three to four tonnes of oil per hectare per year When ripe, its fruits look like lush black berries (each 4-5 cm in length) crowded on short branches and are red at the base of each fruit bunch The oil palm fruit bunches yield two types of oil palm oil and palm kernel oil. Palm oil is extracted from the mesocarp (pulp) of the fruit, which contains 45-55% oil It is reddish-orange in colour due to its high carotene content (> 42,000 μg β-carotene equivalent/100 g fruit), while palm kernel oil is extracted from the kernel of the palm It is pale yellow in colour

The first commercial planting of the oil palm tree in Malaya took place in 1917 Today, Malaysia is the world's largest producer and exporter of palm oil It has 3 4 million hectares of land dedicated to oil palm plantation In 2000, Malaysia produced 10 8 million tonnes of crude palm oil, of which 90% was exported to more than 100 countries

Significance of the study

Several studies have shown that carotenoids have an important role to play in the field of medicine, nutrition, agrochemistry and pharmaceutics This stems mainly from their antioxidant properties Therefore, the high carotene content of palm oil adds to the beneficial aspects of the oil palm and the importance of these micronutrients in oil palm should not go unnoticed Due to its health benefits to humankind, there is a need to further exploit the potential of these useful pigments with the increase in demand from consumers in the future

The information obtained from this study will contribute towards future efforts in metabolic engineering of oil palm fruits to produce value-added products of commercial interests such as palm oil with high lycopene content or high polyhydroxybutyrate (PHBs) content Studies have proven that lycopene has potent antioxidant properties which help prevent cancer in man, hence, palm oil high in lycopene has potential in nutraceutical and pharmaceutical applications, while palm oil rich in PHBs would prove to be a great source for the production of biodegradable plastics.

Objectives of the study

The biosynthesis of carotenoids in plants has been studied since the early 1960s. However, not much effort has been put into studying its synthesis in the oil palm. Therefore in this study, experiments were conducted with the following objectives:

(1) to optimise the extraction of carotenoids from the oil palm fruit

(2) to characterise the carotenoid profiles in the oil palm fruits at different stages of development using TLC and HPLC techniques

(3) to study the biosynthesis of carotenoids in oil palm fruits using various ^{14}C-labelled substrates