UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF PHENOLIC EXTRACTS FROM OIL PALM (*ELAEIS GUINEENSIS*) FRUITS

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By

NEO YUN PING

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

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

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Chair: Azis Ariffin, PhD

Faculty: Faculty of Food Science and Technology

The extracted oil palm fruit phenolics were analysed using spectrophotometry methods to obtain information on the different types of oil palm phenolics and their antioxidative activities. Different methods were used to extract soluble free (SFP), insoluble-bound (ISBP) and esterified (EFP) phenolics for a better understanding of the types of phenolics present. TPC, TFC, ODPI and DPPH of oil palm phenolics were also monitored to investigate the possible relationships between these variables and the degree of maturity/ripeness of the oil palm fruit from 16 to 24 weeks. The antioxidant activities of oil palm phenolic extracts were analysed using different antioxidant assays, namely the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay, the ferric-reducing ability (FRAP) assay, β-carotene bleaching assay (BCB) and the oxidative stability index (OSI). Results showed that oil palm phenolic extracts contained high

antioxidant activities in the order of ISBP > EFP > SFP. Eight different phenolic acids were identified and quantified using a simple reversed-phase high performance liquid chromatography (HPLC) with a diode array detector (DAD) and liquid chromatography/ tandem mass spectrometry (LC/MS/MS). Gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric and ferulic acids were detected in oil palm phenolic extracts. Ferulic, *p*-hydroxybenzoic and *p*-coumaric acid were the dominant phenolic acids found in oil palm fruit extracts and ranged from 55 - 376 µg/g of DW. The results suggested the potent antioxidant activities of oil palm phenolic extracts and the presence of phenolic acids in palm fruits.

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

PENCIRIAN DAN AKTIVITI ANTI-PENGOKSIDAAN EKSTRAK FENOLIK DARI BUAH KELAPA SAWIT (*ELAEIS GUINEENSIS*)

Oleh

NEO YUN PING

JANUARI 2009

Pengerusi: Azis Ariffin, PhD

Fakulti: Fakulti Sains dan Teknologi Makanan

Fenolik yang diekstrak dari buah kelapa sawit telah dianalisis dengan menggunakan kaedah spektrofotometer untuk mengenalpasti jenis sebatian fenolik dan aktiviti anti-pengoksidaannya. Pengekstrakkan fenolik buah kelapa sawit telah dijalani dengan menggunakan dua kaedah yang berbeza. Pembolehubah seperti TPC, TFC, ODPI dan DPPH telah dikaji bagi ekstrak fenolik buah kelapa sawit dari 16 ke 24 minggu untuk mengenalpasti hubungan di antara pembolehubah tersebut dengan kematangan buah kelapa sawit. Keputusannya menunjukkan bahawa tiada gaya tertentu yang dapat dikenalpasti antara pelbagai pembolehubah dan kematangan buah kelapa sawit. Aktiviti antipengoksidaan ekstrak buah kelapa sawit telah dijalankan dengan menggunakan beberapa kaedah seperti 2,2-azino-bis-3-etilbenzotiazolin-6-sulfonik (ABTS), 2,2-difenil-2-pikrahidrazil (DPPH), kemampuan penurunan ferric (FRAP), kemampuan pelunturan β-karotena (BCB) dan indeks kestabilan pengoksidaan (OSI).

Keputusan menunjukkan bahawa ekstrak fenolik bagi buah kelapa sawit mengandungi keupayaan antioksida yang tinggi dalam susunan ISBP >EFP > SFP. Lapan jenis asid fenolik yang berlainan telah dikenalpasti dengan menggunakan kromatografi cecair berprestasi tinggi jenis fasa terbalik yang ringkas dengan pengesan "diode array" (DAD) dan kromatografi cecair /spektrometri jisim (LC/MS/MS). Asid galik, protokatechuik, *p*-hidrosibenzoik, vanilik, kafeik, syringik, *p*-koumarik dan ferulik telah dikenalpasti dalam ekstrak buah kelapa sawit. Asid ferulik, *p*-hidrosibenzoik dan *p*-koumarik merupakan asid fenolik dominan dalam ekstrak buah kelapa sawit bernilai dari 55 - 376 µg/g DW. Keputusan yang diperolehi mencadangkan aktiviti-aktiviti antipengoksidaan yang tinggi di dalam ekstrak buah kelapa sawit dan kehadiran asid fenolik di dalam buah kelapa sawit.

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Yun Ping a.k.a Neo

I certify that an Examination Committee has met on **23 JANUARY 2009** to conduct the final examination of **NEO YUN PING** on her **MASTER OF SCIENCE** thesis entitled "**CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF PHENOLIC EXTRACTS FROM OIL PALM (*ELAEIS GUINEENSIS*) FRUITS** " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the (Name of relevant degree).

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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.

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NEO YUN PING

Date: 23rd January 2009

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LIST OF ABBREVIATIONS

A	absorbance
ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ANOVA	analysis of variance
BCB	β-carotene-linoleic acid bleaching assay
BHA	butylated hydroxyanisole
CE	(+)-Catechin Equivalents
CPO	crude palm oil
DAD	photodiode array detector
DE-EA	diethyl ether: ethyl acetate
DPPH	2,2-diphenyl-2-picrylhydrazyl
DW	dry weight
EC	end-capped
EFP	esterified phenolics
ESI	electrospray ionization
ET	electron transfer
FAE	Ferulic Acid Equivalent
FI	flavonol index
FRAP	ferric ion reducing antioxidant parameter
GAE	Gallic Acid Equivalent
GC	gas chromatography
HAT	hydrogen atom transfer

HCAI	hydroxycinnamic acid index
HPLC	high performance liquid chromatography
ISBP	insoluble-bound phenolics
LC	liquid chromatography
LOD	detection limit
LOQ	quantitation limit
MS	mass spectra
NBDPO	neutralized bleached deodorized palm oil
OIV	Office International de la Vigne et du Vine
ODPI	*o*-diphenol index
ORAC	oxygen radical absorbance capacity
OSI	oxidative stability index
PI	phenol index
POME	palm oil mill effluent
RBDPO	refined bleached deodorized palm oil
RE	Rutin Equivalents
RSD	relative standard deviation
SAR	structure-activity relationships
SD	standard deviation
SFP	soluble free phenolics
SPE	solid phase extraction
TEAC	Trolox Equivalent Antioxidant Capacity
TFC	total flavonoid content

TPC	total phenolic content
TPE	total phenolic extracts
TPTZ	2,4,6-tris-2,4,6-tripyridyl-2-triazine
TRAP	total radical trapping antioxidant parameter
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

LIST OF SYMBOLS

AH	primary antioxidant
$AlCl_3.6H_2O$	aluminium chloride
cm	centimetre
e^-	electron
Fe^{2+}	ferrous cation
Fe^{3+}	ferric cation
$FeCl_3.6H_2O$	ferric chloride
Fe^{2+}-TPTZ	ferrous tripyridyltriazine
Fe^{3+}-TPTZ	ferric tripyridyltriazine
g	gram
hr	hour
$H\cdot$	hydroxyl radical
HCl	hydrochloric acid
i.d.	internal diameter
L	litre
kg	kilogram
M	molar
mg	milligram
min	minute
mL	milliliter
mM	millimolar

N	normality
NaOH	sodium hydroxide
$NaNO_2$	sodium nitrite
nm	nanometre
O_2	oxygen
R·	alkyl radical
RH	unsaturated fatty acid
ROO·	peroxyl radical
ROOH	lipid hydroperoxide
rpm	rotations per minute
PP·	phenoxyl radical
PPH	phenolic antioxidants
ppm	parts per million
v/v	volume/volume (ratio)
°C	degree Celsius
µg	microgram
µL	microlitre
µM	micromolar

1 INTRODUCTION

Oil palm belongs to the genus *Elaeis*. It is a monocotyledon perennial tree crop of the order Spodiciflorae, Palmae family grouped under the Cocoineae tribe. The genus consists of two species, namely *Elaeis guineensis* and *Elaeis oleifera*. *Elaeis guineensis* originated from West Africa whilst *Elaeis oleifera* is from South America. For some 5000 years oil palm has been an important crop for mankind. *Elaeis guineensis* being almost non-existent in the 1950s, has now become the largest cultivated crop in Malaysia and plays a significant role in the socio-economic well being of the country. Currently, South-East Asia, particularly Malaysia and Indonesia are the world's largest producers of palm oil (Malaysian Palm Oil Council, 2009). In 1960 the total planted area of oil palm in Malaysia was 54.700 hectares but by 2007, this has increased to 4.3 million hectares. Prices of all oil palm products registered significant gains and export earnings and increased 41.8% to a record RM 45.1 billion (Malaysian Palm Oil Board, 2008).

Climatic conditions in Malaysia, with a tropical humid climate with temperatures ranging from 24- 32 °C throughout the year, are ideally suited for the cultivation of oil palm. The cross between *Dura* and *Pisifera* known as *Tenera* is the most common planted oil palm cultivar. The oil palm is unique because it produces two types of oil. Palm oil is produced from the pulp (mesocarp) of the fruit and the kernel of the fruit produces palm kernel oil. The mesocarp is fibrous and the kernel has a slight cavity in the center. The oil palm fruit is a drupe varying in shape (from spherical to ovoid