UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF HYDROPHILIC PHENOLIC COMPOUNDS IN PALM OIL AND PALM KERNEL OIL

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FSTM 2009 29

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2009

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

DETERMINATION OF HYDROPHILIC PHENOLIC COMPOUNDS IN PALM OIL AND PALM KERNEL OIL

By

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November 2009

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The quantification of total phenolic content expressed as gallic acid equivalent (GAE) was based on the Folin-Ciocalteau colorimetric method. The total phenolic contents (TPC) of palm oil samples were determined from crude to refined palm and palm kernel oils obtained from palm oil mills. The samples was crude palm oil (CPO), refined palm oil (RPO), refined palm olein (RPOo), crude palm kernel oil (CPKO), refined palm kernel oil (RPKO), and palm kernel olein (PKOo). All samples were utilized to determine the TPC. Quantification of phenolics extracted from palm and palm kernel oils showed the highest level of TPC in CPO and refining reduces the TPC in the oil. The TPC in CPO ranged from 31.73 – 70.18 mg/kg, bleached palm oil (BPO) from 18.36 – 22.25 mg/kg, RPO from 16.90 – 26.89 mg/kg, RPOo from 11.36 – 12.20 mg/kg and palm fatty acid distilled (PFAD) from 1.07 – 5.48 mg/kg. While that the TPC for CPKO ranged from 16.80 –27.25 mg/kg, RPKO from 3.16 – 3.82 mg/kg and PKOo from 2.52 – 8.60 mg/kg. Furthermore, the reduction of phenolic content were obtained in the different stages of the refining step. Results showed that the degummed and bleached oil (BPO) contained a lower

amount of TPC (4.4% - 6.4%) reduction of TPC. In the final refined product, RPO, the TPC has been reduced (9.6% - 14.0%). After the refining process, the TPC was reduced from 9.2% - 11.3% and it showed that a portion of the phenolics end up in the PFAD fraction. From the TPC in respective oils, it can be seen that a significant amount of the phenolics was probably lost through absorption of bleaching earth, volatilization and degradation during the refining process.

Antioxidant activity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays by measuring the decrease in absorbance at 517 nm. Results showed that the effect of antioxidants increase on DPPH radical scavenging activity in order of oil extracts was CPO > CPKO > RPO > RPKO > RPOo > PKOo. Overall it was found that CPO extract exhibited the highest antioxidant activity. This is due to high TPC compared to other extracted oil samples.

Eight different phenolic acids were identified in palm oil and palm kernel oil extracts using a simple reversed-phase high performance liquid chromatography (HPLC) equipped a UV-Visible detector. The phenolic acids are gallic acid, protocatechuic, *p*-hydroxybenzoic, vanillic acid, syringic acid, caffeic acid, *p*-coumaric and ferullic acid. The results showed that most were benzoic and cinnamic acid derivatives with, *p*-hydroxybenzoic acid being the predominant acid present in all sample extracts. In comparison with benzoic acids, cinnamic acids were present in lower concentrations and these were caffeic, coumaric and ferullic acids. The profiling of hydrophilic phenolic compounds would provide information on the possible role of these compounds in oil stability, TPC and other possible beneficial properties. The results

suggested the potent antioxidant activities of palm oil phenolic extracts and the presence of phenolic acids in palm oils and palm kernel oils.

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

KAJIAN TERHADAP SEBATIAN FENOLIK HIDROFILIK DALAM MINYAK KELAPA SAWIT DAN MINYAK ISIRONG

Oleh

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Penentukan jumlah kandungan fenolik (TPC) diungkap sebagai kesamaan asid galik (GAE) adalah berdasarkan kepada kaedah Folin-Ciocalteau Kalorimetrik. Jumlah kandungan fenolik sampel minyak kelapa sawit ditentukan daripada minyak sawit mentah kepada minyak yang telah diproses dan minyak isirong diperolehi daripada kilang penapisan minyak. Sampel-sampel adalah terdiri daripada minyak sawit mentah (CPO), minyak sawit ditapis (RPO), minyak olein (RPOo), minyak isirong mentah (CPKO), minyak isirong ditapis (RPKO), dan minyak isirong olein (PKOo). Kesemua sampel diekstrak untuk menentukan jumlah kandungan fenolik. Kuantitatif ekstrak fenolik daripada minyak kelapa sawit dan minyak isirong ditentukan menunjukkan paras jumlah kandungan fenolik tertinggi dalam CPO dan penapisan mengurangkan jumlah kandungan fenolik di dalam minyak. Kandungan jumlah fenolik dalam CPO adalah lingkungan 31.73 – 70.18 mg/kg, BPO dari 18.36 – 22.25 mg/kg, RPO dari 16.90 – 26.89 mg/kg dan PFAD dari 1.07 – 5.48 mg/kg. Sementara jumlah kandungan fenolik untuk CPKO adalah lingkungan 16.80 –27.25 mg/kg, RPKO dari 3.16 – 3.82 mg/kg dan PKOo dari 2.52 – 8.60 mg/kg. Tambahan lagi, pengurangan kandungan fenolik diperolehi di dalam peringkat yang berbeza dari

langkah penapisan. Keputusan menunjukan bahawa minyak yang diluntur iaitu minyak BPO mengandungi jumlah kandungan fenolik yang rendah (4.4% - 6.4%) pengurangan jumlah kandungan fenolik. Produk penapisan yang terakhir, RPO, jumlah kandungan fenolik telah berkurangan (9.6% - 14.0%). Selepas proses penapisan minyak sawit, jumlah kandungan fenolik telah berkurangan sebanyak 9.2% - 11.3% dan ini menunjukan bahawa sejumlah fenolik berakhir dalam pecahan PFAD. Daripada jumlah kuantiti fenolik dalam minyak masing-masing, menunjukkan bahawa nyata sekali kandungan fenolik adalah berkemungkinan hilang melalui penyerapan peluntur bumi, pemeruapan dan perendahan semasa proses penapisan.

Aktiviti kestabilan pengoksidaan ditentukan menggunakan kaedah 2, 2-difenil-1-pikrahidrazil (DPPH) berdasarkan penurunan dalam penyerapan pada 517 nm. Kesan antioksida terhadap aktiviti pemerangkapan radikal bebas DPPH mengikut urutan peningkatan dalam ujian sampel ekstrak minyak adalah CPO > CPKO > RPO > RPKO > RPOo > PKOo. Secara keseluruhannya didapati bahawa ekstrak CPO dapat memberi ketahanan pada aktiviti antioksida yang tertinggi. Ini adalah kerana kehadiran TPC yang tertinggi berbanding sampel ekstrak minyak yang lain.

Lapan jenis asid fenolik yang berbeza telah dikenalpasti hadir dalam ekstrak minyak sawit dan ekstrak minyak isirong dengan menggunakan kromatografi cecair berprestasi tinggi jenis fasa terbalik yang ringkas (HPLC) dengan pengesan sinaran Ultra Ungu-Nyata. Asid fenolik yang dikesan adalah asid galik, protokatechuik, *p*-hidrosibenzoik, asid vanilik, asid syringik, asik kafeik, *p*-koumarik dan asid ferulik. Kebanyakkan adalah terdiri daripada terbitan asid benzoik dan asid sinamik. Jika

dibandingkan dengan asid benzoik, asid sinamik hadir dalam kepekatan yang rendah dan terdiri daripada asik kafeik, *p*-koumarik dan asid ferulik. Profil sebatian fenolik hidrofilik ini akan memberi informasi terhadap keseluruhan sebatian dalam kestabilan minyak, TPC dan kebaikan kandungan yang lain. Keputusan yang diperolehi mencadangkan aktiviti-aktiviti pengoksidaan yang tinggi di dalam ekstrak minyak sawit dan kehadiran asid folik di dalam minyak sawit dan minyak isirong.

ACKNOWLEDGEMENTS

Firstly, I pray to Almighty Allah Subhanahu wata'ala who gave me the bless, thoughts and guidance throughout my studies.

I wish to express my most sincere gratitude to my supervisors, Associate Professor Abdul Azis b. Ariffin and Associate Professor Yaakob b. Che Man from the Faculty of Food Science and Technology, Universiti Putra Malaysia; Dr. Tan Yew Ai, supervisor of the Malaysian Palm Oil Board (MPOB), Yg. Bhg. Datuk Hj. Basiron, the former Director-General of the Malaysian Palm Oil Board (MPOB) and the Yg. Bhg. Dato' Dr. Mohd Basri b. Wahid, present Director-General of the Malaysian Palm Oil Board (MPOB); for their supervision, guidance and encouraged throughout my research and writing of this thesis.

Thanks would also be extended due to all staff of the Unit Analytical, Product Development and Advisory Services Division, (MPOB) and my colleaguess in Universiti Putra Malaysia for their guidance, valuable comments and useful discussion during my research.

Finally, I am deeply obligated, gratitude and appreciation to my father, my mother, brothers and my beloved daughters for their love with continuously encouraged over the course of this thesis and presented me the most beautiful world.

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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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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FADZLINA BINTI ABDULLAH

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABTS	:	2,2'-azinobis(3-ethylbenzthiazoline-sulphonic acid)
ANOVA	:	Analysis of variance
BPO	:	Bleached Palm Oil
COOH	:	carboxylic acid group
CHD	:	coronary heart disease
CPO	:	Crude Palm Oil
CPKO	:	Crude Palm Kernel Oil
DAD	:	photodiode Array Detector
DPPH	:	2, 2-Diphenyl-1-picrylhydrazyl
DHPE	:	3,4-hydroxyphenylethanol
DNA	:	Deoxyribonucleic acid
EVOO	:	Extra Virgin Olive Oil
FFA	:	Free Fatty Acid
FFB	:	Fresh Fruit Bunch
FRAP	:	Ferric-Reducing Antioxidant Power
GAE	:	Gallic Acid Equivalent
HPLC	:	High Performance Liquid Chromatography
LDL-C	:	low density lipoprotein cholesterol
LDL	:	low density lipoprotein
LSD	:	least significantly difference
OD	:	Optical Density
PFAD	:	Palm Fatty Acid Distillate
PKO	:	Palm Kernel Olein
PTFE	:	Polytetrafluoroethylene

PKOo	:	Spiked Palm Kernel Olein with gallic acid standard
PV	:	Peroxide Value
RBD	:	Refined, Bleached Deoderized
RBDPO	:	Refined, Bleached Palm Deoderized
RPKO	:	Refined Palm Kernel Oil
PKOo	:	Refined Palm Kernel Olein
RPOo	:	Refined Palm Olein
RPO	:	Refined Palm Oil
RT	:	Retention Time
SCOPA	:	Seed Crushers and Oil Processors Association
TAGs	:	triacylglycerols
TBHQ	:	Tertiary-butylhydroxy quinine
TC	:	Total Cholesterol
TPC	:	Total Phenolic Content
TRF	:	tocotrienol-rich fraction
UV-Vis	:	Ultra Violet-Visible

LIST OF SYMBOLS

DPPH•	:	DPPH radical
g	:	gram
L	:	Liter
min	:	minute
mL	:	milliliters
mm	:	millimeters
mg/L	:	milligram per liter
μL	:	microliter
nm	:	nanometer
OH	:	hydroxyl group
OCH3	:	methoxy substitution
^{o}C	:	degree Celsius
ppm	:	part per million
R_2	:	Regression
(vol/vol)	:	volume/volume (ratio)
(w/w)	:	weight/weight (ratio)

CHAPTER 1

INTRODUCTION

Polyphenols are a group of chemical substances found in plants and divided into the division of tannins, lignins, and flavonoids which derived from the variety of simple polyphenolic units derived from secondary plant metabolism of the shikimate pathway (Dewick, 1995). In organic chemistry, phenols, sometimes called phenolics. Phenols are a class of chemical compounds consisting of a hydroxyl functional group (-OH) attached to an aromatic hydrocarbon group (Pokorny *et al*., 2001). Compounds containing phenol moieties can act as free radical scavengers and there are anecdoted evidence regarding its in prevention of premature aging and cancer caused by oxidative stress.

The palm oil is a source of water-soluble phenolics antioxidant (Sambanthmurthi *et al*., 2000). Palm and palm kernel oil are in demand for edible and non edible applications. However, palm oil, like all vegetable oils, will deteriorate when subjected to heat and aeration. Vitamin E, especially helps prevent deteriorate of the oil by capturing free radicals, and preventing the oxidation of the unsaturated fatty acids of the oil, into hydroperoxides, ketones and aldehydes. In palm oil there are a number of minor components including the carotenoids, tocopherols, tocotrienols, sterols, phosphatides, triterpenic, aliphatic alcohols and phenolics compounds. Although these minor components account for less than 1% of the oil's constituents, they nevertheless play significant roles in maintaining its stability and quality (Abushita *et al*., 1997). In addition, some of these minor components especially the

phenolic compounds, carotenoids and vitamin E (tocopherols and tocotrienols) are important nutritionally. The protection that fruit and vegetables provide against several disease has been attributed to the various antioxidants, vitamin C, vitamin E, α-tocopherol, β-carotene and polyphenolic compounds (Abushita *et al*., 1997; Aruoma, 1998; Moure *et al*., 2001).

Solvent extraction is more frequently used for isolation of antioxidants and both extraction yield and antioxidant activity of extracts are strongly dependent on the solvent, due to the different antioxidant potential of compounds with different polarity (Julkunen-Tiito, 1985; Marinova and Yanishlieva, 1997). It is widely accepted also by Pokorny *et al*., (2001), that, antioxidant activity of these extracts depends on the type and polarity of extraction solvent, isolation procedures and purity and identity of active components of extracts from these raw materials. Polar solvents are among the most employed solvents for removing polyphenols from oil. Ivanova *et al*., (2005), proposed that not all phenolic compounds possessed radical quenching activity. The method of extracting the oils with methanolic solvent might give the different results in antioxidant activity from other published reports. The difference would be due to organic solvents used in isolation that extracted only some selective components (Tiwari *et al*., 2001).

Phenolics can act as radical scavengers or radical-chain breakers (Grassmann *et al*., 2002; Gil *et al*., 2000). The antioxidant properties of phenolics are mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Rice-Evans *et al*., 1997). Oxidation caused by free radicals reduced capabilities to combat ageing and serious illness, including cancer,

kidney damage, artherosclerosis and heart diseases (Ames, 1983). Phenolic acids also play an important role in combating oxidative stress in the human body by maintaining a balance between oxidants and antioxidants (Temple, 2000). According to Kaur and Kapoor, (2001), antioxidants neutralize free radicals by donating one of their own electrons thereby ending the electron-stealing reaction. They act as scavengers and play the housekeeper's role by mopping up free radicals before they get a chance to act. Thus, antioxidants may well be defined as the substances that are capable of quenching or stabilizing free radicals.

Radical scavenging is the main mechanism by which antioxidants act in food. The activity is assessed by the scavenging of synthetic radicals in polar organic solvent, e.g., methanol, at room temperature using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to monitor the free radical scavenging abilities (the ability of a compound to donate an electron) of various antioxidant. The free radical scavenging activity of palm and palm kernel oils extracts were carried out according to Brand-Williams *et al*., (1995). This assay is based on the measurement of the reducing ability of antioxidants toward $DPPH^{+}$. The $DDPH^{+}$ radical is one of the few stable organic nitrogen radicals, which bears a deep purple colour due to its impaired electron, and radical scavenging can be followed by spectrophotometrically by the loss of absorbance at 517 nm, as the pale yellow nonradical form is produced. DPPH is a stable nitrogen centered free radical which can be effectively scavenged by antioxidants (Grassmann *et al*., 2002). Hence it has been widely used for rapid evaluation of the antioxidant activity of plant extracts relative to other methods. DPPH is also considered as a good kinetic model for peroxyl radicals (Salah *et al*.,

1995). The ability of palm and palm kernel oil extracts to scavenge DPPH radicals was determined by the decrease in its absorbance at 517 nm.

The test is simple and rapid and needs only a UV-Vis Spectrophotometer to perform, which probably explains its widespread use in antioxidant screening. Prior *et al*., (2005), found good reproducibility with the DPPH assay. DPPH is a stable nitrogen radical that bears no similarity to the highly reactive, transient peroxyl radicals involved in lipid peroxidation and strong oxidizing capacity (Nzaramba, 2004). Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH due to steric inaccessibility. Free radical scavenging is one of generally accepted mechanisms against lipid oxidation. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Baumann *et al*., 1979).

Various methods have been used for identification of phenolics. An HPLC technique was developed for the separation and quantification of the phenolic acids by Wulf and Nagel, (1976). Reversed-phase high-performance liquid chromatography (RP-HPLC) currently is the most popular and reliable technique for the determination of phenolic compounds (Tasioula-Margari and Okogeri, 2001). All polyphenolics absorb in the UV region (Robards and Antolovich, 1997). HPLC methods give specific information on individual compounds and are widely used for examination of fruit and vegetable phenolics (Kim and Lee, 2002).

The phenolic compounds of palm oil and palm products have great potential in the development of health-beneficial foods, feeds, cosmetic and pharmaceutical

preparations (Pokorny *et al*., 2001). Based on the hypothesis that the phenolic compounds present in the palm oil and palm kernel oil (albeit in a small quantities), the objectives of the project were:

(i) to extract and quantify the phenolics from palm oil and palm kernel oil;

(ii) to determine the DPPH scavenging capacity of the different extracts;

(iii) to identify the hydrophilic phenolic compounds in palm oil and palm kernel oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Palm oil and palm kernel oil

Palm oil and palm kernel oil are composed of fatty acids, esterified with glycerol just like any ordinary fat. Both are high in saturated fatty acids, about 50% and 80%, respectively. The 16 carbon saturated fatty acid palmitic acid is the major fatty acid accounting for 44% of the total fatty acid composition found in palm oil followed by the monounsaturated oleic acid (39%) while palm kernel oil contains a high level of lauric acid (Sambanthamurthi *et al*., 2000). Palm oil is the largest natural source of tocotrienol, part of the vitamin E family. Palm oil is also high in vitamin K and dietary magnesium (Faessler, 2004).

2.2 Crude palm oil (CPO) and palm kernel oil (PKO) and their fractions

The compositions weight percentage of these fractions is shown in Table 1.1. Fractionation of CPO and CPKO in the refinery produces the liquid stearin fraction and a solid stearin component. Refined CPO denoted as Refined, Bleached and Deorderized Palm Oil (RBDPO) has similar fatty acid composition to that CPO. RBDO can be further fractionated into the liquid Olein and the solid fraction Stearin. The fatty acid compositions the palm oil products, compared with coconut oil and soy oil are presented in Table 1.1. Palm oil has a balanced ratio of saturated and unsaturated fatty acids while palm kernel oil has mainly saturated fatty acids which are broadly similar to the composition of coconut oil. Compared to soy oil, palm oil