



UNIVERSITI PUTRA MALAYSIA

**A CHARACTERIZATION, ANTIOXIDANT PROPERTIES AND
AUTHENTICATION OF VIRGIN COCONUT OIL**

MARINA BINTI ABDUL MANAF

FSTM 2009 19



**CHARACTERIZATION, ANTIOXIDANT PROPERTIES AND
AUTHENTICATION OF VIRGIN COCONUT OIL**

MARINA BINTI ABDUL MANAF

**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

2009



**CHARACTERIZATION, ANTIOXIDANT PROPERTIES AND
AUTHENTICATION OF VIRGIN COCONUT OIL**

By

MARINA BINTI ABDUL MANAF

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia in Fulfillment of the Requirement for the Degree of Doctor of
Philosophy**

August 2009



ESPECIALLY DEDICATED TO MY BELOVED FAMILY



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

CHARACTERIZATION, ANTIOXIDANT PROPERTIES AND AUTHENTICATION OF VIRGIN COCONUT OIL

By

MARINA BINTI ABDUL MANAF

August 2009

Chairman: Professor Yaakob bin Che Man, PhD

Faculty: Food Science and Technology

A study on chemical properties and authentication of virgin coconut oil (VCO) was conducted. Chemical properties showed that commercial VCO had iodine value of 4.47 to 8.55, peroxide value of 0.21 to 0.57 meq oxygen/kg, free fatty acid of 0.15 to 0.25, saponification value of 250.07 to 260.67 mg KOH/g oil and anisidine value of 0.16 to 0.49. Lauric acid was the predominant fatty acid which ranged from 46.64 to 48.03%. Major triacylglycerol (TAG) were LaLaLa, LaLaM, CLaLa, LaMM and CCLa (La:lauric; C:capric; M:Myristic) which accounted for more than 80% TAG of the oil. Total phenolic content ranged from 7.78 to 29.18 mg GAE/g oil. VCO samples exhibited higher antioxidant activity (49.79 to 79.87%) compared to refined, bleached and deodorized (RBD) coconut oil (49.58%).



Comparison between different processing methods of VCO showed that VCO produced by fermentation method possessed the strongest scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) with the amount of oil necessary to decrease the initial DPPH radical concentration by 50% (EC_{50}) value of 1.24 mg/mL. VCO produced by fermentation method also exhibited the highest antioxidant activity of 71% while the highest reducing power of VCO produced by chilling method was 1.02 at 10 mg/mL. The results revealed that VCO produced by both fermentation and chilling method had higher antioxidant potency than RBD coconut oil. Total phenolic content was strongly correlated with radical scavenging capacity ($r = 0.91$) and reducing power ($r = 0.96$) while no correlation was observed for β -carotene bleaching test. Some phenolic acids found in VCOs were protocatechuic, vanillic acid, caffeic acid, syringic acid, coumaric acid and ferulic acid.

Rapid methods were developed to detect adulteration in VCO. First, Fourier transform infrared (FTIR) spectroscopy was used to detect adulteration of VCO with palm kernel olein. The results showed that FTIR was capable of detecting adulteration down to 1% adulteration level. Discriminant analysis using 10 principal components was able to classify pure and adulterated samples on the basis of their spectra. A partial least square (PLS) calibration demonstrated a good linear regression (R^2) of 0.9875 of actual value against FTIR predicted concentration of palm kernel olein. Discriminant analysis was also capable to distinguish between VCO and other vegetable oils.



Another rapid method, differential scanning calorimetry (DSC) was also used to determine adulteration of VCO with selected vegetable oils, namely soybean oil (SBO) from linolenic acid group, sunflower oil (SFO) from oleic-linoleic acid group and palm kernel oil (PKO) from lauric acid group. The heating curves of SBO and SFO adulterated samples demonstrated adulteration peaks appearing at the lower temperature region starting at 10% adulteration level. Regression analysis using stepwise multiple linear regressions (SMLR) was used to predict the percent of adulterant with R^2 of 0.9390 for SFO and 0.9490 for SBO. No adulteration peak was observed for PKO adulterated oils but a good relationship between the main exothermic peak height of PKO and percentage of adulteration was established with R^2 of 0.9454.

Finally, electronic nose with surface acoustic wave (SAW) sensor was used to detect adulteration in VCO with palm kernel olein. Qualitative analysis was made possible using VaporPrint™, which translated the sensor's response into visualized two dimensional image. Adulteration peaks were identified from chromatogram profile and the best relationship ($R^2 = 0.9093$) was obtained between adulterant peak F and the percentage of palm kernel olein added. Pearson correlation (r) of 0.92 was obtained between adulterant peak F and iodine value while correlation (r) of 0.89 was obtained between peroxide value and adulterant peak F. Principal component analysis (PCA) provided good separation of samples with 74% of the variation accounted for principal component 1 and 17% accounted for principal component 2. Excellent result

was obtained in the differentiation of pure and adulterated samples down to 1% detection limit.

In conclusion, this study provides references on chemical properties as well as presented the antioxidative potential of VCO. New methods were also developed for detection of adulteration of VCO with other oils using rapid analytical techniques.



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

PENCIRIAN, SIFAT ANTIOKSIDA DAN AUTENTIKASI MINYAK KELAPA DARA

Oleh

MARINA BINTI ABDUL MANAF

Ogos 2009

Pengerusi: Profesor Yaakob bin Che Man, PhD

Fakulti: Sains dan Teknologi Makanan

Sifat kimia dan autentikasi minyak kelapa dara telah dikaji. Sifat kimia menunjukkan minyak kelapa dara komersil mempunyai nilai iodine 4.47 hingga 8.55, nilai peroksida di antara 0.21 sehingga 0.57 meq oksigen/kg, nilai asid lemak bebas di antara 0.15 sehingga 0.25, nilai saponifikasi di antara 250.07 sehingga 260.67 mg/KOH minyak dan nilai anisidin di antara 0.16 sehingga 0.49. Asid laurik merupakan asid lemak tertinggi dengan nilai kandungan daripada 46.64 hingga 48.03%. Triasilgliserida (TAG) paling tinggi terdiri daripada LaLaLa, LaLaM, CLaLa, LaMM dan CCLa (La: laurik, C: Kaprik dan M: Miristik) yang mewakili sebanyak 80% daripada keseluruhan TAG. Jumlah kandungan fenolik minyak kelapa dara di antara 7.78 sehingga 29.18 mg GAE/100g minyak. Minyak kelapa dara mempunyai aktiviti antioksidan (49.79 hingga 79.87%) lebih tinggi daripada minyak kelapa komersil (RBD) (49.58%).

Perbandingan di antara kaedah pemprosesan menunjukkan bahawa minyak kelapa dara yang dihasilkan daripada kaedah fermentasi mempunyai kuasa pelunturan ke atas DPPH paling tinggi dengan nilai EC_{50} 1.24 mg/mL. Minyak kelapa dara yang dihasilkan daripada kaedah fermentasi juga menunjukkan aktiviti antioksidan paling tinggi iaitu 71% manakala kuasa penurunan paling baik didapati pada minyak kelapa dara yang menggunakan kaedah pendinginan, dengan nilai pencerapan di antara 1.02 sehingga 10 mg/mL. Hasil kajian menunjukkan bahawa minyak kelapa dara yang dihasilkan daripada kedua-dua kaedah fermentasi dan pendinginan mempunyai potensi antioksidan lebih tinggi daripada minyak kelapa RBD. Korelasi juga menunjukkan jumlah kandungan fenolik mempunyai perhubungan yang kuat dengan kuasa pelunturan DPPH dan kuasa penurunan manakala korelasi tinggi ditunjukkan oleh ujian pelunturan β -karotena dan jumlah asid fenolik. Beberapa asid fenolik yang dijumpai di dalam minyak kelapa dara ialah asid vanillik, asid kaffeik, asid syringik, asid kumarik dan asid ferulik.

Seterusnya, kaedah pantas dibangunkan untuk mengesan penambahan bahan asing ke dalam minyak kelapa dara. Pertama, spektroskopi fourier transform Infra Merah (FTIR) spektroskopi telah digunakan untuk mengesan penambahan olein isirung sawit ke dalam minyak kelapa dara. Hasil kajian menunjukkan FTIR dapat mengesan kehadiran olein isirung sawit sehingga 1%. Analisis diskriminasi menggunakan 10 komponen utama berjaya memisahkan minyak kelapa dara asli dan minyak kelapa dara bercampur olein isirung sawit berdasarkan spektrum

minyak tersebut. Kalibrasi menggunakan 'Partial Least Square' menunjukkan persamaan linear yang baik dengan nilai R^2 bersamaan 0.9875. Analisis diskriminasi juga dapat membezakan di antara sampel minyak kelapa dara dan lain-lain minyak sayuran.

Satu lagi kaedah pantas, kalorimeter pengimbas pembezaan (DSC) juga digunakan untuk mengesan pencampuran di antara minyak kelapa dara dengan minyak sayuran terpilih, iaitu minyak kacang soya, yang merupakan minyak sayuran daripada kumpulan asid linolenik, minyak bunga matahari, yang merupakan minyak sayuran daripada kumpulan oleik-linoleik dan minyak isirung sawit daripada kumpulan asid laurik. Keluk pemanasan sampel yang dicampur minyak kacang soya dan minyak bunga matahari menunjukkan satu puncak baru (puncak pencampuran) muncul pada suhu rendah pada pencampuran 10%. Analisis regresi 'stepwise multiple linear regression (SMLR)' digunakan untuk meramalkan peratus pencampuran dengan nilai R^2 bersamaan 0.9390 dan 0.9490 untuk minyak kacang soya dan minyak bunga matahari. Tiada puncak baru diperhatikan pada minyak kelapa dara yang dicampur minyak isirung sawit tetapi terdapat korelasi yang tinggi di antara ketinggian puncak eksotermik utama dengan peratus pencampuran, dengan nilai R^2 bersamaan 0.9454.

Dalam kajian akhir, hidung elektronik dengan pengesan gelombang permukaan akustik (SAW) telah digunakan untuk mengesan pencampuran minyak kelapa dara dengan olein isirung sawit. Analisis kualitatif dapat diperhatikan dengan

menggunakan VaporPrint™, yang dapat menunjukkan tindak balas pengesanan secara visual. Beberapa puncak yang menunjukkan pencampuran telah dikenalpasti dan perhubungan yang kuat diperolehi di antara puncak F dan peratus olein isirung sawit dengan nilai R^2 bersamaan 0.9093. Terdapat kolerasi yang tinggi (r) 0.92 di antara puncak F dan nilai iodin manakala korelasi (r) di antara puncak F dan nilai peroksida adalah 0.89. Analisis komponen utama (PCA) menghasilkan pemisahan yang baik di antara sampel minyak kelapa dara asli dan sampel yang dicampuri olein isirung sawit dengan 74% variasi diperuntukkan untuk komponen utama 1 dan 17% diperuntukkan untuk komponen utama 2. Tahap minima pengesanan pencampuran adalah sehingga 1%.

Sebagai kesimpulan, kajian ini memberi rujukan tentang sifat kimia dan menunjukkan potensi antioksidan yang terdapat pada minyak kelapa dara. Tambahan pula, kaedah pengesanan pencampuran minyak kelapa dara dengan minyak sayuran lain telah dibangunkan menggunakan teknik analitikal pantas.



ACKNOWLEDGEMENTS

In the name of Allah, Most gracious, Most merciful. Alhamdulillah, with His blessing, I have completed this project and the preparation of this dissertation.

I would like to express my gratitude to my supervisory committee chairman, Professor Dr. Yaakob bin Che Man, who constantly motivated me with his knowledge and insight, throughout the course of my research study. I am also grateful to my members of supervisory committee, Associate Professor Dr. Amin Ismail and Dr. Nazimah Sheikh Abdul Hamid for sharing their expertise and experience.

Appreciation also goes to my friends, Nor Hayati Ibrahim, Syahariza Zainul Abidin, Aida Azrina Azmi, Ahmad Nizam Abdullah, Nur Huda Faujan and Wah Wah Aye. I cannot imagine doing this job without the friendship from all of you.

Acknowledgement also goes to Universiti Sains Malaysia for financing my study. I am forever grateful to my late parents, who have taught me the moral values of lives. Your memories always keep me going. To my husband, thank you for being there for me, supporting, understanding and believing in me. Finally, to my son, you are the source of my inspiration.



I certify that an Examination Committee has met on 11th August 2009 to conduct the final examination of Marina Binti Abdul Manaf on her thesis entitled “Characterization, Antioxidant Properties and Authentication of Virgin Coconut Oil” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be rewarded the Doctor of Philosophy

Members of the Thesis Examination Committee were as follows:

Tan Chin Ping, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Abdul Azis Ariffin, PhD

Associate Professor
Universiti Putra Malaysia
Faculty of Food Science and Technology
(Internal Examiner)

Abdulkarim Sabo Mohammed, PhD

Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Casimir C. Akoh, PhD

Professor
University of Georgia, USA
(External Examiner)

BUJANG KIM HUAT, PhD

Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Yaakob bin Che Man, PhD

Professor
Faculty of Food Sciences and Technology
Universiti Putra Malaysia
(Chairman)

Amin bin Ismail, PhD

Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Nazimah binti Sheikh Abdul Hamid, PhD

Senior lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 16 October 2009



DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions

MARINA BINTI ABDUL MANAF

Date: 24 August 2009



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xix
CHAPTER	
1 GENERAL INTRODUCTION	1
2 LITERATURE REVIEW	4
Coconut oil	4
Introduction	6
Composition of coconut oil	10
Extraction of coconut oil	10
Virgin coconut oil	11
Methods of extraction of virgin coconut oil	13
Wet extraction	13
Chilling, freezing and thawing techniques	14
Fermentation technique	18
Enzymatic extraction technique	20
Antioxidant activity	21
Free radicals	21
Measurement of antioxidant activity	23
Free radical scavenging activity	23
β -carotene bleaching method	24
Reducing power	25
Conjugated diene assay	26
Oxygen radical absorbance capacity (ORAC)	26
Ferric reducing ability of plasma (FRAP)	27



Polyphenols	27
Variability of phenolic content of foods	28
Phenolic content in olive oil	29
Authentication of vegetable oils	31
Indices of admixture of vegetable oils with other vegetable oils	31
Fatty acids	31
Triacylglycerol (TAG)	34
Sterol	36
Unsaponifiable fraction of oil	39
Phenolics and alcohol	41
Tocopherols	42
Authentication and adulteration detection: technique-oriented perspective	43
High performance liquid chromatography (HPLC)	44
Gas chromatography	46
Differential scanning calorimetry (DSC)	49
Electronic nose	53
Fourier transform infrared (FTIR) spectroscopy	57

3 CHEMICAL PROPERTIES OF COMMERCIAL VIRGIN COCONUT OIL

Introduction	65
Materials and Methods	67
Materials	67
Chemical analyses	67
Fatty acid analysis	68
Triacylglycerol analysis	68
Total phenolic content	69
Antioxidant activity	69
Statistical analysis	70
Results and discussion	71
Fatty acid composition	71
Triacylglycerol composition	73
Chemical analyses	75
Iodine value	75
Saponification value	77
Peroxide value	78
Anisidine value	79
Free fatty acid	80
Total phenolic content	80
Total antioxidant activity	81
Conclusion	83



4	ANTIOXIDANT CAPACITY AND PHENOLIC ACIDS OF VIRGIN COCONUT OIL	
	Introduction	84
	Materials and Methods	86
	Materials	86
	Chemicals	86
	Sample preparation	87
	Preparation of polyphenol extract	88
	Determination of total phenolic content	88
	Determination of antioxidant capacity	89
	DPPH radical scavenging activity	89
	β -carotene linoleate bleaching activity	89
	Reducing power	91
	Determination of phenolic compound	91
	Statistical analysis	92
	Results and discussion	93
	Total phenolic content	93
	Phenolic acid contents	95
	Antioxidant capacity	98
	DPPH radical scavenging activity	98
	β -carotene linoleate bleaching activity	101
	Reducing power	102
	Correlation of total phenolic content and total phenolic acids to antioxidant capacity	105
	Conclusion	107
5	ANALYSIS OF ADULTERATION OF VIRGIN COCONUT OIL BY PALM KERNEL OLEIN USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY	
	Introduction	108
	Materials and Methods	110
	Samples	110
	Calibration standard	110
	Discrimination analysis	111
	Instrumentation and spectral acquisition	111
	Statistical analysis	112
	Results and discussion	112
	Spectra	112
	PLS calibration and cross validation	114
	Discriminant analysis	120
	Conclusion	125



6 MONITORING ADULTERATION OF VIRGIN COCONUT OIL BY SELECTED VEGETABLE OILS USING DIFFERENTIAL SCANNING CALORIMETRY

Introduction	126
Materials and methods	128
Materials	128
Blend preparation	128
Thermal analysis	129
Fatty acid analysis	129
Iodine value analysis	130
Statistical analysis	130
Results and discussion	131
General	131
Fatty acid analysis	136
Iodine value	141
Thermogram analysis	142
DSC heating thermogram	142
DSC cooling thermogram	149
Conclusion	155

7 USE OF THE SAW SENSOR ELECTRONIC NOSE FOR DETECTION OF RBD PALM KERNEL OLEIN IN VIRGIN COCONUT OIL

Introduction	156
Materials and methods	158
Materials	158
Blend preparation	159
Chemical analysis	159
Fatty acid analysis	159
Electronic nose analysis	160
Statistical analysis	160
Results and Discussion	161
Fatty acid analysis	161
Electronic nose analysis	162
Chemical tests	168
Principal component analysis	170
Conclusion	174



8	GENERAL CONCLUSION AND RECOMMENDATIONS	
	Conclusion	175
	Recommendations	180
	REFERENCES	181
	BIODATA OF STUDENT	202
	LIST OF PUBLICATIONS	203



LIST OF TABLES

Table		Page
1	Coconut oil composition and physical characteristics	9
2	Sterol in some vegetable oils	38
3	Oil content in some fruits and seeds and content of unsaponifiable, phosphatides and squalene in oils	40
4	Frequencies of bands or shoulders of some edible oils and fats in mid infrared spectra, together with the assigned functional group, mode of variation and the intensity	60
5	Fatty acid composition of virgin coconut oil (VCO)	72
6	TAG composition of virgin coconut oil (VCO)	74
7	Chemical composition of virgin coconut oil	76
8	Total phenolic content and total antioxidant activity of virgin coconut oil	82
9	Concentration of phenolic acids in virgin coconut oil (VCO)	96
10	Free radical scavenging activity (EC_{50}) of virgin coconut oil	101
11	Reducing power of virgin coconut oil (VCO) at various concentrations	104
12	Correlation between antioxidant assays and each of the total phenolic content and total phenolic acid	106
13	Fatty acid composition (%) of virgin coconut oil and palm kernel olein	115
14	Fatty acid composition of virgin coconut oil (VCO), palm kernel oil (PKO), soybean oil (SBO) and sunflower oil (SFO)	132
15	Fatty acid composition of virgin coconut oil (VCO) adulterated with different concentration of palm kernel oil (PKO)	137



16	Fatty acid composition of virgin coconut oil (VCO) adulterated with soybean oil (SBO)	139
17	Fatty acid composition of virgin coconut oil (VCO) adulterated with sunflower oil (SFO)	140
18	Iodine value of virgin coconut oil (VCO) adulterated with sunflower oil (SFO), palm kernel oil (PKO) and soybean oil (SBO)	142
19	Fatty acid composition of virgin coconut oil (VCO) adulterated with palm kernel olein	163
20	The electronic nose data of virgin coconut oil adulterated with different percentage of palm kernel olein	166
21	Chemical test values of virgin coconut oil (VCO) blend with palm kernel olein	169
22	Pearson's correlation between chemical tests and adulterant peaks	170



LIST OF FIGURES

Figure		Page
1	Robledano-Luzuriage process	16
2	Krauss-Maffei for coconut oil extraction	17
3	Mean total phenolic content of virgin coconut oil produced from different methods	94
4	Scavenging effect of virgin coconut oil extracts on radical DPPH	100
5	Antioxidant activity (%) of virgin coconut oil using β -carotene bleaching assay	103
6	The typical spectra of virgin coconut oil (----) and palm kernel olein (—)	113
7	FTIR spectra of virgin coconut oil adulterated with palm kernel olein in the order of (A)50%, (B)40%, (C)30%, (D)20%, (E)10%, (F)0%	116
8	(a) FTIR predicted values for adulteration obtained from the PLS model versus the actual concentration of palm kernel olein	117
	(b) Cross validation of the PLS model by removing one standard at a time	118
9	Root mean square error of cross validation (RMSECV) versus PC factor	119
10	Cooman plot for the classification of pure virgin coconut oils and adulterated samples	121
11	The spectral variation between virgin coconut oil samples near wavenumber 1654 cm^{-1}	123
12	Cooman plot of virgin coconut oils and other vegetable oils	124
13	Cooling thermogram of pure samples; A: sunflower oil, B:soybean oil, C: palm kernel oil and D:virgin coconut oil	133



14	Heating thermogram of pure samples; A: virgin coconut oil, B: palm kernel oil, C: sunflower oil, D: soybean oil	135
15	Differential scanning calorimetry (DSC) heating thermogram of virgin coconut oil (VCO) adulterated with sunflower oil (SFO) at various concentration level	143
16	Differential scanning calorimetry (DSC) heating thermogram of virgin coconut oil (VCO) adulterated with soybean oil (SBO) at various concentration level	145
17	Differential scanning calorimetry (DSC) heating thermogram of virgin coconut oil (VCO) adulterated with palm kernel oil (PKO) at various concentration level	147
18	Differential scanning calorimetry (DSC) cooling thermogram of virgin coconut oil (VCO) adulterated with sunflower oil (SFO) at various concentration level	150
19	Differential scanning calorimetry (DSC) cooling thermogram of virgin coconut oil (VCO) adulterated with soybean oil (SBO) at various concentration level	152
20	Differential scanning calorimetry (DSC) cooling thermogram of virgin coconut oil (VCO) adulterated with palm kernel oil (PKO) at various concentration level	153
21	Peak height of major exothermic peak of palm kernel olein (PKO) versus percentage of adulteration	154
22	VaporPrint™ of virgin coconut oil adulterated with different percentage of palm kernel olein	164
23	Compound concentration versus percentage of adulteration for peak (a) E (b) peak H and (c) peak F	167
24	Principial component analysis (PCA) score plot of virgin coconut oil (VCO) adulterated with different level of palm kernel olein	171
25	Principial component analysis (PCA) loading plot of virgin coconut oil adulterated with different level of palm kernel olein	172
26	Partial least square model of actual versus calculated value of palm kernel olein in virgin coconut oil (VCO)	173

