LIPID AND PHYTOCHEMICALS PROFILES OF NON HEAT TREATED VIRGIN COCONUT OIL

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To my beloved husband, ma, ayah, family members and friends, thank you for your constant encouragement, love and support.

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ABSTRACT

The production of virgin coconut oil (VCO) was carried out using three processing methods; the wet, dry and integrated wet process. The highest processing temperature was set at 50ºC in order to preserve the heat labile components in VCO. In this study, the yield, the chemical composition, minor bioactive components, descriptive sensory analysis and lipid profiles were investigated. The VCO yield (%) from the wet, dry and integrated wet process was 19.82±0.46%, 43.28±2.0% and 36.71±1.6% respectively. VCO extracted by integrated wet process exhibit the highest quality of oil in addition to preservation of the minor bioactive compounds; α-tocopherol (40.0±0.11 ppm) and polyphenol (4.34 ± 0.09mg GAE/g oil). Eventhough the yield were slightly lower than dry process, the concentration of the labile compounds was higher. VCO extracted by wet process appeared colorless and exhibited a slight detectable rancidity. On the other hand, VCO extracted by integrated wet process was clear in appearance, sweet in aroma, taste and flavor. The VCO extracted by dry process was slightly yellow in colour and had nutty aroma and flavor. The lipid profiling study was carried out using Ultra Performance Liquid Chromatography- Electrospray Ionization - tandem Mass Spectrometry (UPLC-ESI-MS/MS). The lipid profiles of coconut oil indicated that the neutral lipids accounted for 17.4% of the total lipid formed. Meanwhile, 82.6% was accounted for polar lipids with the value for phospholipids, glycolipid and sphingolipid of 21.5%, 33.0% and 45.4%, respectively. VCO extracted by integrated wet process contain only triacylglycerols (TAG) while the wet and the dry process exhibit diacylglycerides (DAG), monogalactosyl diacylglycerides (MGDG), suloquinorosyl diaclyglycerides (SQDG) and digalactosyl diacylcerides (DGDG). The present study using UPLC-ESI-MS/MS to profile the lipid content of VCO demonstrated that the integrated wet process is better than the wet and dry process in preserving the minor components and TAG.

ABSTRAK

Penghasilan minyak kelapa dara (VCO) telah dijalankan dengan tiga kaedah pemprosesan: proses basah, kering dan integrasi basah. Suhu pemprosesan tertinggi ialah 50°C untuk mengekalkan komponen meruap dalam VCO. Dalam kajian ini, hasil, komposisi kimia, komponen kecil bioaktif, analisis deskriptif deria dan profil lipid dijalankan ke atas produk. Hasil VCO dari proses basah, kering dan integrasi basah adalah masing-masing 19.82±0.46%, 43.28±2.0% dan 36.71±1.6%. Ekstrak VCO dari proses integrasi basah menghasilkan kualiti minyak yang baik dan turut mengekalkan komponen kecil bioaktif, iaitu α-tokoferol (40.0±0.11 ppm) dan polifenol (4.34 ± 0.09mg GAE/g oil). Walaupun hasilnya adalah agak rendah dari proses kering, kepekatan komponen meruapnya lebih tinggi. VCO yang diekstrak melalui proses basah tidak bewarna dan menunjukkan sedikit kesan bau tengik. Sebaliknya, VCO yang diekstrak melalui proses integrasi basah adalah jernih pada permukaan, mempunyai bau, perasa dan perisa yang manis. VCO yang diesktrak dengan proses kering adalah sedikit kekuningan dan mempunyai aroma dan perisa kekacang. Kajian profil lipid dijalankan menggunakan Ultra Performance Liquid Chromatography- Electrospray Ionization - tandem Mass Spectrometry (UPLC-ESI-MS/MS). Profil lipid minyak kelapa menunjukkan lipid neutral mengandungi 17.4% dari keseluruhan lipid yang terhasil. Manakala, 82.6% merupakan lipid polar dengan nilai untuk pospolipid, glikolipid dan spingolipid masing-masing 21.5% dan 33.0% dan 45.4%. VCO yang diekstrak dengan proses integrasi basah menunjukkan kehadiran triasilgliserol (TAG), sementara yang diekstrak melalui proses basah dan kering mengandungi diasilgliserid (DAG), monogalactosil diasilgliserid (MGDG), suloquinorosil diasilgliserid (SQDG) dan digalaktosil diasilserid (DGDG). Kajian menggunakan UPLC-ESI-MS/MS untuk memprofilkan kandungan lipid VCO menunjukkan proses integrasi basah adalah lebih baik berbanding proses basah dan proses kering untuk mengekalkan komponen kecil dan TAG.

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NOMENCLATURES

ANOVA	-	Analysis of Variance
AV	-	Aniside value
BHA	-	Butylated hydroxyanisole
ESI	-	Electron Spray Ionization
FFA	-	Free Fatty Acid
GC-MS	-	Gas Chromatography Mass Spectrometry
HPLC	-	High Performance Liquid Chromatography
IV	-	Iodine Value
KI	-	Potassium Iodine
LC	-	Liquid Chromatography
LC-MS	-	Liquid Chromatography Mass Spectrometry
LC-MS/MS	-	Liquid Chromatography tandem Mass Spectrometry
NA_2CO_3	-	Sodium carbonate
NAOH	-	Sodium hydroxide
$NA_2S_2O_3$	-	Sodium trisulfate
O_2	-	Oxygen
PCA	-	Principal Component Analysis
PV	-	Peroxide value
SEM	-	Standard error method
SIMCA	-	Soft Independent Modelling of Class Analogies
TIC	-	Total Ion Chromatography
UPLC-MS/MS	-	Ultra Performance Liquid Chromatography Mass Spectrometry
UV	-	Ultra violet
UV/Vis	-	Ultra violet/visible
m	-	meter

mg	-	milligram
mL	-	mililiter
mM	-	milimol
mm	-	milimeter
m/z	-	mass to charge ratio
ppm	-	part per million
v/v	-	volume per volume
T	-	tocopherol
T3	-	tocotrienol
µm	-	micrometer
µL	-	microliter
α	-	alpha
ß	-	beta
g	-	gram
°	-	degree
°C	-	degree celcius
%	-	percent
<	-	less than
>	-	more than
≤	-	more than or equal to

CHAPTER 1

INTRODUCTION

1.1 Background of research

The coconut fruit is actually a simple dry fruit. The hard outer part of the coconut fruit is called husk and the inner white fleshy part of the seed is the coconut meat which is edible. The fresh coconut meat can be grated and the milk pressed out could be used for cooking and other types of food preparation.

Coconut oil play a vital role in our daily diet as it is an important physiological functional food. The health and nutritional benefits that can be derived from consuming coconut oil have been recognized in many parts of the world for centuries (Santos *et al.,* 2005). Coconut oil is derived from the seeds of coconut palm, (*Cocos nucifera).* It contains about 84% tri-saturated, 12% di-saturated-mono-saturated and 4% mono-saturated-di-unsaturated triglycerides. 91% of the fatty acids in the coconut's triglycerides or coconut oil are saturated. The major component of the fatty acids in these triglycerides is the medium chain fatty acid such as the lauric acid 45%, myristic acid 19%, capric 10% and caprylic acid 8%. The high content of tri-saturated triglycerides and their saturated fatty acids renders coconut oil to be chemically stable and less prone to free radical induce oxidation and thermal degradation. These high saturation and medium chain fatty acids content of coconut oil contribute to its unique physical, chemical and biological properties.

The current popular form of coconut oil is the virgin coconut oil (VCO). VCO is a type of coconut oil produced using a fresh kernel by mean of a mild processing condition. The chemistry of the triglycerides and fatty acids content of VCO is similar to the commercial coconut oil. The major difference is in the minor component contents which are preserved in the VCO product. The concept of VCO is taken from the well-known virgin olive oil product whereby the non-oil components are retained. The Philippine National Standard (PNS) defines VCO as the oil obtained from the fresh, mature kernel of the coconut by mechanical or natural means with or without the use of heat, without undergoing chemical refining, bleaching, odorizing and does not lead to the alteration of the nature of the oil. PNS also describe VCO as colorless with natural coconut scent and free from rancid flavor and odors. The moisture content of the oil must be at least or less than 0.1%. The odor and taste of oil is largely due to the presence of small quantities, perhaps less than 150ppm of δ- and γ-lactones. In addition, the mild processing preserves the content of the minor components like provitamin A, vitamin E (α and γ tocopherol), phytosterols and polyphenols. These minor components are believed to contribute to the extra nutritional benefit of the coconut oil.

1.2 Problem Statement

The main issue need to be addressed is how to retain the minor components of virgin coconut oil and what are the parameters of the process that really contribute in retaining the minor component in VCO.

As for the lipid profiling, Gas Chromatography Mass Spectrometry (GC-MS) is widely used for lipidomic studies and provides efficient and reproducible analysis for lipidomic studies. However, it requires sample derivation to create volatile compound that can be separated on the Gas Chromatography (GC) column. Thus, this research presents the alternative method in applying Ultra Performance Liquid Chromatography tandem mass spectrometry (UPLC/MS/MS) for lipidomics studies. It uses columns with smaller particle sizes, has improved resolution and it also

allows a more rapid analysis without loss of resolution (Bruce *et al.*, 2008). This quantitative technique is chosen as rapid and effective method to get the high sensitivity and specificity of the compound detection in the virgin coconut oil extract.

1.3 Objective of the study

Based on the research background and the problem statement, the objectives of this research are:

1. To establish the effect of the extraction process on the virgin coconut oil yield
2. To establish their lipid profiles of non- heat treated virgin coconut oil.

1.4 Scope of the study

This research consists of the following scopes in achieving the objectives above:

1. To evaluate the effect of the different extraction process on the extraction efficiency, yield and sensory quality of non heat treated virgin coconut oil
2. To identify the lipid profiles in non heat treated virgin coconut oil using UPLC-ESI-MS/MS.

1.5 Significance of the study

Coconut oil has been generally labeled as bad fat alongside with beef fat and lard because of its saturated fat property. But, in contrast with these animal fats, coconut oil contains unique lipid profile with proven health benefit. The health benefits of coconut oil include antifungal, antimicrobial and antioxidant properties.

This study will enable a better understanding regarding the lipid profile of virgin coconut oil as well as the better extraction method that retains the minor components.

The main aim of this study is to investigate the use of UPLC-ESI-MS/MS for its ability to produce information rich and informative mass spectra from virgin coconut oil.

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