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Coconut (*Cocos nucifera* L.) Lipids: Extraction and Characterization

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ABSTRACT

Lipids extraction from dried coconut meat is conducted by maceration using **chloroform/methanol (2:1,v/v)** and partition extraction with n-hexane/87% ethanol (3:1), and column chromatography. Lipids are analyzed using FTIR, GC-MS, and TLC. Results reveal that dried coconut meat has 0.04% phospholipids, 0.22% of neutral lipids, and 0.04% of glycolipids and ceramides. Coconut phospholipids consist of phosphatidylcholine (PC), phosphatidylserine (PS), and/or phosphatidylethanolamine (PE) classes for the hydrophilic parts with dodecanoic acid (C12: 0), **hexadecanoic acid (C16:0)** and octadecanoic acid (C18: 0) as the lipophilic parts.

Keywords: Coconut, Phospholipids, Dodecanoic acid, Hexadecanoic acid, Octadecanoic acid.

INTRODUCTION

Phospholipids (PLs) are amphiphilic lipids obtained in cell membranes of all organisms, organized as lipid bilayers. The PLs discovered are mainly glycerophospholipids (GPLs), which are made of fatty acid residues (FAs) attached to a glycerol backbone by ester formation, a phosphate group and a hydrophilic head group (e.g. choline, serine or ethanolamine). Widespread supplies of industrially cultivated PLs are eggs (chicken and fish), bovine milk, soya, rapeseed, sunflower, coconut, sesame¹ and jack bean². Each source has

a unique composition of distinct phospholipid species and accordingly diverse applications in pharmaceuticals³, cosmetics⁴, nutrition, food⁵, and drug delivery^{6,7}.

In aqueous media, PLs molecules form aggregate structures by self-assembly course of action^{8,9}. Spherical bilayers known as vesicles or liposomes are among of these structures^{10,11}. Nowadays liposomes^{7,12-14} and related structures^{15,16} have been actively developed as carriers of bioactive compounds^{13,14,17-19} in various applications^{13,20} since their excellent biocompatibility to natural body environment^{6,12,21}.



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The characteristic of liposomes is greatly influenced by the physicochemical properties of PLs constituent, such as their molecular species²²⁻²⁵. PLs molecular species are determined by their head group (hydrophilic) and hydrocarbon chain types (hydrophobic). These components are diversified base upon their sources³. PLs acquired from various natural sources mostly have unique head groups and acyl chains compositions. A judicious stage in lipidomic study is lipid extraction with a suitable organic solvent combination (solvent system) preceding to MS analysis. The accomplishment of the lipid extraction by a certain solvent modification depends upon the partitioning of the lipids into the organic phase and therefore on the lipid composition^{2,26,27}.

Previously Hudiyaniti *et al.*,²⁵ obtained that crude coconut PLs contain sephaline groups whilst the lipophilic parts comprise of dodecanoic acid (C12:0) and octanoic acid (C8:0). In this study we take further steps by purifying these crude PLs with column vacuum chromatography using several eluents to learn more information about the chemistry of coconut PLs. Our new finding is that crude coconut PLs also contained phosphatidylcholine groups and the lipophilic components were hexadecanoic acid (C16:0) and 9-octadecenoic acid (C18:1) as well. Further details of this new finding is presented in the following discussions.

MATERIALS AND METHODS

Materials

Dried coconut meat, filter paper, silica gel G60 for column chromatography, chloroform, acetone, methanol, aquabidest, NaCl, the solvent for partition was prepared by partitioning ethanol 87% with n-hexane (1: 1, v/v) and the top layer was mark solvent A while the bottom layer was solvent B, TLC plat of silica gel GF254 was washed with chloroform/methanol (1:1 v/v), then impregnate with 2.3% boric acid in absolute ethanol prior used. Spot regents for TLC were dragendorff, ninhidrin, and primulin reagents.

Extraction

Polar lipids from coconut meat was isolated by method previously presented by Hudiyaniti *et al.*, (25). Briefly, this was prepared by the following

procedure. Dried coconut meat powder was macerated with chloroform / methanol (2:1) for 7 days. The filtrate was washed with a 0.9% of NaCl solution. Chloroform layer was evaporated to acquired coconut lipid extract. After that 10 g of coconut lipid extract was dissolved in 45 ml of solvent A and added to 15 ml solvent B in a separating funnel I. The mixture was shaken for 2 minutes. The bottom layer was transferred into a second separating funnel containing 45 ml solvent A and shaken for 2 minutes. The bottom layer containing polar lipids was collected in a vial for further purification while the upper layer was also kept in a separated vial. Fresh 15 ml solvent B was added to the remaining mixture in the first separating funnel and shaken for about 2 minutes. Then the bottom layer is transferred into the second separating funnel containing of fresh 45 ml solvent A. The mixture then shaken and separated as above. The bottom layer and the upper layer were accumulated into the previous separate containers. The procedure was repeated 4-6 times for each 10 g of coconut lipid extract. The polar lipids containing PLs were purified by column vacuum chromatography as described below.

Purification

A column containing 50 g of silica gel G60 was prepared. A solution of the polar lipid/silica gel (1:1, w/w) was carefully placed on the silica gel column. For first elution we used chloroform to elute the remaining neutral lipids. Ten ml eluent was employed for each 10 mg of sample. Second eluent was acetone/methanol (9:1, v/v) to elute glycolipids and ceramides (with ratio: 5 ml eluent for 10 mg of sample) and followed by methanol to elute PLs. The coconut lipids was analyzed using FTIR, GCMS, and thin layer chromatography.

RESULTS AND DISCUSSIONS

Extraction of lipids from dried coconut meat resulted in crude lipids extract, 49.03% of dried coconut meat. Furthermore the crude lipids extract contained 0.22% of neutral lipids, 0.04% of glycolipids and ceramides, and 0.04% of PLs.

The FTIR spectra of coconut crude lipids extract, neutral lipids, glycolipids and ceramides and PLs are presented in Fig. 1. The major absorption

(-CH₂CH₂N⁺H₃) groups. These results suggest phosphatidylcholine (PC), phosphatidylserine (PS), that coconut PLs contain lipid from and/or phosphatidylethanolamine (PE) classes.

Table 1: Acyl chain components in coconut lipids

Acyl chains	Area (%)			
	Crude lipids	Neutral lipids	Glycolipids & ceramides	PLs
1 C6:0 (hexanoic acid)	0.45	-	-	-
C8:0 (octanoic acid)	6.70	17.92	3.93	-
C10:0 (decanoic acid)	6.10	5.45	0.81	-
C12:0 (dodecanoic acid)	45.85	48.13	68.15	90.45
C14:0 (tetradecanoic acid)	19.61	2.13	-	-
C16:0 (hexadecanoic acid)	10.32	1.16	0.61	0.37
C18:0 (octadecanoic acid)	3.34	10.79	0.91	1.58
C18:1 (9-Octadecenoic acid)	6.34	2.01	-	-
C18:2 (9,12-octadecadienoic acid)	1.29	-	-	-



Fig. 2. TLC profile of coconut PLs under UV lamp λ=365 nm

CONCLUSION

Dried coconut meat contain 0.04% phospholipids, 0.22% of neutral lipids, and 0.04% of glycolipids and ceramides. Coconut PLs comprise of phosphatidylcholine (PC), phosphatidylserine (PS), and/or phosphatidylethanolamine (PE) classes with dodecanoic acid (C12: 0),



a. b.

Fig. 3. TLC Profile of coconut PLs (a). Dragendorff stain (b). Ninhydrin stain

octadecanoic acid (C18: 0) and hexadecanoic acid (C16:0) as the lipophilic parts.

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