Phospholipids of Palm Oil (*Elaeis guineensis*)

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ABSTRACT

Mesocarp oil of *Elaeis guineensis* provides 1000-2000 ppm of phospholipids. Thin layer chromatography revealed that the major components are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylglycerol (PG). Minor components are phosphatidic acid (PA), diphosphatidylglycerol (DPG) and lysophosphatidylethanolamine (LPE), and traces of lysophosphatidylcholine (LPC) and phosphatidylserine (PS) are detectable. An artifact from enzymatic transphosphatidylation in methanolic solvents was isolated and characterized as phosphatidylmethanol (PM). Phospholipids are only present at low levels (20-80 ppm) in commercial crude palm oil and they usually account for a minor part of the total elemental phosphorus of the oil. It is desirable to have low levels of phospholipids in the oil to obtain better oxidative stability and bleaching properties.

INTRODUCTION

Palm oil derived from the mesocarp of *Elaeis guineensis* has become a major source of edible oil. Although the composition of the glycolipids of palm oil has recently been reported (1), little is known of the phospholipids. In Malasian palm oil, the phospholipid level has been arbitarily determined by total phosphorus determination in the oil with using the "phospholipid equivalent" conversion factor. Thus, the phospholipid levels have been reported as ranging from "very small quantity" (2) to as high as 1,000 ppm (3). In a preliminary investigation, Tan (4) reported that substantial amounts of phospholipids, which are not normally found in commercial palm oil, can be solvent-extracted from the palm oil fruit mesocarp; an "anomalous" phospholipid component (found to be an artifact) was also observed.

In recent years, attention has been drawn to the undesirable characteristics of phospholipids. It has been reported (5) that the presence of excessive amounts of phospholipids in palm oil can result in some refinery problems such as loss of oil and loss of bleaching power of the bleaching earth. In a recent study of the oxidative stability of crude palm oil, phospholipids have been implicated as one of the causes of oxidation of the oil (6). Similar problems have been found earlier for soybean oil (7-11), although antioxidant and other synergistic properties of phospholipids in other oils and fats have also been reported (12-15). This paper reports a study of the nature of the phospholipids in palm oil and discusses their possible effects on the stability and quality of the oil.

MATERIALS AND METHODS

Materials

Fresh fruits (Dura and Tenera varieties) were obtained from the Palm Oil Research Institute of Malaysia (PORIM) research station, Serdang, Malaysia. Crude palm oil, crude olein, crude stearin, bleached olein, refined, bleached and deodorized olein, neutralized, bleached and deodorized stearin, and spent bleaching earth were commercial samples obtained from Keck Seng (M) Bhd., Masai Palm Oil Refinery, Johore, Malaysia, and Lam Soon Oil and Soap Mfg., Sdn. Bhd., Petaling Jaya, Malaysia. Sludge water and palm fruit fiber waste were obtained from Guthrie Palm Oil Mill, Chemara Research Station, Seremban, Malaysia. Thin layer chromatographic (TLC) plates were purchased from Merck (Darmstadt, Germany). Florisil and commercial phospholipids were from Sigma (St. Louis, MO). Other chemicals were of analytical or reagent grade, supplied by Merck, Sigma, May & Baker (Dagenham, England), Hopkin & William (Essex, England), AJAX Chemicals (Sydney, Australia), and British Drug House Chemicals (Poole, England). Solvents were redistilled before used.

Proton (100 MHz) and carbon-13 (25 MHz) nuclear magnetic resonance (NMR) spectra were recorded on a Jeol JNM-FX100 Fourier Transform NMR spectrometer. Absorbances were taken from a Varian Superscan 3 UV-VIS spectrometer. Gas chromatography (GC) was performed on a Varian Aerograph series 1800 equipped with an FID detector. Centrifugation was done on an MSE swing-out centrifuge, made in London, England.

Extraction of Mesocarp Oil

Fresh fruits (500 g) were sterilized in a boiling-water bath for .5 hr. The mesocarp was peeled off and blended with 500 mL chloroform/methanol (1:2, v/v) mixture. The blended mesocarp was transferred into a large conical flask; a further 1 L of the mixed solvent was added and the mixture was left for 24 hr. The extracts were filtered and the residue was reextracted once with another liter of the same solvent mixture. The combined extracts were washed twice with 0.2 vol of 0.6% NaCl solution. The chloroform layer was then separated and concentrated by rotary evaporation. The oil obtained was pumped free of solvents by a vacuum pump. The yield was 31% based on the fresh weight of mesocarp (water content of the fresh mesocarp was ca. 40% by weight).

Column Chromatography for Polar Lipids

Separation of lipids was by a column packed with acid-treated Florisil in chloroform solution following the method of Carroll (16-18). The eluting solvents were chloroform, acetone, methanol and methanol/acetic acid (95:5, v/v), respectively. Phospholipids were in the methanolic fractions and were analyzed as described later.

Qualitative Analysis on TLC

TLC of phospholipids was performed on a 20 × 20 cm Silica Gel G plates of 0.25 mm thickness. The solvent system used was chloroform/methanol/25% aq ammonia (65:35:4, v/v/v) in the first direction and chloroform/methanol/acetic acid/water (170:25:25:6, v/v/v/v) in the second direction. The spots were detected by staining with specific spray reagents (given next) and identified by comparing with R_f values reported in the literature (19-21), then further confirmed by cochromatography with authentic phospholipid standards. The TLC spray reagents used were: Zinzade's Reagent, modified from that described by Kates (22) into a spray solution; sodium molybdate dihydrate (6.85 g) and hydraxine sulfate (400 mg), dissolved in 100 mL of water with conc sulfuric acid (250 mL) added. After cooling, the mixture was diluted with 300 mL of water. Phospholipids sprayed with this reagent appeared as deep blue spots in a white background; ninhydrin spray reagent was purchased from Sigma and molybdatophosphoric acid was from Merck.

Phosphatidylmethanol

Phosphatidylmethanol was isolated from chloroform/methanol extracts of unsterilized fruit mesocarp by column chromatography on acid-treated Florisil as already described. Phosphatidylmethanol eluted before the other phospholipids. Dimethylphospholipid was obtained by treatment of diazomethane on phosphatidylmethanol as described by Renkonen (23) and purified by column chromatography on acid-treated Forisil.

Extraction of Oil from Other Sources

Spent bleaching earth (10 g) was extracted by 30 mL chloroform/methanol (1:2, v/v) at room temperature with vigorous stirring. The extract was filtered, then the solvent was rotary-evaporated and pumped off by a vacuum pump to give 2.4 g of oil.

Palm fruit fiber waste (75 g) was extracted with 400 mL chloroform/methanol (1:2, v/v) at room temperature. After filtration of the fiber, the filtrate was rotary-evaporated and dried by a vacuum pump to yield 4.6 g of oil.

Sludge water (500 mL) was extracted once with 100 mL chloroform at room temperature. The lower layer was separated, then the solvent was removed by rotary-evaporation and pumped dry to give 0.7 g of oil.

Centrifugation of Crude Palm Oil

Crude palm oil was centrifuged at 15,000 rpm at 30 C for 30 min. The supernatant oil was decanted and the gummy residue was collected. The residue thus accumulated was dried in a vacuum desiccator for about 48 hr. The yield of the residue was 1.1 g/kg of crude palm oil. Later batches of crude palm oil (150 g) were centrifuged in admixture with 27 mL petroleum ether (60-80 C) at 2,000 rpm and 40 C for 25 min.

Extraction of Phospholipids from Palm Oil

Palm oil (5 g) was vigorously stirred magnetically with boiling methanol for 20 min. A second extraction only provided a further 1% more phospholipids. The methanol fraction was concentrated and the phospholipids were analyzed semiquantitatively by TLC or converted to orthophosphate to be analyzed as molybdenum blue according to modified procedure of AOCS official methods (24) (see next section).

Quantitative Analysis of Phosphorus and Phospholipids

Total phospholipids were analyzed semiquantitatively on 1-dimensional TLC using 5 × 10 cm Silica Gel G plates of 0.25 mm thickness and chloroform/methanol/acetic acid/water (170:25:25:6, v/v/v/v) as solvent. The color intensities and spot sizes were matched with the known quantities of phospholipid standards chromatographed on the same plate.

Total phosphate in the palm oil and that in the isolated phospholipid fractions were analyzed by a modified procedure of AOCS official methods (24). Palm oil (6 g) and zinc oxide (1 g) in a crucible was completely charred on an electric hot plate and dry-ashed in a muffle furnace at 550-600 C for 2 hr. After cooling, water (5 mL) and concentrated hydrochloric acid (5 mL) were added, and the mixture was heated to gentle boiling for 10 min. The mixture was filtered and washed thoroughly with about 25 mL of hot water into a 100 mL volumetric flask. After cooling, the filtrate was neutralized with 50% aq potassium hydroxide (indicated by the formation of zinc hydroxide precipitate, just redissolved by adding conc hydrochloric acid) and 2 additional drops of the acid was added. Distilled water was then added to the 100-mL mark. To a 50-mL portion (or an equivalent volume containing about 20 μg P), in a 100-mL volumetric flask, 0.015% hydrazine sulfate (8 mL) and sodium molybdate (2 mL) solutions (prepared according to AOCS methods [24]) were added, in that order. The mixture was heated on a vigorously boiling water bath for 10 min. After cooling to room temperature with running water, the mixture was diluted to 100 mL with distilled water. Absorbance was taken immediately against sample blank at 820 nm. Phosphorus levels were read from a calibration curve that had been determined earlier.

Fatty Acid Composition of Palm Oil Phospholipids

The phospholipid fractions (~100 μg) were transesterified into methyl esters (25). The methyl esters were then analyzed by GC using a 10-ft 10% SP2330 on Chromosorb W column.

RESULTS AND DISCUSSION

Palm mesocarp oil was solvent-extracted from the mesocarp of ripe palm oil fruits after sterilization and the phospholipids were isolated by column chromatography over acid-treated Florisil. Two-dimensional TLC revealed the presence of 9 phospholipid components; the major components were phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylglycerol (PG). Minor components included diphosphatidylglycerol (DPG), phosphatidic acid (PA) and lysophosphatidylethanolamine (LPE), and traces of phosphatidylserine (PS) and lysophosphatidylcholine (LPC) also were detected. The results are summarized in Table I.

In earlier experiments, Tan (4) had observed an anomalous component which constituted up to 45% of the total phospholipids from solvent-extracted mesocarp oil. This component is now found to be the artifact phosphatidylmethanol (PM) which arose from enzymatic transphosphatidylation of natural phospholipids during extention of unsterilized fruits by methanolic solvents. This component is

TABLE I

Phospholipids of Palm Oil

Phospholipid[a]	Mole %	R_f	
		S_1^b	S_2^b
PC	36	25	43
PE	24	35	73
PI	22	12	19
PG	9	35	55
DPG	4	53	90
PA	3	7	81
LPE	2	25	25
PS	tr[c]	20	25
LPC	tr	10	5
PM	0[d]	60	87

[a]PC = phosphatidylcholine, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PG = phosphatidylglycerol, DPG = diphosphatidylglycerol, PA = phosphatidic acid, LPE = lysophosphatidylethanolamine, PS = phosphatidylserine, LPC = lysophosphatidylcholine, PM = phosphatidylmethanol.

[b]TLC on Silica Gel G. Solvent system S_1: chloroform/methanol/28% aq ammonia 65:30:4, v/v/v. S_2: chloroform/methanol/acetic acid/water 170:25:25:6, v/v/v/v.

[c]Trace.

[d]Oil used for analysis was extracted from sterilized mesocarp where PM is not formed. Methanolic solvent extractions of unsterilized mesocarp yield variable amounts of PM, depending on the length of extraction, temperature and solvent composition. Using chloroform/methanol (1:2, v/v) to extract the mesocarp as high as 45% of PM was found in the phospholipid fraction.

absent in commercial crude palm oil and is not formed if the fruits are sterilized at or above 100 C prior to solvent extraction. It was found that boiling methanol/chloroform mixtures was ineffective in destroying the phospholipase activity. Damaged fruits samples also did not show enhanced phospholipase activity as PA was not found to be excessively high. PM was isolated and characterized by proton and ^{13}C NMR spectra. The methoxy protons appeared as a doublet at 3.6 ppm (J_{PH} 10.7 Hz) and the methoxy carbon was observed at 53 ppm. Upon diazomethane treatment, PM was converted to dimethylphospholipid (PMM) which showed 2 pairs of sharp doublets at δ 3.70 and 3.71 (J_{PH} 11.1 Hz) in the proton NMR. This agrees with the observations of Renkonen (23), but the better resolved spectrum shows the magnetically nonequivalent methoxy proton doublets separated by 0.8 Hz. The carbon spectrum also revealed a methoxy doublet at 54 ppm (J_{PC} 6.1 Hz).

The fatty acid composition of the phospholipid fraction is given in Table II and is shown to be more unsaturated with a higher linoleic content compared to the triglycerides. Relatively higher unsaturation in phospho- and glycolipids is not uncommon in plant lipids (27). The determination of the fatty acid composition and phospholipid distribution enabled the computation of the "phospholipid equivalent factor," which is 24. This factor converts the elemental phosphorus content of the oil to phospholipid, assuming that all the analyzed phosphorus is derived from phospholipids. The factors recommended at present for use with palm oil and soybean oil are 25 and 30, respectively (24, 28). The amounts of phospholipids in various grades of Malaysian palm oil were further examined and the results are given in Table III. As shown in the table, commercial grades of palm oil actually contain relatively small amounts of phospholipids and the "phospholipid equivalent" obtained from the analysis of phosphorus, in fact, gave exaggerated high values of phospholipids because nonphospholipid phosphorus, principally inorganic phosphates, accounts for most of the analyzed phosphorus. The low levels of phospholipids in palm oil is understandable because, unlike seed oils which are solvent extracted, palm oil is physically extracted and is separated from aqueous "sludge" during milling. Refining removes considerable amounts of phospholipids through alkali washing or phosphoric acid degumming and bleaching earth finally eliminates most of them. An examination of Table IV shows

TABLE II

Fatty Acid Distribution in Glycolipids, Phospholipids and Glycerides in Palm Oil

Fatty acid	Phospholipids	Glycerides[a]	Glycolipids[b]
14:0	–	1.0-1.5	2
16:0	38.4	41.8-45.0	36
16:1	–	–	tr[c]
18:0	1.2	3.7-5.1	9
18:1	37.4	38.6-40.2	27
18:2	32.2	10.2-11.9	15
18:3	–	–	11

[a]Ref. 26.
[b]Refs. 1,4.
[c]Trace.

that, in the mill, most of the phospholipids of palm fruit mesocarp remain in the sludge water and the fiber waste. In sludge water, phospholipids are directly responsible for oil loss due to micellar and emulsifying properties. Fiber presumably contains membrane material with considerable phospholipid content.

In crude palm oil, in which the major part of the phospholipids is not particularly soluble, most of the phospholipids are expected to remain in reverse micelles, vesicles or emulsion droplets. During long storage, some phospholipid gums precipitate together with suspended insoluble material. Similar precipitation was done by centrifugation in the laboratory and the oily gums obtained were found to contain as much as 1.4% of phospholipids together with relatively high amounts of iron and copper, which are undesirable prooxidants in palm oil (Table III). The iron and copper contents can be attributed to the sequestering action of phospholipids. Micellar action would also cause water-soluble metal ions to be associated with phospholipids whereas hydrated insoluble metal salts could possibly be dispersed by phospholipids in a similar action. Stearin, which is the solid (precipitated) fraction of palm oil, contains larger amounts of phospholipids than the liquid olein fraction (Table III), presumably as a consequence of co-precipitation of phospholipid vesicles with particulate matter. Removal of phospholipid gums by phosphoric acid treatment also leads to the removal of substantial quantities

TABLE III

Phospholipid Levels in Various Grades of Palm Oil

	Phospholipids (phosphorus)[a] (ppm)		Total phosphorus (ppm)
Solvent-extracted palm oil	1000-2000	(42-83)	85.2
Crude palm oil	20-80[b]	(0.8-3.3)	20
Crude olein	7	(0.3)	5.2
Crude stearin	33	(1.38)	10.7
Bleached olein	3	(0.13)	1.00
Refined, bleached & deodorized olein	6	(0.25)	1.05
Neutralized, bleached & deodorized stearin	ND[c]		ND
Recovered oil from spent bleaching earth	10,000	(416.7)	
Centrifuged gummy residue[d]	14,000	(483.3)	6500

[a]Factor 24 was used for conversion of phospholipids to phosphorus, which is shown in the parentheses.
[b]The range of values obtained from 4 samples.
[c]Not detectable.
[d]Residue from centrifugation of crude palm oil; Fe and Cu levels are 5800 and 32 ppm, respectively; crude palm oil normally contains 5 ppm Fe and 0.1 ppm Cu.

TABLE IV

Phospholipids in Palm Oil Milling

	Weight %[a] (tonnes)	Phospholipids (ppm)	Total phospholipids, kg (%)
Crude palm oil	22	80[b]	1.8 (4)
Oil recovered from sludge water	0.67	40,000	26.8 (55)
Oil recovered from fiber waste	0.54	37,000	20.0 (41)
(Total extractable oil[c])	(23.2)	(2,100)	(48.6 [100])
Solvent-extracted oil[d]		2,000	

[a]Based on 100 metric tonnes of fresh fruit bunches.
[b]Values of 20-80 ppm have been analyzed.
[c]Sum of the above values, assuming no other oil losses.
[d]Mesocarp oil extracted by chloroform/methanol, 1:2, v/v.

of iron and copper with a resultant increase of oxidative stability of the oil (6). A relationship in the phosphorus content and the oxidative stability of palm oil therefore seems to exist, not unlike that observed previously for soybean oil (7). Bleaching ability of palm oil was also observed to be significantly improved with phosphoric acid degumming (6).

It is notable that, apart from our discussion of the direct and indirect roles of phospholipids in palm oil, other interesting observations have been documented (7-11,29,30). For example, the antioxidant activity of phospholipids has been taken for granted in many studies involving other lipids (12-14). In palm oil obtained by the present wet milling process, such a beneficial effect would be less certain. Also, it has been reported (31) that it is necessary to reduce (but not totally eliminate) the water content of palm oil to maximize storage quality. This is perhaps an indirect effect of the action of phospholipids which is not adequately understood. Central to these and other problems is the understanding of the multifaceted properties of phospholipids (32,33) which could differ widely among the different component phospholipids themselves. Although these problems will be subject to further study, for practical purposes, it seems that removal of phospholipids would help to enhance stability of the oil.

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