Phospholipids from Palm-Pressed Fiber

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ABSTRACT: Palm-pressed fiber, a by-product of palm oil milling, was extracted successively with hexane and 95% ethanol; the ethanol extracts yielded 46,800 ppm of phospholipids. The phospholipid composition, as analyzed by HPLC coupled with an ELSD, was found to be predominantly PC, PE, phosphatidylglycerol, and PA; as expected, the FA were more unsaturated than the TAG. Palm-pressed fiber is estimated to be able to provide 21,645 tonnes of palm lecithin based on the present total world production of fresh fruit bunches and thus be an alternative source of lecithin, which is normally obtained from soybeans.

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Phospholipids are important structural and functional components of cell membranes. The chemical composition of the various phospholipid classes determines physical properties, which can affect the biological function of membranes (1).

Phospholipids, commercially referred to as lecithin, are common emulsifiers in the food, feed, and pharmaceutical industries. They also have been used as release agents, through their role as surfactants. Phospholipids play a part in a wide range of human metabolic processes as well: fat absorption, cholesterol metabolism, nerve function, biosynthesis of prostaglandins, and others. Over-the-counter lecithin preparations are widely available in the form of capsules or granules and usually contain less than 35% PC. Phospholipids are also used to produce liposomes, which carry moisturizers, vitamins, and fragrances. Liposomes also can be used to deliver drugs directly to targeted sites in the human body and lessen the negative effects associated with drugs by encapsulating the toxic drug until it is delivered to the site of infection.

Lecithins are produced commercially from soybeans and egg yolks, which contain 1.1–3.2 and 10 wt% (2) of lecithins, respectively. Crude lecithin has both weak water-in-oil (w/o) and oil-in-water (o/w) emulsifying properties owing to the constituent phospholipids. To improve properties for specific product applications, crude lecithins are modified, refined, and/or fractionated (3).

Malaysian crude palm oil contains relatively low levels of phospholipids (5–130 ppm), as the wet-milling process reduces the original phospholipid content. Large amounts of phospholipids still remain in palm-pressed fiber and sludge (4). The increased production of oil palm also resulted in increased production of by-products as well as waste. Because phospholipids are high value-added products (commercial lecithins are sold at 2–10 times the price of soybean oil), it is prudent to conduct an in-depth study on the recovery of phospholipids from palm-based products, particularly the palm-pressed fiber, a by-product of palm oil milling.

The present study analyzed phospholipids of palm-pressed fiber extracted with hexane and 95% ethanol. The detailed composition analysis was carried out by HPLC, which had not been done before. FA composition was determined by GC.

MATERIALS AND METHODS

Materials. Palm-pressed fiber was obtained from the MPOB Research Plantation. Florisil and standard compounds (phospholipids and FA) were purchased from Sigma Chemical Company (St. Louis, MO). TLC plates were purchased from Merck (Darmstadt, Germany). All solvents and other chemicals were of HPLC or analytical grade, and were supplied by Merck, Sigma, J.T.Baker (Fairlawn, NJ), or Ajax Chemicals (Sydney, Australia).

Visible absorption spectra were recorded on a Hitachi U-2000 spectrometer in 10-mm cells. A vortex shaker was used for mixing or shaking when required. The HPLC system comprised a Waters 996 model (Waters, Milford, MA) with a 100-μL loop injector, an ELSD (Model 1000; Polymer Laboratories, Shropshire, United Kingdom), a ternary solvent system, and a Millennium Workstation 2000 (Waters). The nebulized air pressure was set at 30 psi, and the gas flow rate was 1.5 SLM. The column used was a 5-μm LiChrosorb (25 cm × 4.6 mm i.d.; Merck).

Methods. Fresh palm-pressed fiber collected from a palm oil mill was stored at 0°C. The fiber was dried in an oven at 60°C, and 300 g of the dried fiber was extracted successively with 3.5 L of hexane and 3.5 L of 95% ethanol, providing hexane (FOHS) and ethanol (FOES) extracts, respectively. Chromatographic purification of the oil extracts was carried out with acid-treated Florisil, prepared according to the method of Carroll with slight modifications (5). Column chromatography was carried out with the prepared Florisil using chloroform, acetone, and methanol as solvents. Acid-treated Florisil mixed in chloroform was packed into the open column. Phospholipids eluted in the methanolic fractions.

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2D-TLC of the phospholipid fraction was performed on a 20 × 20 cm silica gel G plate of 0.25-mm thickness. The solvent system used was chloroform/methanol/25% aqueous ammonia (65:30:4, by vol) in the first direction and chloroform/methanol/acetic acid/water (170:25:25:6, by vol) in the second direction. Phospholipids were detected by modified Zinzade's reagent (6) as blue spots and identified by comparing with the R_f values reported in the literature (4,7) and further confirmed by authentic standards.

Total phospholipids were determined according to Goh *et al.* (8). Modified Zinzade's reagent (4 mL) was added to a sample of the purified phospholipid fraction and shaken for 30 min. Then 5 mL of hexane was added to the contents and shaken for another 1 min to extract the molybdenum-blue complexes formed. A visible spectrum was recorded, and the absorbance was read at 711 nm, the λ_{max} for crude palm oil phospholipids. As a sample reagent blank was found to be negligible, hexane was used directly.

The HPLC analysis was carried out according to Juaneda *et al.* (9) with slight modifications. The solvents used were A, hexane; B, 2-propanol/chloroform (4:1 vol/vol); and C, 2-propanol/water (1:1 vol/vol). The flow rate was 0.9 mL/min; the gradient solvent system is presented in Table 1. The column was re-equilibrated for 10 min before subsequent injections. Solutions of known concentrations of each phospholipid standard were analyzed by HPLC.

FA (as methyl esters) of phospholipids were analyzed by GC (PerkinElmer Autosystem XL with an FID) using a 5 μm, 4.6 mm i.d. × 250 mm length column 10% SP2330 (Merck). Samples of phospholipids (*ca.*100 μg) were transesterified into methyl esters by reaction with 1 M sodium methoxide (1 mL) over 20 min. The methyl esters formed were extracted by hexane (0.5 mL) and used for GC analyses.

RESULTS AND DISCUSSION

Palm-pressed fiber oil was first purified by open-column chromatography over acid-treated Florisil. The isolated phospholipid fractions were then subjected to several analyses. The present study provides a means of obtaining a concentrate of phospholipids based on their different solubilities in hexane and ethanol. As much as 46,800 ppm of phospholipids was found in the ethanolic extract (FOES), whereas only 1,367 ppm of phospholipids and mostly neutral lipids was present in the hexane extracts (FOHS) as shown in Table 2.

TABLE 1
HPLC Solvent System for Phospholipid Analysis[a]

Time (min)[b]	A (%)	B (%)	C (%)
0	42	52	6
25	32	52	16
65	32	52	16
65.1	42	52	6

[a]A, hexane; B, isopropanol/chloroform (4:1, vol/vol); C, isopropanol/water (1:1, vol/vol).
[b]Time elapsed since injection.

TABLE 2
Phospholipid Content in FOHS and FOES

Method	Component	FOHS (ppm)	FOES (ppm)
HPLC-ELSD[a]	PC	763	20,886
	PE	456	12,482
	PG	147	6,077
	PA	ND	7,355
	Total	1,367	46,800
UV[b]		1,233	44,205
2D-TLC[c]		PC, PE, PG	PC, PE, PG, PA

[a]Concentration of phospholipids determined by HPLC-ELSD.
[b]Total phospholipid content determined by a UV spectrophotometer.
[c]Component determined by 2D-TLC and visualized with Zinzade's reagent.
ND, not detected; PG, phosphatidylglycerol; FOHS, phospholipids in hexane extracts; FOES, phospholipids in the ethanolic extract.

2D-TLC revealed the similarity of components in FOHS and FOES, e.g., PC, PE, and phosphatidylglycerol (Fig. 1), that were also found in crude palm oil (4). PI, another major component of phospholipids previously found in crude palm oil, was not observed in the present samples. The identities of the various phopholipids were further confirmed by HPLC as shown in Figure 2. The major phospholipids of FOES were somewhat similar to those of soybean, which consists of 35–46 wt% PC and 25–27 wt% PE, whereas FOHS contains a higher amount of PC. Egg yolk, which is another source of commercial lecithin, contains more PC (10). A noticeable amount of PA in FOES is likely due to hydrolysis during palm oil milling or to prolonged heating during the removal of solvents by rotary evaporation.

The results of quantitative phospholipid analyses by HPLC using ELSD and UV detection are shown in Table 2. UV-vis spectrophotometry gave lower total phospholipid levels than HPLC determinations. Since the absorbance of the molybdenum-blue complexes was only recorded at 711 nm, an overall λ_{max} for crude palm oil phospholipids, variations could arise from each individual species. In fact, the λ_{max} of each phospholipid-molybdate complex can be different from each other, e.g., the λ_{max} for PA- and PC-molybdate complexes are at 700 and 725 nm, respectively. Hence, it is reasonable that the results, though similar, could be slightly different quantitatively.

The FA composition of the phospholipid fractions is given in Table 3. Both FOHS and FOES show similar FA compositions. They are more unsaturated, with a higher linoleic content, as compared with TAG in crude palm oil. The high-linoleic acid content indicates that phospholipids are formed at early stages of development, but as the fruit matures, enzymatic processes provide for TAG with more saturated FA. Higher degrees of unsaturation observed in phospholipids can also be due to differences arising from the statistical distribution of FA in the *sn*-1,3 and *sn*-2 positions of the TAG. Position *sn*-2 of TAG is esterified mainly with unsaturated oleic and linoleic acids (12). Since position 3 of phospholipids has been esterified by the phosphoric acid ester, there will be a statistical reduction in the amount of saturated acids in the molecule.

FA in phospholipids from palm-pressed fiber contain relatively little unsaturated FA compared with soybean phospholipids, which comprise about 60% linoleic acid, 10% oleic

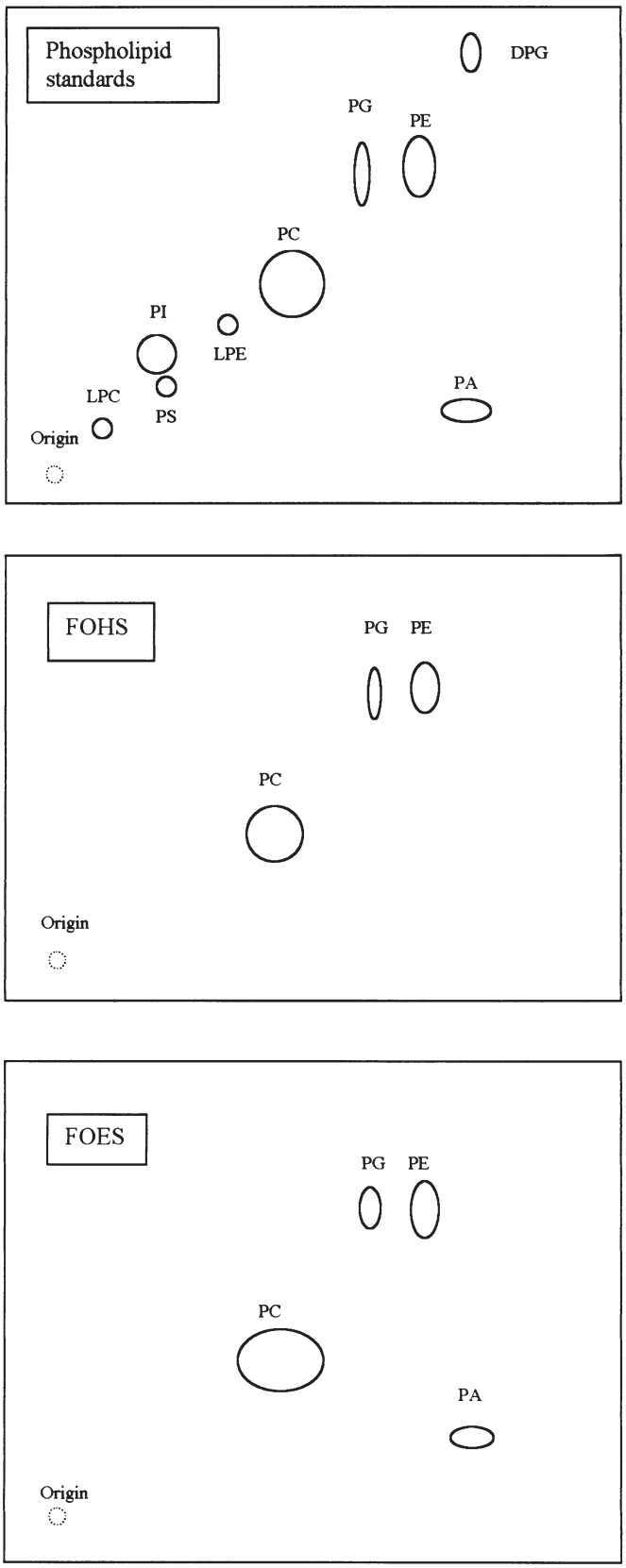

FIG. 1. 2D-TLC of palm-pressed fiber phospholipids on a silica gel plate, FOHS, hexane extract of pressed fiber; FOES, ethanol extract of pressed fiber; PG, phosphatidylglycerol; LPC, lysophosphatidylcholine; DPG, diphosphatidylglycerol; LPE, lysophosphatidylethanolamine.

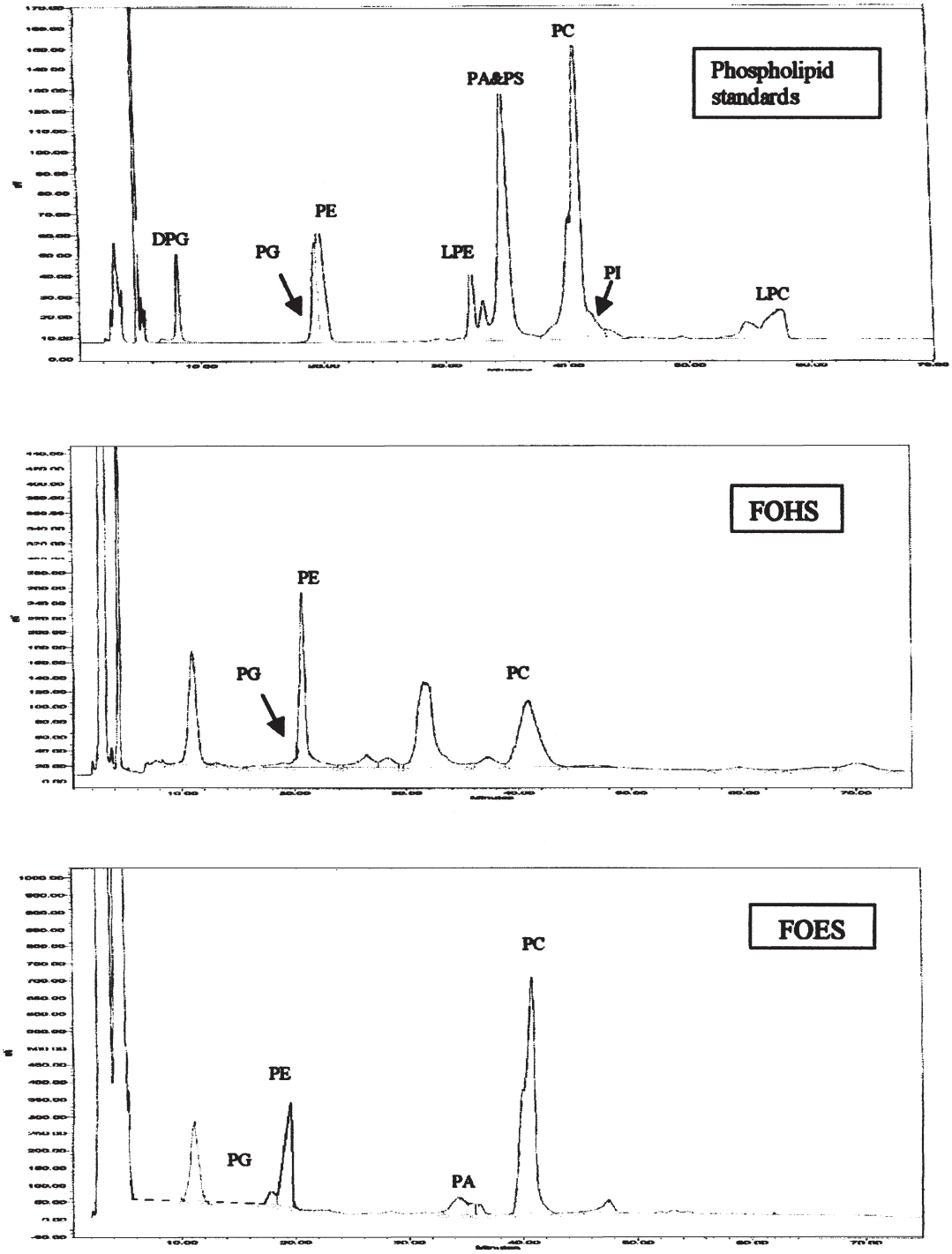

FIG. 2. HPLC chromatograms of phospholipid standards and phospholipid fractions of FOHS and FOES. For abbreviations see Figure 1.

acid, and 5% linolenic acid (13). Thus, owing to their similar composition to phospholipids in soybeans, palm-pressed fiber phospholipids may have greater antioxidant stability than phospholipids in soybeans owing to enhancement with a greater proportion of saturated FA (14).

The high level of phospholipids remaining in palm-pressed fiber is understandable because, unlike seed oils, which are solvent-extracted, palm oil is mechanically extracted without solvents and is separated from an aqueous slurry during milling. Thus, it can be expected that considerable amounts of phospholipids still remain in the fiber. Palm-pressed fiber constitutes about 15% by weight of the fresh fruit bunches (15), and fresh fruit bunch production for 2001 was about 54 million tonnes. Thus, considering the fiber-extracted oil yield of 5.71% (FOES), a potential production of 21,645 tonnes palm lecithin per year based on the total world production of fresh fruit bunches is expected.

TABLE 3
FA Composition (%) of Phospholipids in FOHS and FOES and in TAG of Palm Oil

FA	Phospholipids		Glycerides[a]
	FOHS	FOES	
C14	ND	ND	1.0–1.5
C16	33.71	34.58	41.8–45.0
C18:0	2.41	1.03	3.7–5.1
C18:1	32.91	33.75	38.6–40.2
C18:2	30.76	30.05	10.2–11.9
C20	0.21	0.59	ND

[a]*Source:* Reference 11. For abbreviations see Tables 1 and 2.

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