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CHEMICAL COMPOSITION OF OIL DROPLETS FROM PALM OIL MILL SLUDGE

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ABSTRACT

Oil droplets from the centrifuge sludge of a palm oil mill were separated by high speed centrifugation, dried and extracted with organic solvents, methanol and chloroform. The oil droplets (73 wt %) was solvent extractable. The extract was determined to consist of 84 wt % neutral lipids and 14 wt % of complex lipids (6 wt% glycolipids and 10 wt % phospholipids). The neutral lipids consisted of 83% triglycerides, 8% diglycerides, 0.5% monoglycerides and 8.0% free fatty acids.

Five types of glycolipids were determined and identified as digalactosyl diglycerides (22%), steryl glycosides (17%), cerebroside (9%), monogalactosyl diglycerides (20%) and esterified steryl glycoside (26%). Five types of phospholipids were determined and identified. They were phosphatidylethanolamine (21%), phosphatidylglycerol (37%), phosphatidylcholine (17%) and phosphatidylserine together with phosphatidylinositol at 11%. Palmitic acid (C16:0) and oleic acid (C18:1) were the major fatty acids found in the lipids.

The oil droplets found in the sludge were not unruptured oil droplets inherent in ripe mesocarp or young palm fruits. From the chemical analysis of the lipids associated with the oil droplets, it was deduced that oil droplets from the sludge are indeed formed in the milling process and possibly stabilized by the surface active agents of mainly phospholipids and glycolipids. The relatively high concentration of such biosurfactant in the oil droplets may have commercial potential as a value-added resource from the palm oil milling process.

Keywords: palm oil, sludge, oil droplets, phospholipids, glycolipids.

INTRODUCTION

An interfacial tension of less than 8 mN m^{-1} at the interface of crude palm oil and sludge supernatant from the palm oil mill was determined (Chow and Ho, 2000). This is thermodynamically favourably low enough for emulsification of palm oil in water. The extreme heat, agitation and pumping in the milling process further assist in the formation of these undesirable oil droplets. These oil droplets can be visibly identified by optical microscopy in the sludge from the palm oil mills and they made up 30% of the total amount of solvent extractable oil from the dried sludge (Chow *et al.*, 1987). The diameter of these oil droplets ranges from 1.2-12 microns. These oil droplets are extremely stable as even when separated they would not coalesce to form a homogenous layer of oil.

The stability of these oil droplets can be attributed to a layer of surface active agents covering the surface of the oil droplets. Surface active materials present in the milling process could only be indigenous of the palm fruits as in the milling process no chemicals are used. Botanical constituents of the plant cells, including the surface active agents are solublized in the crude oil slurry at the pressing stage where the plant cells including oil cells are disintegrated and ruptured. The commonly known natural surface active lipids from plants are monoglycerides, phospholipids and glycolipids which are either

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constituents of the cellular membrane or other organelles of the plant cells.

An analysis of the chemical components of these oil droplets including the surface active lipids will give a better understanding of the role of these biological components of the plant cells with respect to the stabilization of oil droplets and thus oil loss in a palm oil mill.

MATERIAL AND METHODS

Separation of Oil Droplets from Centrifuged Sludge

Fresh sludge was collected from a sludge separator of a 60 t fresh fruit bunch hr^{-1} palm oil mill. The sludge was warmed to 60°C and centrifuged at 10 000 rpm for 20 min at 30°C using a Sorvall high speed centrifuge with a fixed angle rotor GSA.

The creamed oil droplets were scooped off from the surface of the sludge. The oil droplets collected from 1 litre of sludge were further washed by shaking with 200 ml of distilled water at 60°C and re-centrifuged to separate the oil droplets. The washing was repeated and the free oil droplets by now would have concentrated to form a dense layer of yellowish cream which could be lifted easily by a flat spatula from the centrifuge tube. The concentrated oil droplets were weighed into round bottom flask and vacuum dried at 60°C. The moisture content was determined.

Extraction of Lipids from Dried Oil Droplets

The above dried oil droplets (1 g) was weighed into a small beaker. Warm methanol (10 ml) was added and stirred for 1 min using a magnetic stirrer followed by 20 ml chloroform and further stirring for another 2 min. The solvent mixture of methanol and chloroform was filtered into a pre-weighed flask.

The partially extracted residual solids were further extracted with 30 ml of a $CHCl_3$: MeOH (2:1) mixture and filtered. Further extraction of the solids were carried out using 20 ml of chloroform and finally another 10 ml of methanol. The combined filtrates consisting of the various solvents were rota-evaporated to remove the solvents. The wt % of extract obtained was determined. The extract was spotted on to TLC plates and developed in a solvent system consisting of ($CHCl_3$:MeOH: CH_3COOH:H_2O) (170:25:25:6 v/v). The plate was visualized with Zinzade and Orcinol spray for preliminary observations (*Figure 1*).

Column Chromatography of Lipid Extract

Separation of the above lipids mixture into simple and complex lipids was carried out using column chromatography. Silicic acid (10 g, previously conditioned at 110°C) were slowly packed into a 1.15 cm x 30 cm glass column and sealed with some glass wool near the teflon stopper.

About 0.3 g of the lipid dissolved in chloroform was eluted stepwise with 200 ml $CHCl_3$ (Fraction 1), 800 ml CH_3COCH_3 (Fraction 2) and 200 ml MeOH (Fraction 3) at a flow rate of 3 ml min^{-1}. Initially, volumes of 10 ml eluant were collected regularly to determine the required volume of each solvent needed to completely elute each class of lipids. The eluant collected was rota-evaporated and the individual dried lipid fraction weighed (*Table 1*).

Thin Layer Chromatography

The dried lipid fraction eluted with chloroform (Fraction 1) was dissolved with a little chloroform and spotted on to silica gel TLC plate. Elution of the plates was carried out in a solvent tank consisting of hexane:diethyl ether:formic acid (80:20:2 v/v) (*Figure 2*).

The dried lipid fraction eluted with acetone (Fraction 2) and with methanol (Fraction 3) were also dissolved in acetone and methanol respectively and spotted on to silica gel TLC plates. Elution of Fractions 2 and 3 were carried individually in separate tanks using the solvent system $CHCl_3$:MeOH: CH_3COOH:H_2O (170:25:25:6 v/v) (*Figure 3*).

Identification of Lipids

Identification of phospholipids and glycolipids from Fractions 2 and 3 was by visualizer sprays (*Figure 3*) and by comparing the retention time with authentic compounds wherever possible. All phospholipids and digalactosyl diglyceride were purchased from Sigma Chemicals. The simple lipids, *i.e.* Fraction 1 were identified by visualizing sprays and also compared with those of palm oil (*Figure 2*).

Visualizer Sprays

(a) Zinzade reagent. The reagent is dark greenish brown and phospholipids are stained blue instantly. Charring visualizes all the compounds present.
(b) Orcinol-sulphuric mixture. Glycolipids appeared as reddish purple spots when heated.
(c) Dragendorf' reagent. Choline containing compounds are stained orange red with this reagent.
(d) The 2', 7'-dichlorofluorescein spray. Simple lipids become obviously fluorescent when viewed under UV light.
(e) Ferric chloride spray. The spray is colourless but stained sterol to pinkish purple.

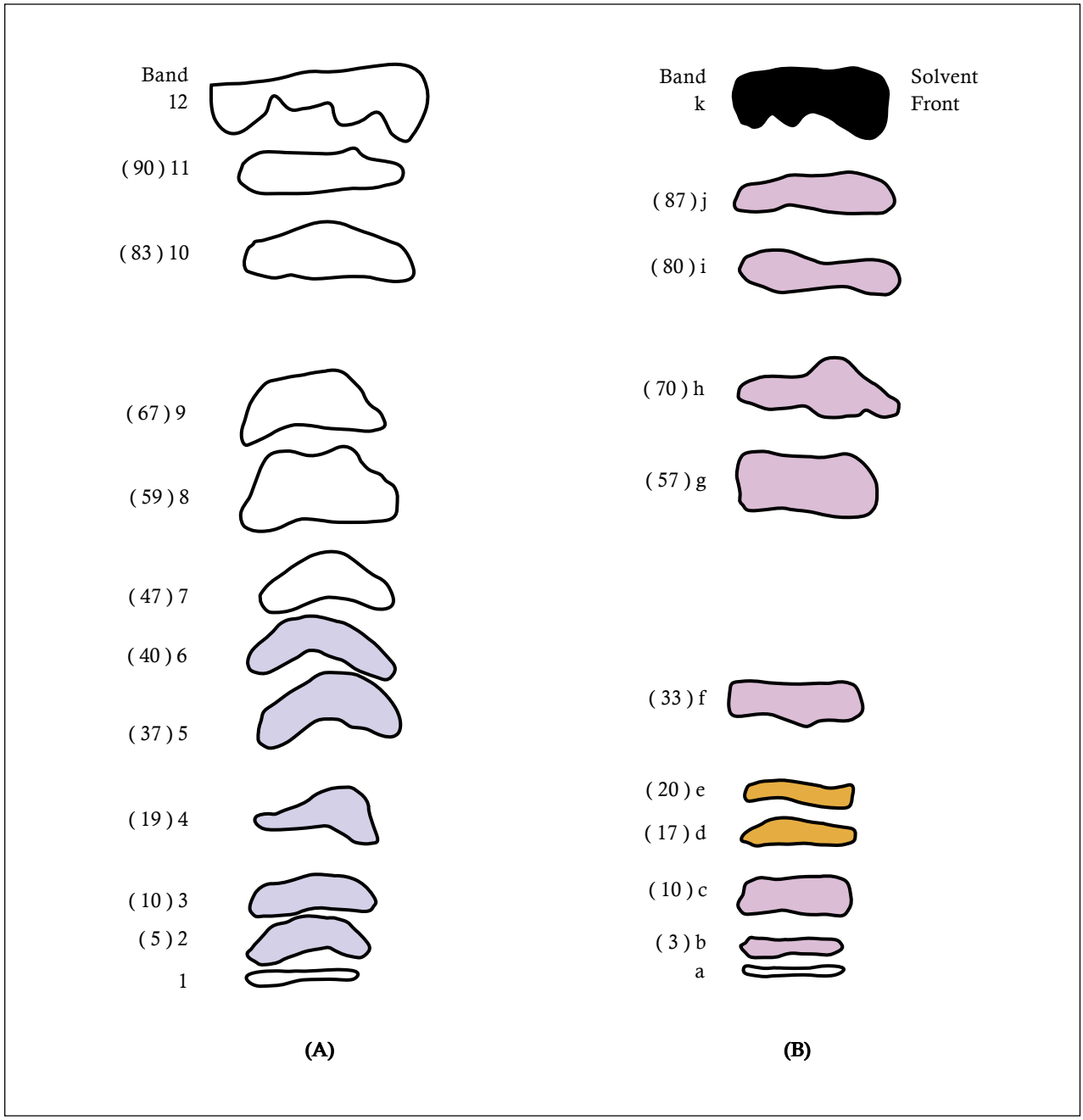

Figure 1. Thin layer chromatography of $CHCl_3$:MeOH extract on silica gel G. Solvent system $CHCl_3$:MeOH:CH_3COOH:H_2O (170:25:25:6 v/v). (A) is visualized with Zinzade reagent. Bands 2,3,4, 5 and 6 are blue before charring. Rest of the bands became visibly black after charring due to concentrated H_2SO_4 in the spray. (B) is visualized with Orcinol-sulphuric acid. All bands appear purplish except d, e and k which are brown and black respectively. ( ) indicates the retention factor.

(f) Ninhydrin spray. Free amino acids are stained orange.

Quantification of Phospholipids and Glycolipids by Colorimetry

For quantifying the separated lipids from the TLC plate, a long band was spotted on the plate. After elution, the plate was covered with a narrower clean glass leaving only a small margin on both sides of the plate. The visualizer was then sprayed on the uncovered part of the plate and heated with a blower, if necessary. The required band was then marked and scrapped for further analysis.

The acetone fraction (Fraction 2) was separated by TLC and visualized with Orcinol. Quantification of the hexose units in glycolipids was according to method of Yamamota and Rouser (1970). The methanol fraction (Fraction 3) was separated by TLC and visualized with Zinzade reagent. Its phosphorus content was determined according to PORIM Test Method (PORIM, 1985).

TABLE 1. LIPID COMPOSITION OF THE $CHCl_3$/MeOH EXTRACT OF THE DRIED OIL DROPLETS BY COLUMN CHROMATOGRAPHY

Lipid	Range (%)	Mean (% wt/wt)
Neutral lipids (chloroform fraction)	74-91	84
Glycolipids (acetone fraction)	3-9	6
Phospholipids (methanol fraction)	5-19	10

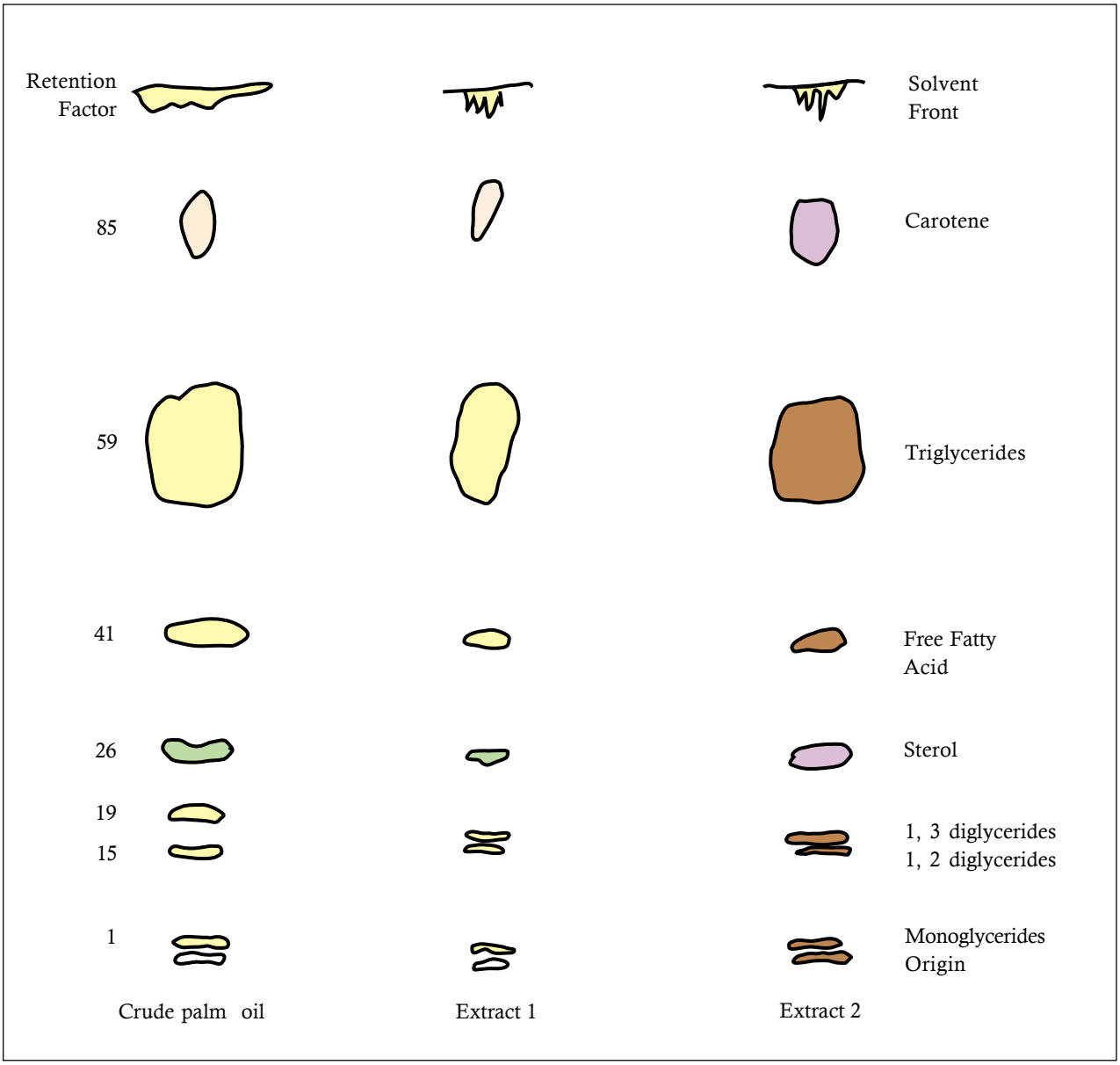

Figure 2. Thin layer chromatography of chloroform fraction. Solvent system hexane:diethylether:formic acid (80:20:2 v/v). Crude palm oil and chloroform extract 1 were visualized with 2,7-dichlorofluorescein and lipids were stained yellow. Chloroform extract 2 was visualized with ferric chloride and lipids were stained brown.

Quantification of Simple Lipids by Gas Chromatography

Silylation of glycerides. About 0.5 g of the simple lipids (Fraction 1) was dissolved in 0.5 ml of dichloromethane. The N_1O-Bis (0.5 ml) (trimethylsily1) trifluoroacetamide (BSTFA) was added. The mixture was capped and heated (40°C-50°C) for 15 min.

Gas chromatography. The silylated compounds (0.1 ml) of the above was injected into a Hitachi G-3000 gas chromatograph fitted with a fused silica capillary column of PEG-20M bonded liquid phase

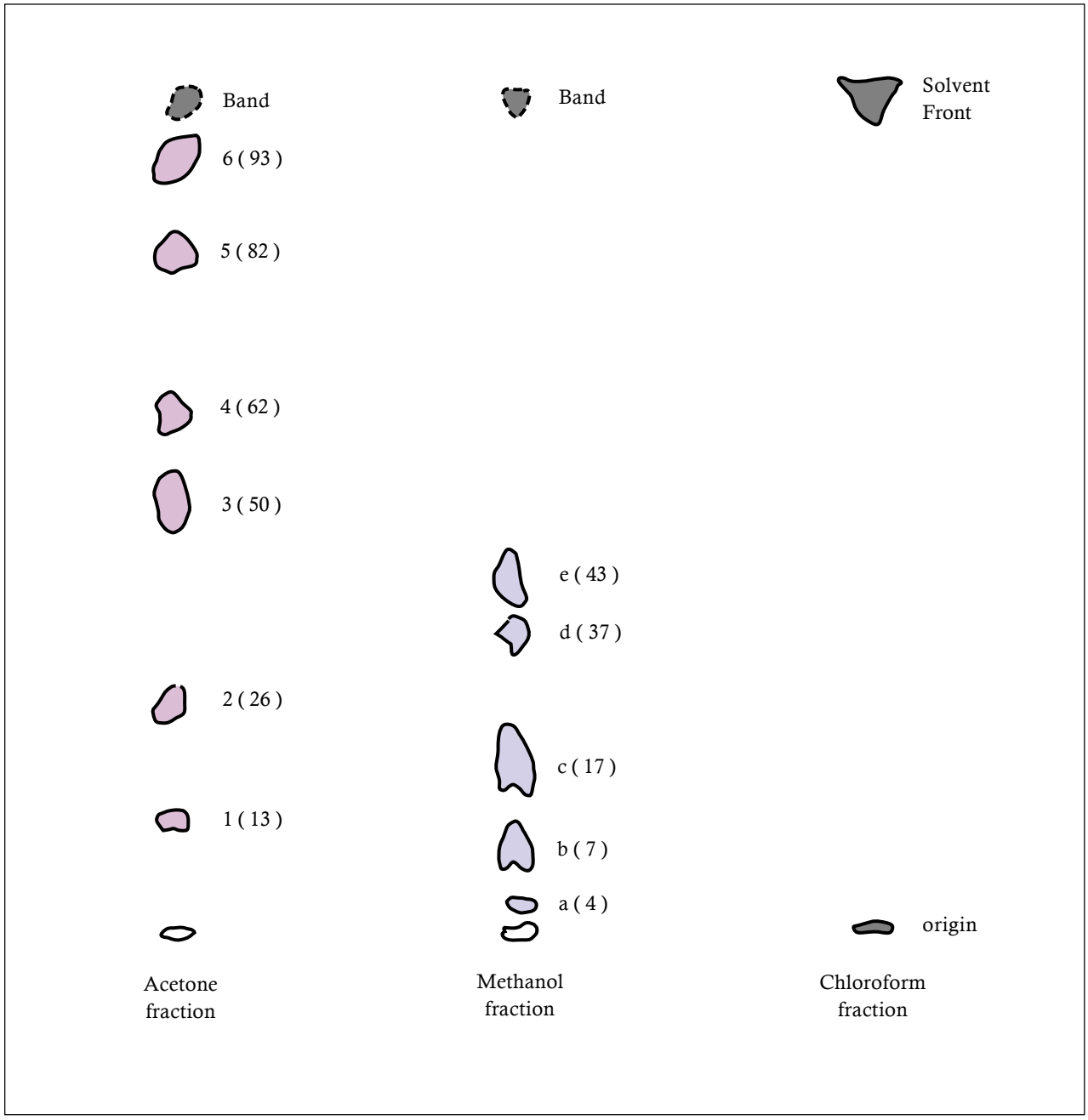

Figure 3. Thin layer chromatography of the three different fractions separated by silicic acid column. Solvent system $CHCl_3$:MeOH:CH_3COOH:H_2O (170:25:25:6). The glycolipids of the acetone fraction were stained purple when visualized with Orcinol-sulphuric acid. The phospholipids of the methanol fraction were stained blue when visualized with Zinzade reagent. The neutral lipids of the chloroform fraction was charred with concentrated sulphuric acid. Note absence of complex lipids indicating good separation. ( ) indicates the retention factor.

(15 m, 0.53 m ID). The injector and detector temperatures were at 270°C and 340°C respectively. The oven temperature was programmed to maintain at 100°C for the first minute and subsequently raised to 320°C within 20 min. The relative percentage of the glycerides (monoglycerides, diglycerides, triglycerides and free fatty acids) was computed with the integrator. Retention time of each component was based on a palm oil standard with an added internal standard, tricaprin.

Fatty Acid Composition Analysis

The individual fractions of simple lipids and phospholipids were scrapped from TLC plates and its fatty acid compostion analysed as follows:

Methylation of lipids. Methanolysis of each separated simple lipids (triglyerides, diglycerides and monoglycerides) was carried out by adding 1 ml of 0.5 sodium methoxide to three drops of the

sample dissolved in 2 ml of hexane. The methyl esters were washed with water and dried with anhydrous sodium sulphate before injecting into the gas chromatograph.

Free fatty acids and phospholipids were converted to the methyl esters of the fatty acid using methanolic boron trifluoride. The lipid was refluxed with 4 ml methanolic sodium hydroxide followed by 5 ml methanolic boron trifluoride. Then 3 ml heptane was introduced into the flask and then floated to the neck of the flask with saturated sodium chloride. The heptane fraction was pipetted off and dried with a little anhydrous sodium sulphate. The purified ester was injected into the gas chromatograph.

Gas chromatograph. Methyl esters were identified using a HP 5890 Series II chromatograph using a SP-2340 fused silica capillary column (60 m, 0.25 m ID). The injector, oven and detector temperatures were at 240°C, 180°C and 240°C respectively. The carrier gas, helium, was regulated at 1 ml min^{-1}.

The relative percentage of each fatty acid as methyl ester was calculated with the HP integrator 3396. Retention time of each component was based on a secondary standard of palm oil glycerides analysis. The primary standard used was RM6 from Sigma Chemicals. Summary of analytical techniques used in the various lipid characterization of oil droplets is as illustrated in *Appendix 1*.

RESULTS AND DISCUSSION

Sludge from the sludge separator in the clarification station of a palm oil mill is viscous and dark brown in colour. When it is centrifuged at high centrifugal force of at least 7600 G, oil droplets will float to the surface as a yellowish cream. These flocculated droplets need to be further water washed to remove the minute fragmented plant cell.

The water washed oil droplets contained a high percentage of water (*Table 2*). The main constituents are $CHCl_3$/MeOH extractable. Very fine residual plant cells and gums contributed to the non-lipid solids. These solids were determined to contain 934-1083 ppm of phosphorous. Separation of the $CHCl_3$ /MeOH extract on TLC plates and charring indicated the presence of at least 12 compounds (*Figure 1*). Five were positive to Zinzade spray and seven positive to Orcinol indicating the presence of phospholipids and glycolipids respectively. Two unknown bands appeared brown and black respectively when sprayed with Orcinol.

Column chromatography of the $CHCl_3$/MeOH extract separated it into three fractions according to the polarity of eluant used (*Table 1*). Neutral lipids as eluted by chloroform (Fraction 1) made up the major portion of the extract phospholipids (Fraction 2) and glycolipids (Fraction 3) were eluted by the more polar solvent of methanol and acetone respectively. Further separation and identification of these individual fractions were carried out using thin layer chromatography.

The chloroform fraction (Fraction 1) consisted of seven compounds which were identified by visualizer sprays and comparison with lipids of crude palm oil (*Figure 2*). The lipids of palm oil is known (Ooi and Chow, 1989). Carotene and sterols were identified but not quantified. Gas chromatography of the chloroform fraction confirmed the usual glycerides of triglycerides, diglycerides, monoglycerides and free fatty acids found in crude palm oil (*Table 3*).

The fatty acid composition of the various lipids of the chloroform fraction was determined by gas chromatography (*Table 4*). Palmitic (C16:0) and oleic (C18:1) made up the major acids of the triglyceride fraction, similar to those of commercial palm oil. The other partial glycerides consisted of mainly C16:0 and C18:1 fatty acids too. However, the diglycerides are more unsaturated than the monoglyceries and fatty acids. Oleic acid and palmitic acid constitute the major fatty acids in the diglycerides and monoglycerides respectively.

The acetone fraction (Fraction 2) consisted of six types of glycolipids being positive to Orcinol sprays

TABLE 2. COMPOSITION OF WATER-WASHED OIL DROPLETS

Constituent	Range (%) on dried wt. basis	Mean (%)
Moisture	43-70	55
$CHCl_3$/MeOH extract	62-82	33(73)*
Residual non-lipids solid (%)	18-28	12(27)*

Note: ( )* Indicates weight % of $CHCl_3$/MeOH extract to residual non-lipid solids on dried basis.

TABLE 3. LIPID COMPOSITION OF CHLOROFORM FRACTION DETERMINED BY GAS CHROMATOGRAPHY

Lipid	*Relative (%)
Triglycerides	83.5
Diglycerides	8.0
Monoglycerides	0.5
Free fatty acids	8.0

Note: The relative (%) of each lipid was based on the peak area of the chromatogram.

(*Figure 3*). Digalactosyldiacylglycerol (DGDG) was confirmed with authentic sample spotted side by side on the same plate. The calculated retention factors of the other four glycolipids were comparable to those obtained by Yamaoko *et al.* (1988) from oil palm leaf (*Table 5*) except that of sulfoguinovosyl diglyceride which had a relatively very different retention factor. Further work is required to confirm the presence of this particular compound in the oil droplets. The various glycolipids present in the sludge droplets were also similar to those extracted from the palm oil from spent earth used in the refining of crude palm oil (Yamaoko *et al.*, 1989). The major ones from the oil droplets were esterified steryl glycoside (ESG) and digalactosyl diglycerides (DGDG).

The methanol fraction (Fraction 3) consisted of five different types of phospholipids which were visualized with Zinzade reagent (*Figure 1*). Bands e and b were stained orange with Ninhydrin indicating the presence of free amino group while band c was positive to Dragendrof reagent indicating presence of phosphatidylcholine.

The various phospholipids were further confirmed with authentic phospholipids spotted on TLC plates beside the methanol fraction. The retention factors are shown in *Table 6*. Bands a, b, c, d and e were identified as phosphatidylserine (PS), phosphatidylinisitol (PI), phosphatidylcholine (PC), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), respectively. Bands a and b were usually very close and were thus quantified as one phospholipid type.

The main fatty acids of the phospholipids associated with the oil droplets were C16:0 and C18:1 (*Table 6*). Similar findings were reported by Goh *et al.* (1982) and Kulkarni *et al.* (1991).

Leonard and Hodges (1980) found that the fatty acid composition of the individual phospholipids may vary from different parts of the plants and also on the types of organelles from which they are extracted but generally C16:0, C18:2 and C18:3 are the main fatty acids. In a compilation of the fatty acids composition of various seed phospholipids and triglycerides, it was noted that the fatty acids composition of these two lipids are usually in the same proportion (Cherry *et al.*, 1981). This is in agreement with the present results where C16:0, C18:1 and C18:2 being the major fatty acids of both the triglycerides in crude palm oil are also the major fatty acids of the phospholipids extracted from the oil droplets.

Different quantities of phospholipids had been extracted from palm mesocarp, crude palm and centrifuged gummy residue (Goh *et al.*, 1982) George and Arumughan (1982), working on the lipid profile of the various streams during milling, found relatively high concentrations of phospholipids and

TABLE 4. FATTY ACID COMPOSITION (%) OF THE NEUTRAL LIPIDS (chloroform fraction)

	Lipids					
***Fatty acid**	**C14:0**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C20:0 &18:3**
Triglycerides	1.1	45.2	3.7	39.9	9.7	0.4
1,2 Diglycerides	0.5	36.8	3.4	49.2	10.0	0.1
1,3 Diglycerides	0.5	42.2	3.3	44.7	9.3	0.5
Monoglycerides	1.4	67.6	1.5	23.7	5.0	0.8
Free fatty acid	1.5	51.0	10.9	34.2	2.2	0.2
Commercial palm oil	1.2	42.1	4.2	39.9	10.8	0.5

Note: *The relative (%) of each fatty acid was based on the peak area of chromatogram run under the same conditions.

TABLE 5. GLYCOLIPIDS (wt/wt %) (acetone fraction) AS SEPARATED IN SOLVENT SYSTEM ($CHCl_3$:MeOH: CH_3COOH:H_2O) 70-25:25:6 (v/v)

Band No*	Rfx100**	Rfx100***	%	Glycolipid
1	26	13	6	Sulfoguinovosyl diglyceride (SQDG)
2@	27	26	22	Digalactosyl diglyceride (DGDG)
3	47	50	17	Steryl glycoside (SG)
4	57	62	9	Cerebroside without hydroxy fatty acid (CU)
5	81	82	20	Monogalactosyl diglyceride (MGDG)
6	87	93	26	Esterified steryl glycoside (ESG)

Notes: * Refer *Figure 3* for band numbering. ** Retention factor determined by Yamaoka *et al.* (1988) in solvent system $CHCl_3$:MeOH:CH_3COOH:H_2O (85:5:10:3 v/v). *** Retention factor determined in study. @DGDG confirmed with authentic sample.

TABLE 6. FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM $CHCl_3$/MeOH EXTRACT OF DRIED OIL DROPLETS

*Phospholipid band	Rf x 100	Phospholipid identified	% wt/wt (phosphorus analysis) on total extract	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	Others
a	4	Phosphatidylserine, PS		0.5	1.2	45.3	5.0	37.3	0.4	1.0
b	7	Phosphatidylinositol, PI	32**	3.0	1.4	55.7	6.1	24.8	0.5	4.1
c	17	Phosphatidylcholine, PC	27	1.3	1.1	41.1	2.7	42.9	0.4	1.9
d	37	Phosphatidylglycerol, PG	20	0.6	1.1	53.1	5.9	34.1	0.4	2.3
e	43	Phosphatidylethanolamine, PE	21	3.0	2.1	51.0	2.1	26.9	0.2	8.4

Notes: Refer *Figure 3* for band labeling. ** PS and PI quantified as one lipid type.

glycolipids from the lipid extracts of the components of the palm fruits when they were separated. The press fibres contained the highest concentration of glycolipids and phospholipids at 2.6 wt % and 3.0 wt % respectively of the total lipids extracted. In the present study, the $CHCl_3$/MeOH extract contained even higher amount of glycolipids (6 wt %) and phospholipids (10 wt %) with respect to the total lipid extracted.

In view of the highly variable amount and composition of the phospholipids obtained and the lack of reports regarding the specific biochemical composition of palm oil organelles in cells, membranes and fibres, the origin or source of the phospholipids found associated with the oil droplets could not be exactly identified. The milling process involves mainly the rupturing of the oil bearing cells by mechanical force to release the oil when the digested fruit mash is screw pressed. The fate of the fragmented mesocarp cells and membranes have not been biochemically characterized but since phospholipids are generally known to be constituents of membranes and organelles of plant cells, their origin in the sludge droplets has to be from the mesocarp. In the intimate mixing of the aqueous and oil phases during processing, the amphiphilic phospholipids are thermodynamically more favourably orientated and concentrated at the oil/water interface of the oil droplets.

Views differ as to whether oil droplets from various oil seeds and plants are surrounded by a membrane. In the early stage of development of the palm fruit, spherical oil bodies in the mesocarp are sparsely distributed throughout the cytoplasm. In mature fruits, the cells are completely filled with oil and become the only predominant organelle of the cell (Ariffin *et al.*, 1990). The spherical configuration could be a result of the phenomenon of cohesion where the surface area is a minimum in relation to the minute volume. But very often the oil was also observed to follow the contour of the cell wall suggesting a sort of flexible membrane surrounding them; otherwise the bigger oil drop will become very irregular in shape. There is no known publication on the nature of the surface of oil droplets found inside the palm oil cells. Harwood *et al.* (1971) in his work on oil bodies of *Ricinus communis* endosperm and Rest and Vaughan's (1972) work on *Sinapis alba L.* seed suggested there was a sharply defined interface with the cytoplasmic material. They postulated that this was a monolayer of phospholipids. Yatsu *et al.* (1971), through electron microcopy, identified a single-line membrane of 20-30 Å thick surrounding the isolated oil bodies from peanuts and cabbage. Jacks *et al.* (1967) attributed this layer to the phospholipid and protein associated with the oil bodies. As the membrane is only one molecular thick, the phosphorus to oil would be extremely low. Phospholipids (0.58%-1.15%) by weight has been calculated from one or two layers surrounding oil bodies of peanuts 1.5 or 3.1 μ diameter respectively.

Thus, small oil droplets found in the sludge could possibly originate from two sources. They could be oil droplets from the ripe or unripe oil cells which may be covered by a membrane made of phospholipids. Oil droplets could be formed during the turbulent pumping at the various stages of the milling process. The surfactants of the cellular fragments favourably adsorbed at the interface of these oil droplets and further stabilized them.

The high concentration of 10% phospholipids found associated with the neutral lipids in the $CHCl_3$/MeOH extract cannot be accounted for only by a monolayer of phospholipids on the droplets. Based on the surface area of the oil droplets determined at 1.166 m^2 g^{-1} of oil droplets (data derived from particle size distribution) only 0.49% phospholipids is required for a monolayer. If they could be covered by multilayers of phospholipids as Larsson (1994) has postulated in the stabilization of oil droplets by bilayer formation, more phospholipids indeed can be present.

The fatty acid composition of the palm oil at different periods of maturity are significantly different (Ariffin *et al.*, 1990). Young fruits before maturity (approximately 14 weeks) have a high level

of C18:2 (>19.3%) and C18:3 (72.7%). In this study, the triglycerides composition of the oil droplets was similar to that of commercial palm oil with 9.7% of C18:2 and insignificant amount of C18:3. Thus, it is deduced that the majority of the oil droplets found in the sludge did not originate from unripe oil cells but were emulsified from the expressed oil during the milling process.

CONCLUSION

The composition of the major lipids found in the oil droplets separated from the centrifuge sludge is similar to that of commercial palm oil, except that it consists of a higher concentration of surface active compounds of phospholipids (10 wt %) and glycolipids (6 wt %). These natural surfactants stabilized the oil droplets rendering oil irrecoverable from the sludge as a homogeneous phase. Phospholipids are commonly used as food emulsifiers but the use of glycolipids is yet widespread due to its limited availability.

The relatively high quantity of these natural products may have commercial implication as a value-added resource by-products from the palm oil milling process.

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Appendix 1

SUMMARY OF ANALYTICAL TECHNIQUES IN LIPID CHARACTERIZATION OF OIL DROPLETS