PHOSPHOLIPIDS AND GLYCOLIPIDS IN THE OIL FROM SOME VARIETIES OF *Elaeis guineensis* IN INDIA

Keywords: Phospholipids; Glycolipids; *Elaeis guineensis;* Chromatography; Fatty acids.

A S KULKARNI, R R KHOTPAL AND H A BHAKARE*

* Department of Oil Technology,
Laxminarayan Institute of Technology,
Nagpur University, Nagpur-440010, India

The phospholipid and glycolipid compositions of oil from two varieties of the oil palm, Elaeis guineensis, *namely* dura *and* pisifera, *are reported. The oil was extracted from mesocarp with chloroform-methanol (2:1, v/v) and the total phospholipids and total glycolipids were isolated from it by silicic acid column chromatography using chloroform, methanol and acetone as the eluting solvents. The individual components of total phospholipids were identified after thin layer chromatography by comparison with authentic standards and using specific spray reagents. The major components were found to be phosphatidyl choline PC, 34% to 35%, phosphatidyl ethanolamine PE, 22% to 26%, phosphatidyl inositol PI, 21% to 25%, cardiolipin CL, 7% to 8%, phosphatidyl glycerol PG, 5% to 7%, and unidentified substances 4% to 6 per cent. The predominant fatty acids in all these components were palmitic, stearic, oleic and linoleic acids.*

Thin layer chromatography revealed that the major components of total glycolipids were monoglycosyl diglyceride (MGDG), 22% to 25% diglycosyl diglyceride (DGDG), 42% to 45%, steryl glycoside (SG)13% to 14% and acylated steryl glycoside (ASG) 14% to 15%, with, unidentified substances making up to 2% to 9 percent. The predominant fatty acids of the glycolipids were palmitic, stearic, oleic, linoleic and linolenic acids. The SG and ASG fractions showed the presence of β-sitosterol, stigmasterol, campesterol and brassicasterol; β-sitosterol being the major component. For SG the ratio of sugar:sterol was 1:1 and for ASG the ratio of sugar:sterol:fatty acids was 1:1:1.

INTRODUCTION

If phospholipids are present in excessive amounts in palm oil, they present refining problems (Lezaic, 1973) and they have also been reported to be one of the causes of oxidation of palm oil (Chooi and Ko, 1981). There appears to be insufficient information available on the phospholipids and glycolipids of palm oil.

In this paper, the phospholipid and glycolipid compositions of oil from the *dura* and *pisifera* varieties of the oil palm *Elaeis guineensis*, are reported.

EXPERIMENTAL

Extraction of Phospholipids and Glycolipids

The palm fruits were obtained from Oil Palm (India) Ltd., Kottayam, Kerala State, India and the mesocarp was extracted with chloroform and methanol (2:1, v/v) by the procedure of Folch *et al.* (1957).

The neutral lipids, glycolipids and phospholipids were separated by column chromatography on silicic acid (200–300 mesh), using chloroform, methanol and acetone as eluents respectively, according to the method of Cārroll (1963). The authentic phospholipid and glycolipid standards were provided by Analabs, U.S.A. and Applied Science Laboratories, State College, Pennsylvania, U.S.A.

Quantitative Separation of Phospholipids

The total phospholipids were separated into the individual components by preparative TLC on silica gel G plates (0.25 mm thickness). 25 mg of total phospholipids applied to the plate using a chromatocharger. The solvent system used was chloroform – methanol – 25% ammonia (65:15:4, v/v/v) in the first direction and chloroform-methanol and acetic acid-water (170:25:25:6, v/v/v/v) in the second direction. The spots were visualized with iodine vapours and the areas corresponding to the separated fractions were scraped off the plate, eluted with acetone and then weighed.

Identification of Phospholipids

a) *By quantitative TLC.* The spots were detected by staining with specific reagents such as; iodine vapours, ammonium molybdate-perchloric acid (Skipski *et al.*, 1962), ninhydrin (Skipski *et al.*, 1962) and Dragendorff's reagent (Mangold, 1961), and were identified by comparing with Rf values reported in the literature (Christie, 1973; Nicholas, 1964; Roughen *et al.*, 1978); the identifications were confirmed by chromatography with authentic standards.

b) *Phosphorus content estimation.* Phosphorus was estimated according to the procedure of Harris and Popat (1954). The phosphorus content was 0.6% and 0.8% for both the varieties in phospholipids, respectively.

c) *Hydrolytic products*

   i) *Bases.* Strong acid hydrolysis (Malkin and Poole, 1953) was carried out for the liberation of bases by heating the sample (4.9 mg) with HCl (1–2 ml, 6N) for 12 hours at 100°C in a sealed tube. The fatty material was removed using chloroform and the aqueous layer was subjected to TLC analysis using the procedure of Kaufmann *et al.*, 1965.

   ii) *Glycerol and inositol.* The sample (4–9 mg) was heated in a sealed tube with HCl (1–2 ml, 6N) for 24 hours at 100°C (Malkin and Poole, 1953). The fatty material was removed with petroleum ether. The aqueous layer was subjected to TLC analysis using the solvent system, n-propanol – ethyl acetate – water – 25% ammonia (50:10:30:10, v/v/v/v/) and the TLC plate was sprayed with meta-periodate-benzidine reagent (Yasuda, 1931).

   iii) *Fatty acid composition of phospholipids.* Fatty acid methyl esters were prepared by acid-catalysed transmethylation of the phospholipid fraction by the method of Christie (1973). The methyl esters were analysed by GLC using a flame ionization detector. The column was packed with 15% EGSS-X on Chromosorb W, 40–60 mesh. The conditions were: Chart speed 60 cm/hr., injection port temperature 300°C, column temperature 200°C and nitrogen flow rate 60 ml minute. The peak area and the percentage of fatty acid methyl ester were obtained with a disc integrator. The component of each peak was identified on the basis of retention data compared with those of the authentic standards.

Quantitative Separation and Identification of Glycolipids

Quantitative separation of glycolipids. The total glycolipids were separated into individual components (MGDG, DGDG, SG and ASG) by preparative TLC using the solvent system chloroform – methanol – 28% ammonia (70:20:2, v/v/v) (Kates, 1972). The spots were visualized using iodine vapours and appropriate areas were scraped off and eluted with acetone. All the fractions were weighed.

i) *Identification of glycolipids.* The glycolipids separated on the TLC plate as described above were detected by staining reagent (Yasuda, 1931) and identified by comparing their Rf values with those reported in the literature (Christie, 1973, Nicholas, 1964; Roughen *et al.*, 1978), then the identifications were confirmed by chromatography with authentic standards.

ii) *Identification of sugars.* Sugars were identified by hydrolysing the sample (5–10 mg) with sulphuric acid (1–2 ml, 0.5 N) for 12 hours at 100°C (Malkin and Poole, 1955). TLC was carried out on alusyl plates (Stahl, 1962). Sugars were specifically identified by the method of Yasuda (1931), and quantitatively estimated by the method of Miyazawa (1974).

iii) *Analysis of components of SG and ASG.* The components of SG and ASG were analysed according to Miyazawa *et al.* (1974). The fatty acids and glycerol were analysed as in the case of phospholipids. The sterols were analysed by GLC after their conversion into trimethylsilyl derivatives (Pierce, 1968).

RESULTS AND DISCUSSION

The results on phospholipids are presented in *Table 1* and those for glycolipids in *Tables 2* and *3*.

TABLE 1. PHOSPHOLIPID COMPOSITION AND FATTY ACID DISTRIBUTION IN PHOSPHOLIPIDS OF OIL PALM VARIETIES

Variety	Component of Phospholipid[a]	Weight per cent	Fatty acids (Wt. %)[b]				
			16:0	18:0	18:1	18:2	18:3
Dura	PC	34.1	39.2	18.3	18.8	20.1	3.6
	PE	22.2	30.2	13.5	38.2	17.3	0.8
	PI	24.8	24.2	21.3	37.2	16.0	1.3
	PG	6.2	24.2	24.0	37.0	14.2	0.6
	CL	7.5	24.3	20.5	40.0	14.0	1.3
	LPC	trace[c]	–	–	–	–	–
	LPE	trace	–	–	–	–	–
	Unidentified	5.2	–	–	–	–	–
Pisifera	PC	35.0	41.2	15.0	14.5	28.1	1.2
	PE	25.3	30.5	12.5	28.1	22.3	6.6
	PI	21.3	23.2	21.3	40.0	15.5	–
	PG	5.5	21.3	22.3	37.7	16.2	2.5
	CL	8.0	22.5	20.2	42.9	12.3	2.1
	LPC	trace	–	–	–	–	–
	LPE	trace	–	–	–	–	–
	Unidentified	4.9	–	–	–	–	–

[a] PC – Phosphatidyl choline, PE – Phosphatidyl ethanolamine, PI – Phosphatidyl inositol, CL – Cardiolipin, PG – Phosphatidyl glycerol, LPC – Lysophosphatidyl choline LPE – Lysophosphatidyl ethanolamine.
[b] All values are means of triplicate analysis.
[c] 'trace' means < 0.5 percent.

TABLE 2. GLYCOLIPID COMPOSITION AND FATTY ACID DISTRIBUTION IN GLYCOLIPIDS OF OIL PALM VARIETIES

Variety	Component of glycolipid[a]	Weight %	Fatty acids[b] (Wt. %)						
			U1	16:0	18:0	18:1	18:2	18:3	U2
Dura	MGDG	22.2	–	36.2	8.3	25.2	16.3	11.1	2.9
	DGDG	42.1	trace[c]	33.5	4.1	30.5	19.2	4.1	8.9
	SG	13.2	–	–	–	–	–	–	–
	ASG	14.1	trace	38.5	11.1	20.0	14.4	11.2	4.8
	Unidentified	8.4	–	–	–	–	–	–	–
Pisifera	MGDG	24.8	–	35.9	8.0	24.1	15.3	7.3	9.4
	DGDG	44.1	trace	34.2	8.9	22.6	18.7	8.6	7.0
	SG	14.0	–	–	–	–	–	–	–
	ASG	14.8	trace	38.2	10.9	21.3	13.8	10.2	5.6
	Unidentified	2.3	–	–	.	–	–	–	–

[a] MGDG – Monoglycosyl diglyceride DGDG, Di–glycosyl diglyceride, SG – Steryl glycoside ASG – Acylated steryl glycoside.
[b] All values are means of triplicate analysis.
[c] 'trace' means < 0.5 percent.
U_1 – lower fatty acids.
U_2 – higher fatty acids.

The composition of the phospholipids in the oil of the two varieties of palm is presented in *Table 1*. The major phospholipids in both cases are phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl inositol. This agrees with the results published earlier on palm oil (Goh *et al.*, 1982; Kulkarni *et al.*, 1987) as well as those on karanja oil (Kulkarni and Bhakare, 1984) and behada oil (Khotpal *et al.*, 1984). In addition, cardiolipin and phosphatidyl glycerol were found in traces in oil from both the varieties and unidentified phospholipids were observed to the extent of 4% to 6 percent.

The fatty acid compositions of individual phospholipids are also reported in *Table 1*. High proportions of palmitic, stearic, oleic and linoleic acids were found in all the components of the phospholipids regardless of variety. Linolenic acid was also found in all the component phospholipids of both varieties. The *dura* variety showed comparatively higher proportions of stearic and oleic acids in PG and PE components than did *pisifera*. The pattern of component fatty acids in these varieties is similar to that reported earlier for those of three varieties of rice bran (Hemavathy and Prabhakar, 1987).

The glycolipid composition of oil from the two varieties of palm is reported in *Table 2*. The major glycolipids were monoglycosyl diglyceride (MGDG), diglycosyl diglyceride (DGDG), steryl glycoside (SG) and acylated steryl glycoside (ASG). The extract from *pisifera* variety contained comparatively higher proportions of MGDG and DGDG components than those from *dura* variety. Similar pattern for MGDG and DGDG components has also been reported earlier for palm oil by Kulkarni *et al.* (1987) and for rice bran from three varieties of rice by Hemavathy and Prabhakar (1987). The extracts from *dura* and *pisifera* varieties also showed some unidentified glycolipids. The sugar in all the four main components of the glycolipids was glucose. The fatty acid analysis of the glycolipids (*Table 3*) showed that ASG component contained higher proportions of palmitic, stearic and linolenic acids followed by MGDG and DGDG components for both the varieties. However, MGDG did not show the presence of lower fatty acids in either of the varieties.

TABLE 3. STEROL COMPOSITION AND MOLAR RATIOS OF SG AND ASG COMPONENTS OF OIL PALM VARIETIES

Variety	Component of glycolipid	Sterols[a]	Weight[b]	Molar Ratios		
				Sterol	Sugar[c]	Fatty acids
Dura	SG	I	71.0	1.00	1.00	–
		II	5.3			
		III	18.7			
		IV	5.0			
	ASG	I	71.3			
		II	3.1	1.01	1.02	1.02
		III	20.4			
		IV	5.2			
Pisifera	SG	I	71.1			
		II	5.7	1.02	1.01	–
		III	18.3			
		IV	4.9			
	ASG	I	72.0			
		II	4.1	1.02	1.02	1.00
		III	19.3			
		IV	4.6			

[a] I: β-Sitosterol (RRT = 1.00), II: Stigmasterol (RRT = 0.88) III: Campesterol (RRT = 0.81) IV: Brassicasterol (RRT = 0.71) RRT = Relative Retention time.
[b] Values based on weight percentages.
[c] Sugar = glucose

Table 3 shows the sterol composition of the steryl glycoside and acylated steryl glycoside fractions, which are similar, β-sitosterol being the major component. The ratio of sugar:sterol: fatty acids was 1:1:1 for ASG and the ratio of sugar:sterol was 1:1 for SG as shown in *Table 3*. The glycolipid composition of oil from these two varieties of palm is similar to the general pattern of other seeds. (Miyazawa *et al.*, 1976; Smith and Wolff, 1966; Hemavathy and Prabhakar, 1987: Osagie and Kates, 1984).

REFERENCES

CĂRROLL, K K (1983). *J. Amer. Oil Chem. Soc.*, *40*, 413.

CHOOI, S Y and KO, H F (1981). *Palm Oil Product Technology in the Eighties*. Incorporated Society of Planters, Kuala Lumpur.

CHRISTLE, W W (1973). *Lipid Analysis*. Pergamon Press, Oxford. p. 53.

FOLCH, J; LEES, M and STANLEY, G H S (1957). *J. Biol. Chem.*, *226*, 497.

GOH, S H; KHOR, H T and GEE, P T (1982). *J. Amer. Oil Chem. Soc.*, *58*, 295.

HARRIS, W D and POPAT, J J (1954). *J. Amer. Oil Chem. Soc.*, *31*, 124.

HEMAVATHY, J and PRABAKHAR, J V (1987). *J. Amer. Oil Chem. Soc.*, *64*, 1016.

KATES, M W (1972). *Techniques in Lipidology*. Work, T S and Work, E (ed.). North Holland Publishing Co., Amsterdam. pp. 578.

KAUFMANN, H P; WESSELS, H and BANDOPADHAYA, C (1983). *Fette Seifen Anstrichmittel, 65*, 543.

KHOTPAL, R R; KULKARNI, A S and BHAKARE, H A (1984). Paper presented at the ISF-OTAI World Congress, New Delhi.

KULKARNI, A S and BHAKARE, H A (1984). Paper presented at the ISF-OTAI World Congress, New Delhi.

KULKARNI, A S; KHOTPAL, R R and BHAKARE, H A (1987). *Proceedings of 1987 International Oil Palm/Palm Oil Conference - Technology.* Kuala Lumpur, Malaysia.

LEZAIC, J (1973). *Bilt. Bill Ulja, 1*, 3.

MALKIN,T and POOLE, A G (1953). *Jap. Chem. Soc.*, 3470.

MANGOLD, H K (1961). *J. Amer. Oil Chem. Soc., 38*, 708.

MIYAZAWA,T; IBA,S and FUZINA, Y (1976). *Ceraal Chemistry, 51*, 623.

NICHOLAS, B W (1964). *New Biological Separations.* James, A T and Morris, L J (ed). D. Van Nostrand, London.

PIERCE, A E (1968). *Silyation of organic compounds.* Pierce Chemical Company, Rockford, Illinois.

OSAGIE, A V and KATES, M (1984). *Lipids, 19*, 958.

ROUGHEN, P G; SLACK, C R and HOLLAND, R (1978). *Lipids, 13*, 497.

SKIPSKI, V P; PATERSON, R F and BARCLAY, J J (1966). *J. Lipid Res., 1*, 123.

STAHL, E (1962). *Thin Layer Chromatography.* Academic Press, London, pp. 465.

YASUDA, M J (1931). *J. Biol. Chem., 94*, 401.