Composition of Polar Lipids in Rapeseed

F. SOSULSKI[1], R. ZADERNOWSKI[2] and K. BABUCHOWSKI[2], [1]University of Saskatchewan, Saskatoon, Canada, and [2]University of Agriculture, Olsztyn, Poland

ABSTRACT

Phosphatidylcholine, -inositol and -ethanolamine represented 48, 18 and 8%, respectively, of the total phospholipids in medium and low erucic acid rapeseed. Oleic acid was the principal fatty acid in phosphatidylcholine and -ethanolamine but all components contained high levels of linoleic and saturated acids. There were eight components in the glycolipid fraction of which digalactosyl diglyceride, monogalactosyl diglyceride, sterol glycoside, esterified sterol glucoside and cerebrosides were identified. Defatted flours contained 2.0-3.5% of bound lipid which was composed primarily of phospholipids and glycolipids whereas neutral lipids were also a major component in the bound lipids of protein concentrates. The storage properties of these protein products would be adversely affected by the polyunsaturated fatty acids in these bound fractions.

INTRODUCTION

Lipoxygenase action on polyunsaturated fatty acids and oxidation of polar lipids such as phosphatidylcholine have been identified as factors responsible for bitter flavors in soybean (1). Polar lipids are the principal contaminants in crude oils and variable proportions remain in the meal to contribute off-flavors during storage (2).

The composition of rapeseed lecithin or gums from high erucic acid (HEAR) oils has been determined (3-5), but less information is available on the identification, yield and fatty acid composition of other polar lipid components, especially in medium (MEAR) and low (LEAR) erucic acid cultivars. Litchfield and Reiser (6) have identified phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG), sterol glucoside and esterified sterol glucoside in HEAR polar lipids. Fatty acid analyses of some of these lipid components have demonstrated the presence of relatively high levels of polyunsaturated fatty acids as compared to the neutral lipids (7).

The objectives of this study were to obtain quantitative data on the yield and fatty acid composition of the various phospho- and glycolipid components in MEAR and LEAR oils. In addition, the proportions of bound lipids in rapeseed meal, flour, hulls and protein concentrate were determined.

MATERIALS AND METHODS

The rapeseed samples were obtained from paired plots of Sinus, a MEAR cultivar from Sweden, and Janpol, a Polish LEAR cultivar, grown at six locations in Poland (8). Analyses were conducted in triplicate on bulked samples of mature, dried seed of each cultivar. The total lipids were extracted by repeated 1-hr extractions with chloroform/methanol (2:1, v/v) and, after removal of solvent by evaporation in a rotary evaporator under vacuum and nitrogen at 50-55 C, the lipids were stored at -18 C under nitrogen. Chloroform/methanol was selected over solvent systems which contained water because the water-containing solvents extracted nonlipid contaminants that were difficult to separate from the lipids.

Total polar lipid were separated from nonpolar fractions by adsorption chromatography using 100-mesh silicic acid (Mallinchrodt Chemical Works, St. Louis, MO) that had been activated by heating at 120 C for 24 hr. A 25-g sample of lipid was dissolved in 300 ml of chloroform, shaken for 20 min with 100 g of silicic acid and poured onto a fritted disc funnel. The nonpolar lipids were eluted with 1,500 ml of chloroform using low vacuum, and discarded after removal of solvent and weighing. Polar lipids were eluted with 1,000 ml of methanol. The eluents were dried at reduced pressure under nitrogen at 50 C, and the lipid fractions were weighed and stored at -18 C under nitrogen. The yield of the combined nonpolar and polar fractions was 99.4% of the original lipid in the chloroform/methanol extract.

The polar lipid fraction was purified of nonlipid components by gel filtration on a Sephadex G-25 column (9) using chloroform saturated with methanol and water as the eluent, and nitrogen pressure to maintain a flow rate of 1 ml/min. The first 50 ml of eluent contained 99.2% of the original polar lipid sample. The purified polar lipids were then separated into the two major components on 4 x 30 cm chromatographic columns of activated silicic acid (10). Polar lipids were applied to the column with chloroform/methanol (2:1, v/v); glycolipids eluted with 1,500 ml of acetone and phospholipids with 800 ml of methanol. The rate of polar lipid fractionation on the column was monitored to the 1,500-ml change by the two-stage thin layer chromatographic (TLC) technique described next. The eluted components were dried under vacuum and nitrogen at 40 C and weighed before storage under nitrogen at -18 C.

The main components of phospholipid and glycolipid fractions were separated on TLC plates coated with Silica Gel G (Merck, Darmstadt, W. Germany) at a depth of 0.25 mm for identification and 0.50 mm for quantitation. The chromatograms were developed by the two-dimensional technique (for identification) and the two-stage procedure (for quantitation and GLC analysis of constituent fatty acids) using chloroform/methanol/28% ammonium hydroxide/water (70:25:3.5:1.5, v/v) followed by chloroform/methanol/acetic acid/water (80:15:2:0.75, v/v) (2, 11). General detection of phospholipids was by spraying the plates with molybdic acid whereas phospholipids containing choline, inositol or amino groups were identified with Dragendorff's reagent, silver nitrate or ninhydrin solutions, respectively. α-Naphthol was used to visualize the glycolipid components whereas antimony trichloride served to locate sterol glycosides and glycoside esters. In addition, phospho- and glycolipid components were identifed by comparing the R_f values with chromatograms of standards and literature values (2,11,12). The TLC plates were also scanned for sulfolipids by the procedures of O'Brien and Benson (13).

Chromatograms used for analyses of fatty acids in the various lipid components were visualized by spraying with a 0.1% solution of 2,7-dichlorofluorescein in methanol. After comparison with standards on the same plates, the spots were scraped off, transferred to a fritted disc funnel and washed three times with methanol to elute the lipid components. The eluents were concentrated and dried under vacuum and nitrogen before weighing and gas liquid chromatographic (GLC) analysis or mineralization for phosphorus determination. Samples (10-50 mg) of the phospho- and glycolipids were dissolved in 1.5 ml of chloroform/methanol/sulfuric acid (100:100:1, v/v), transferred into 2-ml pharmaceutical vials and sealed hermeti-

TABLE I

Weight Percentage Composition of MEAR and LEAR Lipids and Phospholipid Components

Lipid classes	Phospholipid components	MEAR		LEAR	
		Total lipid	Phospholipid	Total lipid	Phospholipid
Neutral lipids		95.8 ± 0.4		95.5 ± 0.4	
Polar lipids					
Phospholipids		3.3 ± 0.3		3.6 ± 0.3	
	Phosphatidylcholine		49.2 ± 0.6		48.1 ± 0.6
	Phosphatidylinositol		17.2 ± 0.4		19.5 ± 0.4
	Phosphatidylethanolamine		7.7 ± 0.5		8.9 ± 0.6
	Other phosphorus compounds				
	X_1		11.6 ± 0.7		11.0 ± 0.3
	X_2		6.5 ± 0.5		5.5 ± 0.4
	X_3		7.9 ± 0.6		7.0 ± 0.6
Glycolipids		0.9 ± 0.1		0.9 ± 0.1	

cally under nitrogen (14). The hydrolysis and methylation of the fatty acids were carried out by heating the vials in a water bath at 90 C for 4 hr. After cooling, the tubes were opened and the sulfuric acid was decomposed with zinc powder. The methyl esters were dried in a stream of nitrogen, purified by hexane extraction (14), and quantitated by GLC on a Pye Unicam Model 104 gas chromatograph with a hydrogen flame detector. Glass columns, 210 cm x 4 mm, were packed with 10% DEGS on 60-80 mesh Chromosorb W. The column temperature was maintained at 195 C with the argon carrier gas flow at 60 ml/min. Peaks were identified by comparison with standards and by referring to relative retention times in the literature (3,7).

Quantitative determination of the phospholipid components (1-40 mg) was by mineralization of the eluted TLC fractions using 1 ml of 70% perchloric acid at 130 C for 30 min. Inorganic phosphorus was determined spectrophotometrically at 820 nm (15).

The sugar moieties in the glycolipid components were determined by waterless methanolysis using 10 ml of 0.5 N hydrochloric acid in methanol/8 mg lipid sample. Methanolysis was done under pressure in an atmosphere of nitrogen for 24 hr at 95 C. The products were extracted three times with 20 ml hexane, which was discarded, and the methanol/water layer was condensed to dryness under nitrogen. The sugars in the dry mixture were silylated with 0.5 ml of Tri-Sil-Z (Pierce Chem. Co., Rockford, IL) by incubation for 1 hr at 65 C (16). The trimethylsilyl (TMS) derivatives of the sugars were fractionated by GlC on 80-100 mesh Chromosorb W (HP) coated with 3% OV-1 and were identified by comparison with TMS derivatives of authentic standards.

Rapeseed flour, hulls and protein concentrate were prepared from hexane-extracted meal to determine the proportions of bound lipids in the protein products of each cultivar. Ground seed was defatted by extraction with diethyl hexane for 14 hr in a Soxhlet extractor, regrinding the meal and extracting a second time for 14 hr. After removal of solvent, the meal was fractionated into flour and hulls by air classification and the protein concentrate was prepared by washing the soluble constituents from the flour with two 1-hr extractions using 50% methanol. The bound

TABLE II

Fatty Acid Composition of the Principal Phospholipid and Glycolipid Components in Weight % Methyl Ester

Components[a]	16:0	16:1	16:3	17:0	18:0	18:1	18:2	18:3	20:1	22:1
Phospholipids										
Phosphatidylcholine										
MEAR	10.1	–	0.8	–	47.7	33.1	5.4	–	1.5	1.4
LEAR	8.7	0.8	1.2	–	55.8	30.9	1.9	0.2	0.5	--
Phosphatidylinositol										
MEAR	25.6	1.4	3.0	--	30.4	35.9	3.4	0.3	--	--
LEAR	21.8	0.8	1.9	--	33.6	38.1	3.6	0.2	--	--
Phosphatidylethanolamine										
MEAR	19.2	0.6	2.6	--	41.4	30.6	2.9	--	1.4	1.3
LEAR	17.7	1.8	2.0	--	47.7	27.3	2.7	0.3	0.5	--
Glycolipids										
Digalactosyl diglyceride (DGDG)										
MEAR	10.9	1.8	0.9	2.2	2.2	14.0	48.4	10.3	2.5	7.2
LEAR	20.2	3.4	tr	3.4	8.4	23.2	31.9	9.3	--	--
Monogalactosyl diglyceride (MGDG)										
MEAR	21.3	3.7	--	1.0	11.4	30.9	12.8	3.6	4.7	10.3
LEAR	19.1	9.9	--	--	6.7	43.3	15.9	9.5	--	--
Esterified sterol glucoside										
MEAR	22.6	1.9	0.6	0.8	5.9	43.0	11.4	3.9	3.7	7.4
LEAR	17.0	5.7	--	0.9	8.9	47.9	14.0	6.1	--	--

[a]Given as number of carbon atoms: number of double bonds.

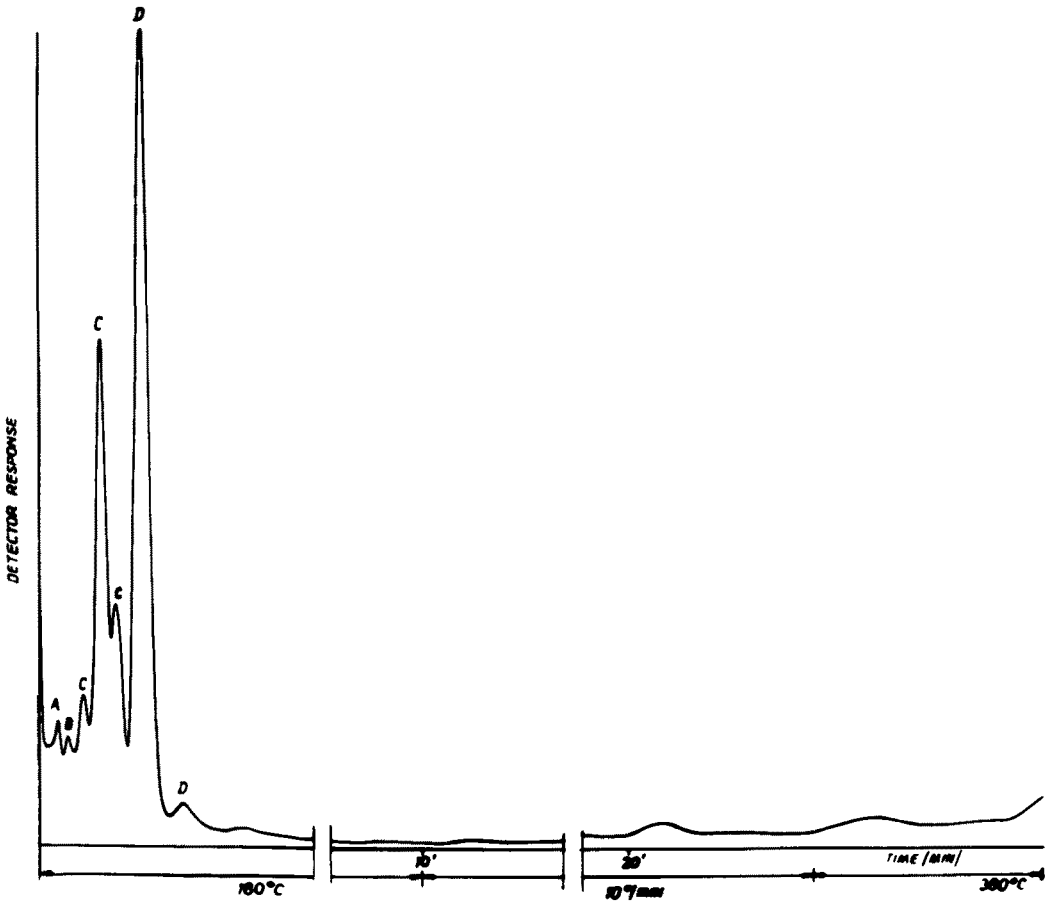

FIG. 1. Chromatogram of oxime TMS derivatives of sugars in glycolipids from rapeseed. Arabinose (A), mannose (B), galactose (C) and glucose (D).

lipids were extracted from the protein products with chloroform/methanol (2:1, v/v), concentrated under nitrogen and fractionated into polar and nonpolar lipid classes as was done for the total lipids. Protein content (N x 6.25) was determined by a micro-Kjeldahl procedure.

RESULTS AND DISCUSSION

Neutral lipids including triglycerides, diglycerides, monoglycerides and sterols represented 95.5-95.8% of the chloroform/methanol-soluble lipids in the two cultivars of rapeseed (Table I). Phospholipids constituted the bulk of the polar lipids (3.3-3.6%), but glycolipids also occurred in significant quantitites (0.9%). Generally, lower yields of total polar lipids (0.5-1.0%) are reported in the literature (3,7,12) because hexane is inefficient in solubilizing these free and bound polar compounds, especially in the absence of water. Despite the high recovery of polar compounds in our study, there was no evidence of sulfo-lipids in the TLC chromatograms.

Phosphatidylcholine, -inositol and -ethanolamine were the major phospholipids, representing ca. 48, 18 and 8%, respectively, of this class of lipid in each oil (Table I). Unidentified phospholipids accounted for ca. 25% of the total phosphorus-containing compounds found in the TLC chromatograms. Weenink and Tulloch (4) found that HEAR gums contained 22, 18 and 15%, respectively, of phosphatidylcholine, -inositol and -ethanolamine. Unknown phospholipids accounted for 16% of the rapeseed gums in their study and 9% was glycolipid which contained primarily long chain fatty acids (C22 and C24) in the molecule. Zajac and Niewiadomski (17) reported that the proportions of phospholipid components in HEAR gums were ca. 40:32:16, respectively, of phosphatidylcholine, -ethanolamine and -inositol.

Total saturated fatty acids in each phospholipid component were relatively high (Table II) and exceeded the levels found in triglycerides or other nonpolar components (8). Low concentrations of short chain fatty acids (12:0 and 14:0) were evident in most components.

Oleic acid was the principal fatty acid in phosphatidylcholine and -ethanolamine, but all components contained high levels of linoleic acid (Table II). The high levels of linoleic acid have been previously reported in rapeseed gums (3,4,7). Linolenic acid levels in the phospholipid components ranged from 1.9 to 5.4% (Table II). Small quantities of long chain unsaturated fatty acids (20:1 and 22:1) were found in two components of the MEAR lipids.

The MEAR and LEAR glycolipids separated into eight fractions in the TLC chromatograms. Five fractions were

TABLE III

Composition of Protein and Bound Lipids in Rapeseed Protein Products (Average of MEAR and LEAR Samples)

Protein product	Protein content (%)	Total bound lipids (%)
Defatted meal	39.6	2.5 - 3.0
Dehulled flour	46.6	2.0 - 3.5
Hulls	18.7	1.5 - 2.0
Protein concentrate	64.5	1.8 - 2.3

TABLE IV

Weight Percentage Composition of Flour and Concentrate Lipids and Phospholipid Components

Lipid classes	Phospholipid components	Flour		Concentrate	
		Total bound lipid	Phospholipid	Total bound lipid	Phospholipid
Neutral lipids		21.1 ± 0.5		46.7 ± 0.1	
Polar lipids					
	Phospholipids	47.5 ± 0.6		37.4 ± 0.6	
	Phosphatidylcholine		38.9 ± 0.6		48.3 ± 0.5
	Phosphatidylinositol		26.9 ± 0.5		22.3 ± 0.4
	Phosphatidylethanolamine		15.1 ± 0.4		13.6 ± 0.4
	Other phosphorus compounds				
	X_1		6.4 ± 0.2		7.5 ± 0.7
	X_2		8.5 ± 0.2		4.9 ± 0.6
	X_3		4.2 ± 0.5		3.3 ± 0.3
	Glycolipids	31.4 ± 0.8		15.8 ± 0.6	

identified as the glucoside diglycerides (MGDG, DGDG), sterol glucosides, esterified sterol glycosides and cerebrosides, but the yields were too low for accurate quantitation.

Fatty acid distributions were determined on three fractions and these were found to contain a wide range of fatty acids, especially in the MEAR cultivar (Table II). As in the phospholipid components, saturated fatty acids (16:0, 17:0, 18:0) represented 15.3-33.7% of the total fatty acids in the three glycolipid components. Linoleic acid was the principal fatty acid in DGDG, but oleic acid predominated in MGDG and the steryl glucoside. The DGDG component also contained a high level of linolenic acid and was much more polyunsaturated than MGDG or the steryl glucoside. In each MEAR fraction, long chain fatty acids occurred in relatively high concentration but were absent in the LEAR fractions.

Galactose and glucose were found to be the principal sugars in the glycolipid fraction with traces of mannose and arabinose also being detected (Fig. 1). The presence of anometric forms resulted in a triple peak for galactose and a double peak for glucose. The other components with long retention times could not be identified on the basis of known standards and may be hydrolytic products of sphingosine.

The levels of bound lipids in rapeseed meal and fractionated products were not constant and depended on seed sample, method of grinding and extraction procedure. Average values ranged between 2.5-3.0% for rapeseed meal and between 2.0-3.5% for rapeseed flours (Table III). Rapeseed hulls contained fairly high levels of protein and bound lipid so that flour was not greatly enriched in either constituent by the dehulling step that removed almost one-third of the meal material.

Phospholipids represented nearly one-half of the bound lipids in the defatted flour whereas the glycolipid level exceeded 30% (Table IV). The recoveries of phospholipids indicated that ca. 40% of the seed level was extracted in the crude oil, with the remainder plus the glycolipids being bound with these meal components. The phospholipids could complex with proteins through acidic groups whereas glycolipids may be associated with meal components through hydrophilic or hydrophobic bonds.

The neutral lipids represented almost 50% of the total bound lipids in the protein concentrates (Table IV). The lower proportions of phospholipids and glycolipids in the concentrates suggested that these components were partially extracted by the aqueous methanol solvent. The composition of phospholipid components in the flour and protein concentrate were 39-48% phosphatidylcholine, 22-27% phosphatidylinositol and 14-15% phosphatidylethanolamine. Compared to the total lipids (Table I), the latter two components were enriched relative to phosphatidylcholine.

High levels of polyunsaturated fatty acids in the polar lipids (Table II) and in the triglycerides (8) of MEAR and LEAR cultivars suggest that oxidative rancidity would be a problem during storage of rapeseed flour and protein concentrate. While freshly prepared protein concentrates are similar to the flour in flavor, they develop a strong, bitter taste within a few weeks of storage. This phenomenon may be associated with the high proportion of neutral lipids in rapeseed concentrate, as well as the generally high level of bound lipids.

REFERENCES

1. Rackis, J.J., D.J. Sessa and D.H. Honig, JAOCS 56:262 (1979).
2. Chapman, G.W., and J.A. Robertson, Ibid. 54:195 (1977).
3. Persmark, U., in "Rapeseed," edited by L.A. Appelqvist and R. Ohlson, Elsevier Publishing Co., Amsterdam, 1972, p. 288.
4. Weenink, R.O., and A.P. Tulloch, JAOCS 43:327 (1966).
5. Appelqvist, L.A., Ibid. 49:151 (1972).
6. Litchfield, C., and R. Reiser, Ibid. 42:757 (1965).
7. Appelqvist, L.A., in "Rapeseed," edited by L.A. Appelqvist and R. Ohlson, Elsevier Publishing Co., Amsterdam, 1972, p. 123.
8. Zadernowski, R., and F. Sosulski, JAOCS 55:870 (1978).
9. Wutheir, R.E., J. Lipid Res. 7:557 (1966).
10. Rouser, G., T.A. MacMurray and W.R. Morrison, J. Chromatogr. 47:227 (1970).
11. McKillican, M.E., and J.A.G. Larose, JAOCS 47:256 (1970).
12. McKillican, M.E., Ibid. 43:461 (1966).
13. O'Brien, J.S., and A.A. Benson, J. Lipid Res. 5:432 (1964).
14. Zadernowski, R., and F. Sosulski, JAOCS 56:1004 (1979).
15. Harris, W.D., and P. Popot, Ibid. 31:124 (1954).
16. Sabir, M.A., F.W. Sosulski and N.W. Hamon, J. Agric. Food Chem. 23:16 (1975).
17. Zajac, M., and H. Niewiadomski, Acta Aliment. Polon. 1:63 (1975).

[Received March 4, 1980]