Hard Red Spring and Durum Wheat Polar Lipids. I. Isolation and Quantitative Determinations[1]

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ABSTRACT

Quantitative analysis of both total and individual classes of lipids extracted from hard red spring (HRS) wheat flour and durum wheat semolina was conducted. Preparative thin-layer chromatography was used to determine the distribution of nonpolar and polar lipid fractions. Of the total lipids, HRS wheat flour yielded the highest amount of polar lipids with an average of 49.0%; durum wheat semolina, 37.3%; HRS whole wheat, 29.8%; and durum whole wheat, 26.4%. DEAE-cellulose column, thin-layer, gas-liquid, and paper chromatography were used to determine the identity, composition, and concentration of individual components of wheat polar lipids extracted from three HRS wheat flours, Chris, Selkirk, and Red River 68, and two durum semolinas, Leeds and Mindum. Sixteen polar lipid components were studied and quantitated for each variety. Esterified monogalactosyl monoglyceride, which had not been reported previously, was tentatively identified. Of the polar lipids, the average ratio of glycolipids to phospholipids was 1.5:1.0 for the HRS wheat varieties, and 1.3:1.0 for the durum varieties. Among the glycolipids, digalactosyl diglyceride (9.0 to 17.6% of the total lipids) and monogalactosyl diglycerides (3.1 to 5.4%) were the major components, with the semidwarf variety, Red River 68, containing the highest amounts. Cerebrosides were present in the next highest concentration, with a range of 1.8 to 3.0%. Chris flour contained the greatest amount of the lyso galactolipids. Of the phospholipids, phosphatidyl choline, *N*-acyl phosphatidyl ethanolamine, and their lyso analogs were the major components and represented 92 to 94% of the total phospholipids in all varieties investigated.

Although advances in chromatographic methods for lipid analysis have been remarkable in the past decade, quantitative analyses of wheat lipids are frequently confined to the determination of fatty-acid composition.

Recent studies by MacMurray and Morrison (1) and Clayton et al. (2) have reported 23 lipid classes in a single sample of spring wheat flour, 16 of which were polar. Three unidentified components were present (2).

This paper is a report on a study conducted on the polar lipids in three varieties of hard red spring (HRS) wheat flour and two varieties of durum semolina. One of the unidentified components reported by Clayton et al. (2) was tentatively identified. In addition, the distribution of nonpolar and polar fractions in the lipid extracts of 33 samples of ground whole wheat, flour, and semolina also was determined.

MATERIALS AND METHODS

Wheat Samples

Nineteen samples of HRS wheat (nine varieties) and 19 samples of durum wheat

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(nine varieties) grown at Carrington, Fargo, Langdon, and Minot in North Dakota during the crop years 1968, 1969, and 1970 were used. All samples were milled in different ways, depending on the need of the experiment. For ground whole wheat, HRS and durum wheats were ground on a Labconco mill; for flour, all varieties of HRS wheat were milled on a Buhler mill, with the exception of Chris grown in 1968, which was milled on a pilot Miag mill. Durum wheat samples were milled on a Buhler mill equipped with corrugated rolls and a Miag laboratory purifier to produce semolina.

Quantitative Analysis of Total Polar Lipids in Wheats

Twenty-gram samples of ground whole wheat, flour, and semolina were used. All samples were extracted with 100 ml. of water-saturated *n*-butanol (WSB) on a Burrell shaker for 30 min. After filtration, the extracts were evaporated to dryness at 45°C. The lipid residue was dissolved in chloroform-methanol (2:1 v./v.) and washed with distilled water (3). The chloroform phase was evaporated to dryness, and the residue was dissolved in chloroform. After centrifugation at 39,080 × g to remove the insolubles, the lipid solution was evaporated to dryness, and the dried material was weighed. This was redissolved in benzene-ethanol (4:1 v./v.) (4), flushed with nitrogen, and stored at −15°C. for further separation into nonpolar and polar fractions.

Nonpolar and polar fractions were separated on preparative thin-layer chromatography (TLC) plates (20 × 20 cm.) coated with silica gel G, 0.75 mm. thick. The lipid (80 to 100 mg.) was streaked on a TLC plate about 2 cm. from the bottom edge. Purified esterified steryl glucoside (ESG) was spotted as the reference lipid. After development in diethyl ether-acetic acid (100:1.5 v./v.) the plates were visualized briefly by iodine vapor. The bands above the ESG band were removed as the nonpolar fraction, while the remainder of the chromatogram, including the ESG band, was collected as the polar fraction.

To remove the polar lipids from the silica gel, 50 ml. of a chloroform-methanol-water-formic acid (97:97:4:2 v./v./v./v.) mixture (5) was added to the polar fraction in a 225-ml. centrifuge jar. The contents of the jar were stirred under nitrogen and centrifuged (1,130 × g). The supernatant was removed, and the extraction was repeated twice. The combined supernatants were evaporated to dryness. The nonpolar fraction was eluted by the same procedure, except that chloroform-methanol (1:1 v./v.) was used as the extraction solvent. Both the dried nonpolar and polar lipid fractions were redissolved in 20 ml. of chloroform and centrifuged at 39,080 × g for 10 min. to remove fine particles and insolubles. The supernatant was removed, evaporated to dryness, and weighed.

Quantitative Analysis of Individual Polar Lipids in HRS Wheat Flour and Durum Wheat Semolina

Thirty-five-gram samples of HRS wheat flour and durum semolina were extracted with 165 ml. of WSB mixture, as described previously.

The total lipids were fractionated on a DEAE-cellulose (Whatman Column Chromedium DE 11, W & R Balston Ltd., London) column prepared as described by Rouser et al. (6). The fractionation system was a modification of that by Galliard (4). Eight fractions were obtained. Each fraction was evaporated to dryness *in vacuo*, and the residue was dissolved in a determined volume of benzene-ethanol (4:1 v./v.), flushed with nitrogen, and stored at −15°C. for analysis. Fractions containing ammonium acetate were dried and dialyzed by the method of O'Brien et

al. (7) to remove the salt. After dialysis, only the chloroform layer was evaporated to dryness. The dried residue was redissolved in a determined volume of benzene-ethanol (4:1 v./v.), flushed with nitrogen, and stored at -15°C. for analysis.

The first fraction (neutral lipids) obtained from the DEAE-cellulose column separation was weighed directly, and no other analysis was performed. The other fractions were assayed for total sugar by the method of Galanos and Kapoulas (8), and total phosphorus by the method of Dodge and Phillips (9), with modifications. To analyze sugar content in the fraction, triplicate aliquots of lipid solution were pipetted into test tubes and dried. To each test tube was added 1 ml. of 95% ethanol and 1 ml. of phenol solution (6% in 95% ethanol). This was mixed, then 5 ml. of 95% sulfuric acid was added. After 10 min., the tubes were placed in a water bath at 20° to 30°C. for 20 min. Absorbances were measured at 490 nm. against the blank. Galactose was used as the standard.

This method could not be used for sterol-containing glycolipids, since hydrolysis of the lipids and complete removal of free sterols was necessary (8). An aliquot of this particular fraction was hydrolyzed 4 hr. with anhydrous 5% methanolic HCl in a sealed tube at 100°C. The residue was dried, dissolved in 2 ml. of water, and extracted three times with about 5 ml. of petroleum ether. The aqueous hydrolysate was analyzed for galactose, according to the phenolsulfuric acid procedure of Dubois et al. (10). To determine phosphorus, duplicate aliquots of lipid solution were dried completely in micro-Kjeldahl flasks. After addition of 1.1 ml. of 70% perchloric acid and a Pyrex glass-bead, the mixture was digested 15 min. and cooled, and 3 ml. of distilled water was added. The mixture was transferred to a 20-ml. test tube calibrated to the 10 ml. position. The following reagents were added to the tube, and the mixture was shaken after each addition: 3.0 ml. of 1% ammonium molybdate; 0.3 ml. of Fiske-Subbarow reagent; and water to the 10-ml. mark. The tube was then heated at 100°C. for 10 min. and cooled. The absorbance was read at 830 nm. against a reagent blank. Calcium glycerophosphate phosphorus (10 to 70 γ) was used as the standard phosphorus.

For the analysis of polar lipid fractions obtained from the DEAE-cellulose column, which contained more than two classes of glycolipids or phospholipds, it was necessary to use preparative TLC and to analyze each component separately. All lipids separated on preparative TLC plates were located with iodine vapor and marked. The lipid spots were scraped individually into test tubes and analyzed for sugar in the presence of silica gel. The sterol-containing glycolipids were hydrolyzed to free sterols in the presence of silica gel. After hydrolysis, the silica gel was removed by centrifugation. The supernatant was treated and analyzed as described. The phospholipid absorbed on the silica gel was extracted (5) and analyzed for phosphorus.

To avoid errors inherent in the application of the sample in the preparative TLC, ratios of components obtained from plates were related to the total amount of sugar or phosphorus determination for the original fraction. All the polar lipids described could be analyzed by sugar or phosphorus content. The data obtained in terms of micromoles of sugar or phosphorus were accordingly converted to micromoles of glycolipid or phospholipid.

Errors in the above determinations were within ±3% of average values. Each sample was analyzed in triplicate.

TLC

Silica gel G TLC plates of 0.25 mm. or 0.75 mm. were prepared. The former were used for class identification and separation, while the latter were used for isolation and quantitative analysis of the individual polar lipids found in the lipid fractions obtained by DEAE-cellulose column fractionation. The solvent systems used for the fractions are given in Table I.

Lipid spots on TLC plates were detected by charring with 50% sulfuric acid or were detected with specific spray reagents: 20% perchloric acid for all glycolipids (11); ninhydrin for amino-containing phospholipids (12); Dragendorff for choline-containing phospholipids (12); molybdenum blue reagent for all phospholipids (13). Lipid spots or bands on preparative TLC plates for fatty-acid analysis and purification purposes were sprayed with water and visualized under UV light.

TABLE I. SEPARATION OF DEAE-CELLULOSE COLUMN FRACTIONS BY TLC

Fraction	Major Components	Abbreviation	TLC Solvent System
1	Neutral lipids	---	---
2	Monoglyceride	MG	$CHCl_3$:MeOH:H_2O (80:8:1)
	Esterified steryl glucoside	ESG	
	Monogalactosyl diglyceride	MGDG	
3	Steryl glucoside	SG	$CHCl_3$:MeOH:H_2O (95:20:2.5)
	Esterified monogalactosyl monoglyceride	EMGMG	
	Free fatty acid	FFA	
	Phosphatidyl choline	PC	
4	Ceramide monoglucoside	CMH	$CHCl_3$:MeOH:HoAc:H_2O (170:25:25:4)(4)
	Monogalactosyl monoglyceride	MGMG	
	Digalactosyl diglyceride	DGDG	
	Phosphatidyl choline	PC	
	Lysophosphatidyl choline	LPC	
5	Digalactosyl monoglyceride	DGMG	$CHCl_3$:MeOH:HoAc:H_2O (170:25:25:4)(4)
	Phosphatidyl ethanolamine	PE	
6	Lysophosphatidyl ethanolamine	LPE	$CHCl_3$:MeOH:7N NH_4OH (65:30:4)(4) $CHCl_3$:MeOH:NH_4OH:H_2O (65:35:5:2.5) (2)
7	*N*-acyl phosphatidyl ethanolamine	APE	$CHCl_3$:MeOH:7N NH_4OH (65:30:4)(4)
	N-acyl lysophosphatidyl ethanolamine	LAPE	$CHCl_3$:MeOH:NH_4OH:H_2O (65:35:5:2.5)(2)
	Phosphatidic acid	PA	
8[a]	Phosphatidyl inositol	PI	$CHCl_3$:MeOH:NH_4OH:H_2O (65:35:5:2.5)(2)
	Phosphatidyl serine	PS	

[a]PI and PS were separated on Adsorbosil-3 rather than on silica gel G.

TABLE II. TOTAL, NONPOLAR, AND POLAR LIPIDS OF WHEAT

Type of Sample	Number of Samples		Total Lipids[a] %	Nonpolar Lipids[b] %	Polar Lipids[b] %	Recovery from TLC Plate %
HRS whole wheat	6	Range	1.40–1.89	68.8–71.2	28.8–31.2	88.3–95.6
		Average	1.55	70.2	29.8	92.5
Durum whole wheat	6	Range	1.26–2.02	71.4–76.3	23.3–28.7	85.4–96.2
		Average	1.60	73.6	26.4	91.5
HRS wheat flour	10	Range	0.93–1.22	46.7–57.1	42.9–53.3	84.8–96.7
		Average	1.06	51.0	49.0	90.6
Durum wheat semolina	11	Range	0.85–1.32	55.7–66.3	33.7–44.3	85.3–95.8
		Average	1.06	62.7	37.3	90.9 ,

[a]Single determination for each sample expressed on a dry basis.

[b]As percent of recovered lipids.

Gas-Liquid Chromatography (GLC)

Analyses of normal fatty acids, α-hydroxy fatty acids, and sterols were performed by GLC. Glass columns (3.5 mm.) were used, and detection was by flame ionization. The carrier gas was nitrogen with a flow rate of 35 ml. per min. Peak areas were determined by triangulation.

Normal fatty acids were converted to esters before analysis by the procedure of Metcalfe et al. (14). Separation was in a 6-ft. column packed with 15% diethylene glycol succinate on 100–120-mesh Gas Chrom P, and maintained at a temperature of 190°C. The α-hydroxy fatty-acid methyl esters were prepared from cerebrosides by the method of Morrison and Smith (15). The methyl esters were purified by preparative TLC (1) before analysis. A 4-ft. column, packed with 3% SE-30 on 80–100-mesh Gas Chrom Q, was used at 225°C. The free sterols liberated from the sterol-containing glycolipids by hydrolysis were analyzed by GLC, using a 6-ft. column packed with 3% SE-30 on 80–100-mesh Gas Chrom Q and maintained at 235°C.

Paper Chromatography

Glycolipids isolated from flour were hydrolyzed with 5% methanolic HCl (16) under nitrogen in sealed tubes at 100°C. for 4 hr. After drying, the residues were extracted with petroleum ether and distilled water. Aliquots of the aqueous extracts were applied to sheets of Whatman No. 1 paper. Descending paper chromatography was used with ethyl acetate:pyridine:water (10:4:3 v./v./v.) (17) as a solvent system. Polyhydroxy compounds were detected by the alkaline silver nitrate spray reagent (18).

RESULTS AND DISCUSSION

Total Lipid Extraction

As shown in Table II, the total lipid content of HRS or durum whole wheat was higher than that present in HRS wheat flour or durum semolina. The amount of total lipids extracted with the WSB in this study was slightly lower than that reported by Pomeranz et al. (19,20). The lower extraction yield may have been a result of the slightly different extraction procedure used, as well as the loss of lipids during the Folch washing procedure (3). Fisher et al. (21) and Bloksma (22) have

reported that the washing procedure may lead to loss of lipid material. However, the WSB extraction technique was considered the best solvent system (1,23).

Quantitative Analysis of Total Nonpolar and Polar Lipids Extracted from Wheats

Wheat lipids have been conventionally fractionated by silicic acid column chromatography into two categories, nonpolar and polar fractions. The latter contains mainly glycolipids and phospholipids. However, such a technique is laborious and time-consuming, particularly when such separation is required for many small samples. The present study presents a simple, rapid procedure by preparative TLC to separate the nonpolar and polar lipid fractions in the total wheat lipids. Figure 1 shows that, with the solvent system described, all polar lipids except ESG and monogalactosyl diglyceride (MGDG) remained at the origin on the TLC plates, while the nonpolar lipids were distributed across the remainder of the chromatogram. The separation of the polar and nonpolar lipids was taken between the ESG and monoglyceride (MG) components.

The amounts of nonpolar and polar lipid fractions in whole wheat, flour, and semolina are given in Table II. Lipid recovery from preparative TLC was in the range of 84.8 to 96.7%, with an average of 91%. These values are slightly lower than

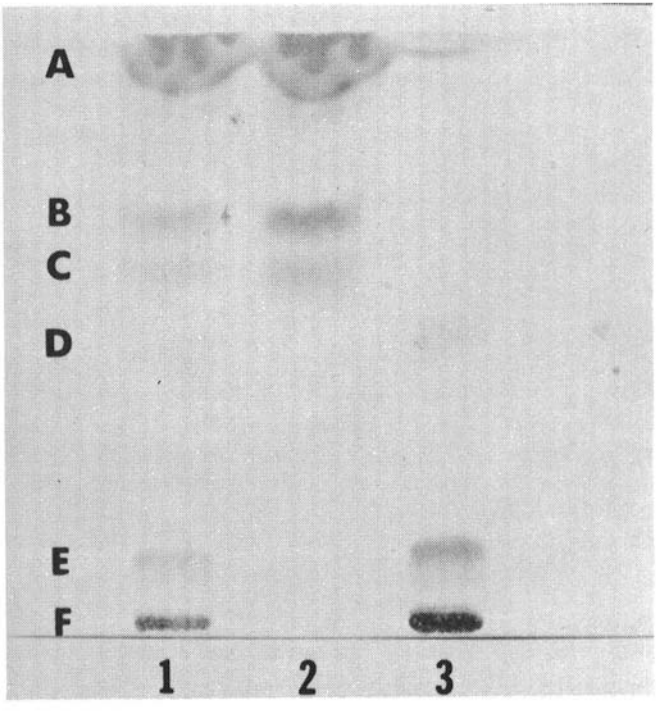

Fig. 1. A thin-layer chromatogram showing the separation of nonpolar and polar lipid fractions. Solvent system, diethyl ether-acetic acid (100:1.5 v./v.). 1, total flour lipids; 2, nonpolar fraction; 3, polar fraction; A, neutral lipids; B, diglycerides and sterols; C, MGs; D, ESG; E, MGDGs; F, origin (polar lipids).

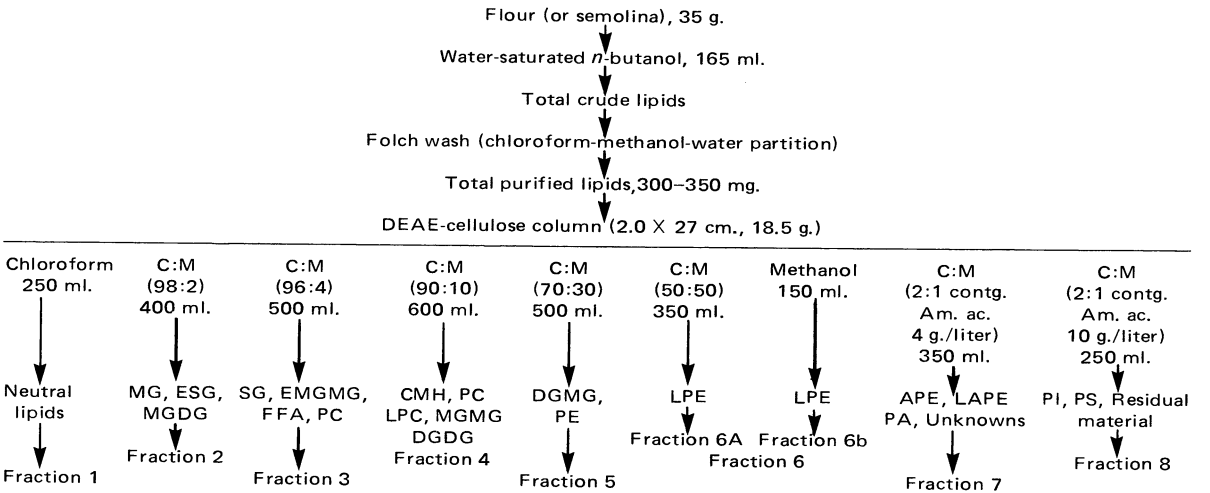

Fig. 2. Diagram of DEAE-cellulose column chromatography of wheat flour and semolina lipids. C = chloroform; M = methanol; Am. ac. = ammonium acetate.

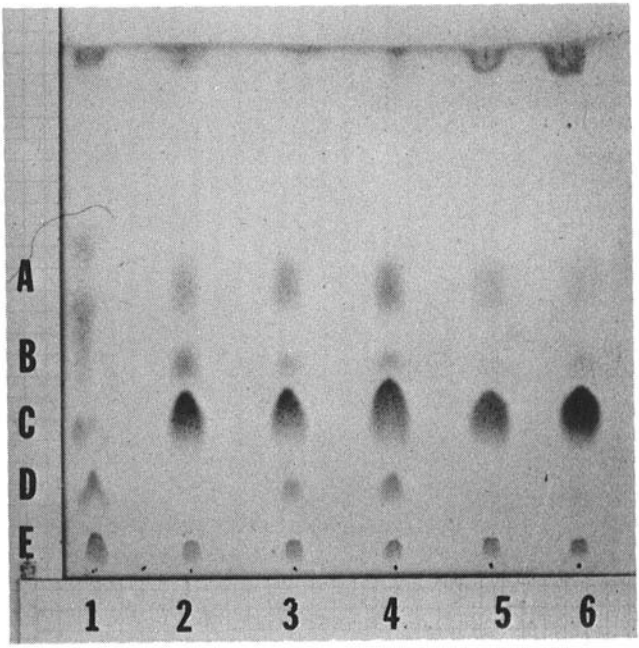

Fig. 3. Thin-layer chromatogram of fraction 4. Solvent system, chloroform-methanol-acetic acid-water (170:25:25:4 v./v./v./v.). Spray reagent, 20% perchloric acid. A, CMH; B, MGMG; C, DGDG; D, PC; E, LPC; 1, standard bovine CMH (two spots), DGDG, PC, and LPC; 2, Chris flour; 3, Selkirk flour; 4, Red River 68 flour; 5, Leeds semolina; 6, Mindum semolina.

those recovered from silicic acid column chromatography (20). However, the technique was rapid and particularly useful for separation of small quantities of nonpolar and polar lipids.

Of the total lipids, HRS wheat flour yielded the greatest amount of polar lipids with an average of 49.0%, followed by durum wheat semolina, 37.3%, HRS whole wheat, 29.8%, and durum whole wheat, 26.4%. In general, these data agreed with those reported by Pomeranz et al. (20).

Preliminary Fractionation of Wheat Flour or Semolina Lipids

A preliminary separation, using DEAE-cellulose column chromatography as developed for resolution of potato lipids by Galliard (4), was adopted. However, the original method was modified by adjusting the elution solvent and elution volume to give better separation of wheat lipids. Figure 2 shows the elution scheme used and the various lipid components obtained in each fraction. The neutral lipids were eluted with chloroform, while the glycolipids, phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), and their lyso analogues were eluted with various combinations of chloroform and methanol. The acidic lipids, such as *N*-acyl phosphatidyl ethanolamine (APE) and its lyso analogue (LAPE), phosphatidyl inositol (PI), and phosphatidyl serine (PS) were not eluted with the chloroform-methanol mixture unless salt, such as ammonium acetate, was added.

In the fractionation of wheat flour lipids on a DEAE-cellulose column

MacMurray and Morrison (1) failed to elute APE in a single fraction. These workers abandoned this procedure only because all of the phospholipid fractions eluted contained APE and LAPE. However, this problem did not occur in the present study. In general, the fractionation of wheat lipids by DEAE-cellulose column chromatography was found to be highly satisfactory and reproducible. The recovery of lipid from such a column averaged 99.2%.

Identification of Polar Lipids

The well-characterized lipids, such as MG, MGDG, PC, PE, PI, PS, CMH, DGDG, LPC, LPE, PA, and FFA were identified by TLC in the solvent systems given in Table I with known standards using specific spray reagents for detection. Identification of other lipids is described below.

ESG (6-o-acyl steryl glucoside). This lipid gave a characteristic pink spot on the TLC plate with 20% perchloric acid spray. It also gave a positive Lieberman-Burchard (24) test. Acid hydrolysis liberated free sterols, glucose, and FFA.

Esterified Monogalactosyl Monoglyceride (6-o-acyl-monogalactosyl monoglyceride) (EMGMG). This new class of lipid was tentatively identified in the present study. Clayton et al. (2) reported 23 lipid classes present in wheat flour; however, they did not identify a component which was marked as an unknown on their thin-layer chromatogram developed in chloroform-methanol-ammonium hydroxide-water (60:35:5:2.5 v./v./v./v.) mixture. Separation by the same solvent in the present study indicated that the R_f value of the spot believed to be EMGMG was the same as that of the unknown spot of Clayton et al. (2). This new lipid component gave a reddish brown spot on the TLC plate with 20% perchloric acid spray, which is a characteristic color of glycolipids. Acid hydrolysis of the lipid produced galactose, glycerol, and FFA. No sterols or α-hydroxy fatty acids were detected by GLC. Furthermore, the lipid contained 72% fatty acids, which is comparable to the theoretically calculated value of monogalactosyl dilinolein (73.1%). The compound migrated more slowly than the MGDG in the majority of the solvent systems investigated. Therefore, the indications are that this new lipid material is the lyso analogue of the well-characterized esterified monogalactosyl diglyceride, or 6-*o*-acyl monogalactosyl monoglyceride.

Steryl Glucoside. This lipid component gave a positive Lieberman-Burchard reaction. It also gave a pink spot on TLC plates with 20% perchloric acid spray. Acid hydrolysis liberated free sterols and glucose.

Cerebroside (ceramide monoglucoside) (CMH). This material gave a reddish pink spot on the TLC plate when sprayed with 20% perchloric acid. Acid hydrolysis liberated glucose, long chain bases, and FFA. However, no further identification of the long chain bases from the hydrolysate was attempted.

Standard bovine-brain CMH gave two spots on the TLC plate, whereas the wheat CMH showed only a single spot (Fig. 3). Moreover, the wheat CMH migrated closer to the slower-moving component of the pair of the standard. After investigating the human-serum CMH, Svennerholm and Svennerholm (25) explained that the faster-moving component of the pair contained normal fatty acids, while the slower component contained α-hydroxy fatty acids. In fact, after acid hydrolysis of the wheat CMH, more than 90% α-hydroxy fatty acids and less than 10% normal fatty acids were present.

Monogalactosyl Monoglyceride (MGMG). This isolated lipid gave a reddish brown spot on the TLC plate with 20% perchloric acid spray. Acid hydrolysis liberated FFA, glycerol, and galactose. When the R_f value of this compound was compared with that of the one identified by Clayton et al. (2), the component appeared to be the same as the one identified by the above workers, which was MGMG (Fig. 3).

Digalactosyl Monoglyceride (DGMG). This component gave a characteristic reddish-brown color with 20% perchloric acid spray on the TLC plate. Acid hydrolysis produced FFA, glycerol, and galactose. By comparing the R_f value of this component with those in the literature (1,2), this lipid component was tentatively identified as DGMG, a lyso analogue of DGDG.

The individual glycolipid components on the TLC plate for each fraction were removed and respotted. Figure 4 shows eight of the identified glycolipid components.

APE and LAPE. These two components (Fig. 5) were identified by comparing their R_f values with those reported by Clayton et al. (2). Both gave a negative ninhydrin reaction but a strong positive molybdenum blue test.

Normal Fatty-Acid Compositon of Polar Lipid Components

The distribution of the major fatty acids for each individual polar lipid class of three HRS flours, Chris, Selkirk, and Red River 68, and of two durum

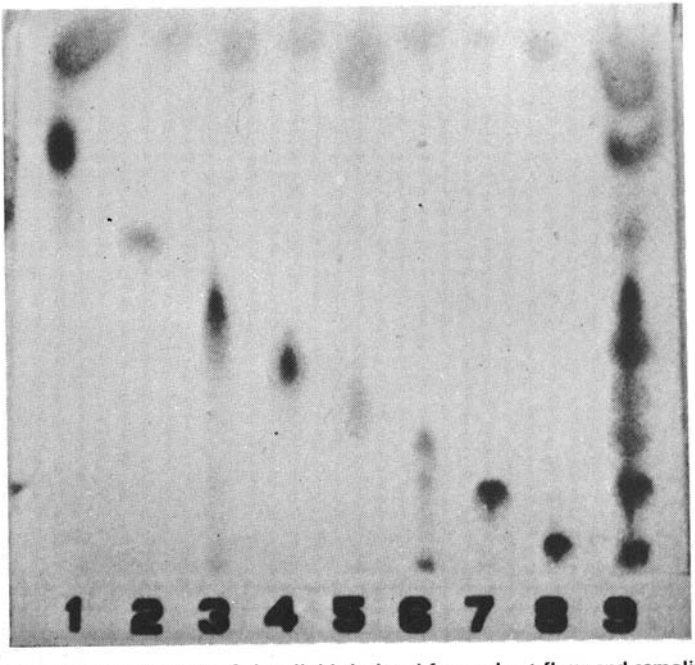

Fig. 4. Thin-layer chromatogram of glycolipids isolated from wheat flour and semolina. Solvent system, chloroform-methanol-water (95:20:2.5 v./v./v.); spray, 20% perchloric acid. 1, ESG; 2, MGDG; 3, EMGMG; 4, SG; 5, CMH; 6, MGMG; 7, DGDG; 8, DGMG; 9, mixture of these glycolipids.

semolinas, Leeds and Mindum, was determined. Only that of the two varieties, Chris and Leeds, is shown in Tables III and IV, respectively.

The major fatty acids in ESG for Selkirk, Red River 68, Leeds, and Mindum were palmitic (38.3 to 45.2%) and linoleic (23.3 to 29.6%), which were slightly different from those for Chris (Table III). In no instance was the fatty-acid distribution of the ESG of any sample similar to that reported by Myhre (26), but the results were closer to the values given by MacMurray and Morrison (1).

Regardless of the sample investigated, the galactosyl glycerides, such as EMGMG, MGMG, DGMG, and DGDG, had a relatively high content of linoleic acid, followed by palmitic and oleic acids. The fatty-acid composition of EMGMG varied considerably among varieties. Chris contained 7.7% palmitic, 13.0% oleic, and 74.6% linoleic acid; Selkirk, 22.6, 12.2, and 55.2%, respectively; and Red River 68, 17.7, 11.3, and 64.8%, respectively. Fatty-acid analysis of EMGMG has not been reported previously by other workers.

The fatty-acid analysis of MGMG showed that the four varieties analyzed contained palmitic (16.3 to 20.3%), oleic (11.9 to 21.0%), and linoleic acid

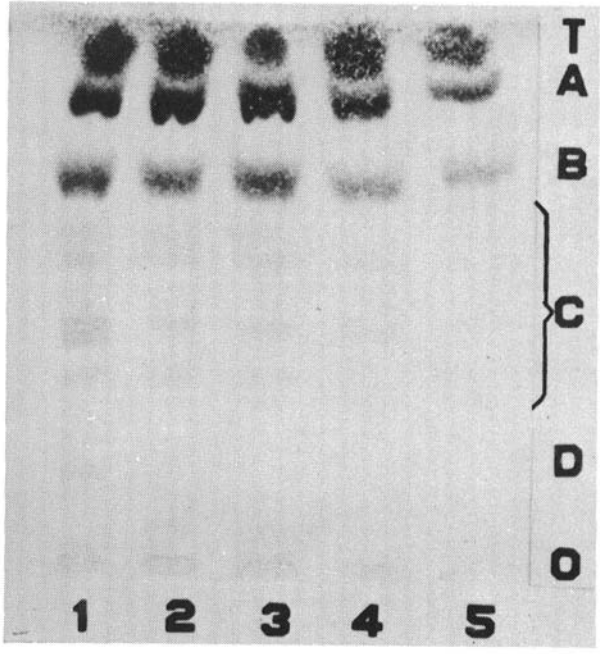

Fig. 5. Thin-layer chromatogram of fraction 7. Solvent system, chloroform-methanol-7N ammonium hydroxide (65:30:4 v./v./v.). Spray reagent, 50% sulfuric acid. T, pigments; A, APE; B, LAPE; C, unknown phospholipids (between B and D); D, LPE; O, material at origin; 1, Chris flour; 2, Selkirk flour; 3, Red River 68 flour; 4, Leeds semolina; 5, Mindum semolina.

(50.1 to 59.5%); however, Selkirk had a higher amount of linoleic acid (73.1%) and a lower amount of palmitic acid (8.8%).

The principal fatty acids in MGDG were palmitic (9.1 to 9.7%), oleic (9.2 to 13.5%), and linoleic acid (71.5 to 75.4%) for all varieties except Chris. These results were different from those reported by Carter et al. (27), who found that MGDG contained 13.9% palmitic acid and 57.0% linoleic acid, but were closer to those reported by MacMurray and Morrison (1).

DGDG contained principally palmitic (10.6 to 16.0%), oleic (6.0 to 9.3%), and linoleic acid (68.2 to 78.4%) for all varieties analyzed except Red River 68, which contained 29.4, 12.8, and 54.1%, respectively, of the above acids. DGDG was the only polar lipid component of all varieties investigated that did not contain palmitoleic acid. These results, except those obtained from Chris, agreed with those of MacMurray and Morrison (1). However, none of the results came close to the values of 41.6% palmitic acid and 29.3% linoleic acid reported by Carter et al. (27).

TABLE III. FATTY-ACID COMPOSITION OF INDIVIDUAL POLAR LIPIDS IN HRS WHEAT FLOUR (CHRIS)[a]

Lipid Class	Fatty Acid							
	$C_{12:0}$ %	$C_{14:0}$ %	$C_{16:0}$ %	$C_{16:1}$ %	$C_{18:0}$ %	$C_{18:1}$ %	$C_{18:2}$ %	$C_{18:3}$ %
ESG	0.3	1.2	31.4	1.4	3.0	13.8	46.2	2.7
MGDG	0.3	1.0	15.4	1.2	2.0	14.4	63.8	2.0
EMGMG	0.1	0.4	7.7	0.3	0.9	13.0	74.6	3.0
CMH	1.8	4.5	32.9	3.4	4.8	14.8	36.0	1.8
MGMG	0.7	1.4	17.2	0.9	1.7	21.0	56.0	1.1
DGDG	0.2	0.6	15.0	---	1.6	9.2	69.9	3.5
DGMG	---	0.2	10.1	---	0.8	8.4	78.5	1.8
APE	0.4	1.4	22.8	0.7	1.2	9.5	62.8	1.2
LAPE	0.7	2.4	25.4	2.3	2.1	12.5	53.3	1.3
PC	0.5	1.0	25.1	0.9	3.1	14.1	54.1	1.3
LPC	0.5	1.2	34.3	---	1.7	7.5	53.3	1.5
PE[b]	2.5	9.1	42.7	7.7	10.8	21.8	5.4	---
LPE	3.3	13.5	40.7	8.6	7.3	21.1	4.7	0.6
PI	4.3	8.9	33.0	5.6	8.6	16.5	21.3	1.8
PS[b]	2.3	4.6	31.1	3.6	19.8	27.7	9.1	1.7

[a]1968 crop year composite flour from several locations.

[b]Composite lipid of the two varieties, Chris and Selkirk.

In the varieties analyzed, the major phospholipids, such as PC, LPC, APE, and LAPE, had a relatively high content of linoleic acid, followed by palmitic acid and oleic acid, with some varietal variation in amounts. The saturated fatty acids were predominant in the minor phospholipids, such as PE, LPE, PI, and PS.

α-Hydroxy Fatty-Acid Composition of CMH

In common with most plant tissue (4,28), wheat CMH contained mainly α-hydroxy acids, more than 90% of the total fatty acids. As shown in Table V, the major acids in all varieties except Red River 68 were palmitic and arachidic α-hydroxy acids with lesser amounts of stearic and nonadecanoic

α-hydroxy acids. The analysis differed from that of Carter et al. (29,30), who found stearic α-hydroxy acid to be the principal acid in wheat CMH. However, similar values were reported by MacMurray and Morrison (1).

Sterol Composition of ESG and SG

The composition of sterols in these two lipids is given in Table VI.

GLC analysis showed that three peaks which individually corresponded to cholesterol, campesterol, and β-sitosterol were consistently present in all chromatograms for ESG and SG of all samples. The cholesterol peak would contain also the saturated sterol, cholestane-3-β-ol, since it had the same retention time as cholesterol. No report, however, has been given for the presence of this saturated sterol in wheat. Identification of cholesterol was based solely on its retention time. The addition of cholesterol to free sterols liberated from a wheat steryl glycolipid increased the area of the peak labeled as cholesterol proportionally. Berry et al. (31) indicated that wheat sterols contained a small amount of cholesterol, whereas McKillican (32) and MacMurray and Morrison (1) did not report the presence of cholesterol in either wheat steryl glycolipid. On the basis of GLC data obtained in this study, cholesterol was found to be comparable in amount to campesterol present in ESG and SG of most varieties. Although the relative amount of the different sterols varied with both variety and lipid component, β-sitosterol was the predominant component in all varieties and for both sterol-containing glycolipids. This result agreed with those in other reports (1,32). MacMurray and Morrison (1) also reported the presence of C_{28}- and C_{29}-saturated sterols. These two components were not investigated in the present study.

Composition of Lipid Classes from HRS Wheat Flour and Durum Wheat Semolina

All analytical results obtained on the individual lipid components, except direct weighing, which was used for the neutral lipids and a calculated weight

TABLE IV. FATTY-ACID COMPOSITION OF INDIVIDUAL POLAR LIPIDS IN DURUM WHEAT SEMOLINA (LEEDS)[a]

Lipid Class	Fatty Acid							
	$C_{12:0}$ %	$C_{14:0}$ %	$C_{16:0}$ %	$C_{16:1}$ %	$C_{18:0}$ %	$C_{18:1}$ %	$C_{18:2}$ %	$C_{18:3}$ %
ESG	1.0	3.9	40.9	5.3	6.3	14.1	26.7	2.0
MGDG	0.5	1.1	9.1	1.0	1.6	9.6	72.7	4.4
CMH	4.5	6.2	33.4	6.3	6.8	17.3	23.4	2.1
MGMG	1.2	4.2	20.3	3.7	3.9	13.1	50.1	3.6
DGDG	0.1	0.4	16.0	---	1.8	7.5	69.1	5.1
DGMG	---	0.2	12.8	---	1.4	9.6	71.7	4.3
APE	0.1	0.6	21.4	0.8	1.8	10.7	62.4	2.1
LAPE	0.7	1.7	27.1	0.8	2.5	10.6	54.9	1.7
PC	0.5	1.6	31.0	2.0	2.8	19.0	41.8	1.3
LPC	4.5	9.5	36.0	9.4	8.7	23.3	8.6	---
PE[b]	3.8	14.7	40.2	6.8	8.9	21.1	4.6	---
LPE[b]	2.6	8.4	32.8	3.3	8.0	19.1	24.3	1.6
PI	0.8	2.4	35.4	1.9	1.6	16.5	39.4	2.1
PS[b]	2.9	9.1	27.1	4.1	7.2	21.9	25.5	2.2

[a]1969 crop year from Minot, N. Dak.

[b]Composite lipid of the two varieties, Leeds and Mindum.

TABLE V. COMPOSITION OF α-HYDROXY FATTY ACIDS IN WHEAT CMH (CERAMIDE MONOGLUCOSIDE)

α-Hydroxy Acid	HRS Wheat Flour			Durum Semolina	
	Chris %	Selkirk %	Red River 68 %	Leeds %	Mindum %
$C_{16:0}$	34.8	40.7	29.3	38.3	41.3
$C_{18:0}$	8.7	9.4	25.8	11.2	10.6
$C_{19:0}$	7.7	9.4	22.2	5.9	6.5
$C_{20:0}$	39.9	40.5	22.8	43.5	41.7
Others	8.9	---	---	1.2	---

TABLE VI. COMPOSITION OF STEROLS IN WHEAT STERYL GLYCOLIPIDS

Lipid Class and Sterol	HRS Wheat Flour			Durum Semolina	
	Chris %	Selkirk %	Red River 68 %	Leeds %	Mindum %
6-*O*-acyl steryl glucoside (ESG)					
Cholesterol	17.8	21.2	40.5	13.0	1.6
Campesterol	20.1	21.1	12.0	19.4	15.2
β-sitosterol	62.1	57.6	47.5	67.6	83.2
Steryl glucoside (SG)					
Cholesterol	20.3	17.3	26.2	15.7	15.5
Campesterol	16.5	15.9	15.8	23.9	22.3
β-sitosterol	63.3	66.7	58.0	60.4	62.2

obtained for monoglyceride and free fatty acids, were expressed on a molar concentration basis. To compare all polar lipid components on a weight basis, the results obtained in terms of micromoles of lipids were converted to weight, using a calculated molecular weight derived from the composition of each lipid component of each wheat variety. Finally, each polar lipid component was expressed as percentage of total lipids. The results are shown in Table VII. Among the five varieties tested, an average of 94.5% of the total lipids were recovered, with the remaining portion (5.5%) representing unknown components, experimental loss, and material not quantitatively analyzed.

The polar lipid content present in HRS wheat flour was found to be about 10 to 15% higher than in durum semolina. In all varieties investigated, the glycolipids were present in a higher percentage than phospholipids. The ratio of glycolipids to phospholipids was 1.7:1.0 for Chris and Red River 68; 1.2:1.0 for Selkirk; and 1.3:1.0 for Leeds and Mindum. The average ratio of glycolipids to phospholipids for the samples studied was higher for HRS wheat flour (1.5:1.0) than for durum wheat semolina (1.3:1.0).

Of the glycolipids, MGDG and DGDG have been considered the major constituents. Acker et al. (33) found that the polar lipids contained about one-third galactolipids and included up to 10% MGDG. MacMurray and Morrison (1) reported that 8.7% of polar wheat lipids was MGDG, or 4.9% of the total lipids. The present study agreed with the above workers. The content of DGDG in the varieties as shown in Table VII also agrees with results of MacMurray and Morrison (1).

TABLE VII. COMPOSITION OF LIPID CLASSES FROM HRS WHEAT FLOUR AND DURUM WHEAT SEMOLINA

Lipid Class	HRS Wheat Flour			Durum Semolina	
	Chris %	Selkirk %	Red River 68 %	Leeds %	Mindum %
Neutral lipids	32.3	41.7	39.0	53.2	53.5
MG	1.2	1.8	0.9	1.1	1.5
FFA	18.8	7.8	8.7	9.5	9.9
Total nonpolar lipids	52.3	51.3	48.6	63.8	64.9
6-*O*-Acyl SG	1.7	1.2	0.8	1.3	1.5
MGDG	3.7	4.8	5.4	3.3	3.1
6-*O*-Acyl MGMG	2.5	0.7	0.6	0.3	0.5
SG	1.2	1.2	1.0	1.1	1.0
CMH	2.6	2.5	3.0	2.1	1.8
MGMG	1.9	0.5	0.8	0.5	0.6
DGDG	10.7	11.3	17.6	9.0	9.6
DGMG	2.1	0.6	0.8	0.2	0.1
Total glycolipids	26.4	22.8	30.0	17.8	18.1
PC	2.5	4.5	3.9	3.3	2.4
LPC	6.2	5.2	4.0	3.0	2.5
PE	0.2	0.4	0.4	0.3	0.3
LPE	0.3	0.5	0.2	0.2	0.2
APE	3.8	6.1	6.3	4.1	2.7
LAPE	2.7	2.5	3.0	2.1	1.8
PI	0.1	0.1	0.1	0.1	0.1
PS	0.1	0.1	0.1	0.2	0.2
Total phospholipids	15.9	19.4	18.1	13.4	10.1
Unresolved lipids	5.6	6.5	3.3	5.0	6.9

CMH, which constituted 1.8 to 3.0% of the total lipids or 6 to 7% of the total polar lipids, was next highest to MGDG in amount, followed by ESG and SG.

Chris flour contained higher amounts of EMGMG (2.5%) and mono- and digalactosyl monoglycerides (1.9 and 2.1%, respectively) than any other variety. The fact that the Chris flour had been stored for 2 years under ambient temperature before lipid extraction may have influenced these results. Hydrolysis of certain lipid components may have taken place during this storage period. It has been reported that some degree of selectivity occurs in the types of esterified fatty acids that are hydrolyzed in flour storage (1,2). The unusually high concentration of FFA in Chris flour might also indicate that some hydrolysis occurred during the prolonged storage of this flour.

Phospholipids, as a group, constituted less than 40% of the total polar lipids in all varieties, except Selkirk, in which they accounted for 45.2% of the total polar lipids. PC, LPC, APE, and LAPE constituted about 94% of total phospholipids in HRS wheat flour, and 92% in durum semolina. PC and LPC were present in nearly equal amounts in all varieties, except Chris flour, where the LPC was 2.5 times as much as PC. Again, the high amount of LPC in Chris flour may be the result of a hydrolytic process on PC during the long storage.

PA was detected in small amounts within the acidic phospholipid fraction, but was not quantitatively analyzed.

PE, LPE, PS, and PI, when combined, accounted for about 1% of the total lipids in all varieties.

Durum semolina lipids contained relatively lower amounts of major polar components, as compared to HRS wheat flour lipids. However, no varietal differences in the classes of polar lipid components were found in these two types of wheat. In general, the composition of HRS wheat flour lipids studied in the present work was found to be similar to the results reported by MacMurray and Morrison (1), although each variety had its own compositional pattern.

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