⁂Physicochemical Characterization of Galactosyldiglycerides and Their Quantitation in Wheat Flour Lipids by High Performance Liquid Chromatography

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ABSTRACT

A high performance liquid chromatographic (HPLC) method was developed for analyzing digalactosyldiglycerides (DGDG) and monogalactosyldiglyceride (MGDG) in polar lipids fractionated from lipid extracts of wheat or flour. Wheat lipid samples were prepared by solvent extraction, then fractionated on a silica gel packed open column. A Spherisorb ODS (octadecyl silane) column with methanol/water elution system was used for separation of glycolipids in the polar lipid fractions. The detection limit of the refractive index detector with interferometric optics was 0.25 μg for both DGDG and MGDG. Separating on nonpolar bonded phase columns permitted us to differentiate, based on fatty acid composition and position, among components within the specific glycolipid classes. Semipreparative HPLC on analytical columns was used to subfractionate the polar lipids. The glycolipids were collected for functional group characterization. Approximately 35% of each DGDG subfraction was accounted for as carbohydrate. The absence of phosphorus precluded phospholipids. Fatty acid analysis by gas chromatography showed the first DGDG to be linoleic acid, whereas the second DGDG peak was composed of linoleic, oleic and palmitic acids. Mass spectrometric analysis of the first DGDG peak showed linoleic acid in both the SN-1 and 2 positions. Mass spectrometric analysis revealed that palmitic or oleic acid in the second peak was preferentially located on the SN-1 position; linoleic acid was on the SN-2 position.

INTRODUCTION

The structure, composition and distribution of lipids in the wheat kernel have been the focus of several studies (1-6). Attempts also have been made to define the function of lipids in dough development and bread-making quality (7-9). Investigations have shown that the free wheat flour glycolipids, particularly digalactosyldiglycerides (DGDG), significantly enhance baking quality (7,10-12). Recently, it was reported that there were highly significant correlations between loaf volumes of hard red winter (HRW) wheat flours and polar lipid or galactose contents of wheat or flour free lipids extracted with petroleum ether (13,14).

Thin layer chromatography (TLC) has been used to qualitatively identify lipid classes (15-17). Lipid quantitation, to be effective, generally has required a combination of two or more chromatographic techniques (column, thin layer, gas) together with chemical analyses of certain elements or organic groups. More recently, lipid extracts have been analyzed by high performance liquid chromatography (HPLC) with a specially constructed, transport detector (pyrolysis moving-wire flame ionization) and a silica gel column (18,19). Because of the flour lipid link with baking quality, it was desirable to perform routine quantitative analysis of glycolipids in wheat and wheat flour by HPLC with a nonselective commercially available detector. Such analytical information may allow wheat breeders to predict eventual end use quality to some degree and thus make selections accordingly at earlier stages of developing new HRW varieties.

The analytes of interest, DGDG and monogalactosyldiglycerides (MGDG), which occur in wheat flour, were found in the polar fraction (eluted from a silica gel packed open column with methanol) of the petroleum ether (PE) lipid extract. Semipreparative separation and elucidation of components of the petroleum ether polar fraction (PE polar) were required.

Applications of the HPLC galactosyldiglyceride determination on wheat directly, to infer quality characteristics of the flour, provides preliminary evaluations in early generations without milling.

EXPERIMENTAL

Wheat and Flour Samples

Regional Baking Standard (RBS-75) was used for developing the analytical HPLC method of analyzing wheat flour glycolipids and for characterizing the HPLC subfraction of polar lipids. RBS-75 was untreated, straight-grade flour, experimentally milled (Allis) from a composite grist of many HRW wheat varieties harvested throughout the Great Plains in 1974. RBS-75 flour contained 0.42% ash and 12.4% protein (N × 5.7, 14% moisture basis, mb). A HRW wheat flour, experimentally milled from Shawnee (C.I. 14157) harvested in 1975, was used for isolating DGDG and MGDG for the HPLC quantitation standards.

The 20 HRW wheats and flours used for quantitation of DGDG and MGDG were as described elsewhere (14). Whole wheat samples were ground on a modified Udy-Weber hammer mill (U-D Corp., Boulder, CO, 0.024 in round hole sieve). Twenty straight-grade flours were experimentally milled (Allis) from the corresponding wheats. The wheat ash content ranged from 1.32 to 1.77%, and the protein content ranged from 11.6 to 15.7% (N × 5.7, 14% mb).

Chemicals

Chemicals were reagent grade. Water was deionized and distilled. Anhydrous methanol for HPLC use was distilled over Grignard reagent according to Vogel (20). Other solvents were HPLC grade (Fisher Scientific Co., Pittsburgh, PA). Ratios of all solvent systems are stated by volume. Reference lipids for TLC and gas chromatography were purchased from Applied Science Laboratories, Inc., State College, PA, and were stored at -18 C.

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[1] Contribution no. 80-207J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS. Part of a dissertation submitted by T.N. Tweeten in partial fulfillment of the PhD degree. Honored Student Award Presentation at AOCS annual meeting, San Francisco, April 1979.

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Preparation of Quantitation Standards for HPLC Wheat Galactosyldiglyceride Determination

The purchased DGDG and MGDG were not satisfactory for HPLC determination of wheat flour glycolipids because they produced multiple peaks, had differing fatty acid compositions, and thus different retention times from wheat flour glycolipids. As a necessity for quantitative determination, reference DGDG and MGDG were isolated from wheat flour. Lipids were extracted from HRW wheat (Shawnee) flour with Skellysolve B on a large Soxhlet extractor and fractionated on a silica gel packed open column. After the nonpolar lipids were eluted with chloroform, the crude MGDG fraction was eluted with chloroform/acetone (1:1, v/v), and the crude DGDG fraction was eluted with acetone. A spot check was run by TLC to verify the individual components were eluted before the next solvent system was introduced. The crude wheat MGDG and DGDG lipid fractions were subfractionated for physicochemical characterization by semipreparative HPLC with methanol/water 88:12, v/v) on a Spherisorb ODS column. Compositional homogeneity was verified by examination of analytical HPLC and direct inlet electron impact mass spectra of the semipreparative HPLC isolated subfractions of DGDG and MGDG.

Care had to be taken in concentrating the collected subfractions of crude DGDG and MGDG. Although the solvent composition of each subfraction was methanol/water (88:12, v/v), it was changed to ca. 1:1 due to faster evaporation of methanol than water during rotary evaporation at 30 C. Glycolipids were extracted with chloroform from the concentrated subfraction (methanol/water, 1:1, v/v), the aqueous layer was discarded and the residual moisture in chloroform layer was dried over anhydrous sodium sulfate overnight to minimize ester bond hydrolysis. The chloroform layer was then decanted, and the solvent removed on a rotary evaporator (ca. 30 C). Tared receiver vials were taken to constant weight after the lipid was added. Duplicate standards for each DGDG subfraction were prepared by separate fractionations, to compare response factors by the HPLC method.

Wheat and Flour Lipid Sample Preparation for HPLC

Lipids were extracted on a large Soxhlet extractor from 450 g (dry basis) RBS-75 flour with 2.5 ℓ of petroleum ether (bp: 35-60 C) or Shawnee flour with Skellysolve B for 24 hr. For determining DGDG and MGDG content, lipids were extracted from 10 g (dry basis) sample (wheats or flours) with 150 ml petroleum ether on a small Soxhlet extractor for 16 hr at a solvent condensation rate of 2-3 drops/min. Lipids were fractionated by silica gel packed open column chromatography into nonpolar and polar lipids with chloroform and methanol, respectively, as eluting solvents. Wheat or flour PE polar lipid fractions were preconcentrated on a rotary evaporator, then dried under a stream of nitrogen at 60 C to constant weight, redissolved in one part chloroform, gently agitated before one part methanol was added and stored at -18 C until analyzed. The polar lipids, dissolved in chloroform/methanol (1:1, v/v), were filtered through a Swinney filter adaptor (Millipore Corporation, Bedford, MA) fitted with a 0.5-μ Fluoropore filter (FHLP 01300). Samples injected before and after filtering showed no loss of glycolipid peak response due to sample clean-up.

High Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a high pressure pump (Model M6000A, Waters Associates, Inc., Milford, MA), an injector, a column, a detector and a recorder.

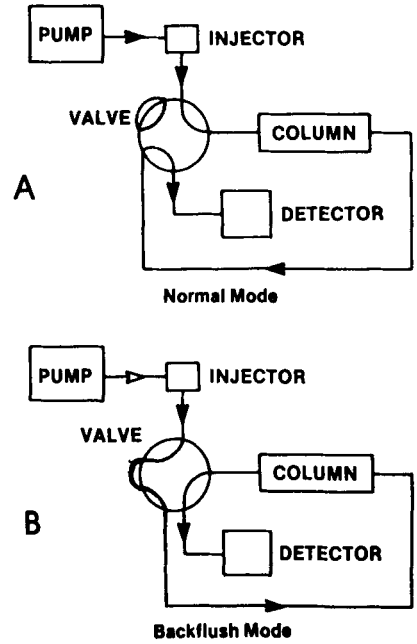


FIG. 1. Configuration of solvent flow: (A) normal direction of flow; and (B) reversed direction of flow through column.

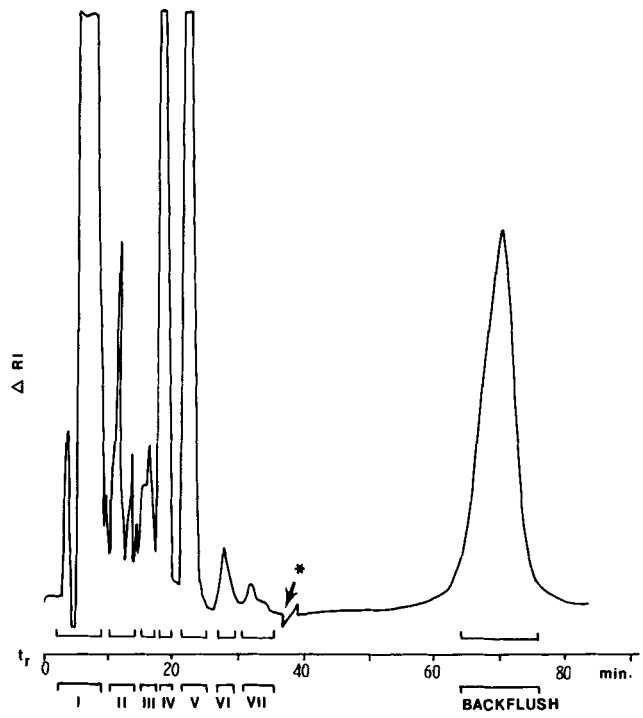


FIG. 2. HPLC fractionation of polar lipids separated on silicic acid open column from RBS-75 flour lipids extracted with petroleum ether. Subfractions I to VII were collected in the forward flow direction and the backflush was collected after the flow direction was reversed (*). HPLC conditions: Spherisorb ODS column, methanol/water (90:10), 1.2 ml/min, and RI detector.

The differential refractive index (RI) detectors used for comparison of glycolipid quantitative response were: (a) Model 0015-18-00 (Varian Instrument Co., Walnut Creek, CA) with water bath Model 001490 (Varian Instrument Co.); (b) Model 401 (Waters Associates, Inc.); and (c) Multiref 912 with a 10-mm path, 15-μl cell (Optilab AB BO Philip Instrumentation, AB, Vallingby, Sweden; US sales: Lachat Chemicals, Inc., Mequon, WI). Solvent tubing, column, and detector head were insulated for protection

from draft to minimize temperature fluctuations of the entire instrument.

An Omniscribe strip chart recorder (Model 5211, Houston Instrument, Austin, TX) equipped with a preamplifier for a 1-mV, full scale response was used to record detector output. Samples were introduced by a low pressure syringe (10 or 25 μl, Hamilton Co., Reno, NV) through a septumless, stop-flow injection system. Two high pressure valves were used after the pump: the first, to stop flow to the injector and the detector reference cell; the second, to control flow only to the detector reference cell.

The columns (25 cm × 4.1 mm id LiChroma 316 SS, Handy and Harman Tube Company, Norristown, PA) were milled out in the exit end to accept a 2-μ, stainless steel frit (0.1875 in od × 0.125 in. thick, Mott Metallurgical Corp., Farmington, CT) and fitted with zero dead volume, 1/16 in to 1/4 in. reducing unions (Swaglock 316 SS). A thin, porous (5-μ) teflon film (480P-5-55-1/8 in., Fluoro-Plastics, Inc., Philadelphia, PA) was sandwiched onto the top of each column to protect the packing material from particulate contamination. Columns used for the backflush were equipped at both ends with 5-μ SS frits (P.J. Colbert Assoc., St. Louis, MO) in the fittings. Column packing material was 10-μ Spherisorb ODS (Spectra Physics, Santa Clara, CA, Regis Chemical Co., Morton Grove, IL). Preparative HPLC of wheat DGDG and MGDG reference materials was done on a Silica Gel-60 (Size B) prepacked column (E.M. Laboratories, Inc., Elmsford, NY).

All the analytical columns used for semipreparative separations and quantitative analysis were packed by a slurry (balanced-density) technique (21). The packing material was dispersed in a volume of tetrachloroethane and 1,4-dioxane (3:1) with an ultrasonic bath. The slurry was rapidly extruded from the slurry reservoir (25 ml volume) into the column by hexane at a pressure of 10,000 psig. A pneumatic amplifier pump (Model DSTV-122/CP4, Haskel Engineering Products, Div., Burbank, CA) was used. Following slurry packing, columns were rinsed with 20-30 column volumes of a series of solvents (22) and tested with 2,6-xylenol/phenol, 1:1) eluted with methanol/water (60:40) at 1.0 ml/min. Only columns with 10,000 or more theoretical plates/m were used.

The previously described backflush technique (23) was used for quantitative recovery of all polar lipid components. A six-port valve (Model 70-10, Rheodyne, Berkeley, CA) was used with solvent flowing through the column forward (Fig. 1, A) to elute individual lipid peaks. Flow in the column was then reversed (Fig. 1, B) to elute the remaining material injected as one large peak.

Chemical Analyses

Phosphorus content was determined by wet chemical digestion of individual HPLC subfractions followed by complex formation with molybdate reagent (24) with dipalmitoylphosphatidylcholine as a standard. Carbohydrate analysis of the HPLC subfractions was accomplished by first reacting on the lipids in a hydrochloric acid/methanol mixture to form hydrolysis products (21). The carbohydrate, partitioned into an aqueous layer, was determined colorimetrically at 490 nm by the phenol-sulfuric acid method (22), with galactose as a standard. Protein, ash and moisture contents were determined for wheats and flours by AACC Approved Methods (25).

TABLE I

Phosphorus and Carbohydrate Contents of Subfractions[a] or Parent Fraction of RBS-75 Flour Polar Lipids

	Phosphorus or carbohydrate content[b] (%) of subfraction								
Component	I	II	III	IV	V	VI	VII	Backflush	Parent fraction
Phosphorus	0.53	2.78	–[c]	–[c]	–[c]	–[c]	0.63	1.74	1.12
Carbohydrate	24.00	13.40	24.9	35.6	31.6	27.9	30.30	9.70	19.30

[a]Subfractions were obtained by HPLC separation on Spherisorb ODS column.

[b]Average of 3 determinations, as galactose.

[c]No measurable amount.

TABLE II

Fatty Acid Composition of Subfractions or Parent Fraction of RBS-75 Flour Polar Lipids[a]

	Fatty acid[b] (%)				
Polar lipid subfractions	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
I	18.3	2.6	7.3	69.3	2.5
II	56.3	9.3	15.6	17.6	1.1
III	17.6	2.1	4.2	59.9	16.3
IV	6.4	2.4	1.7	88.0	1.7
V	25.4	1.6	8.7	64.8	0.3
VI	27.1	2.8	26.2	43.6	0.4
VII	29.9	11.4	12.5	45.2	1.1
Backflush	25.9	3.3	12.5	56.6	1.8
Parent fraction	21.2	1.1	6.6	68.5	2.8

[a]Polar lipids were obtained by a silicic acid open column separation and subfractions were obtained by HPLC separation on Spherisorb ODS column.

[b]Average of 5 determinations.

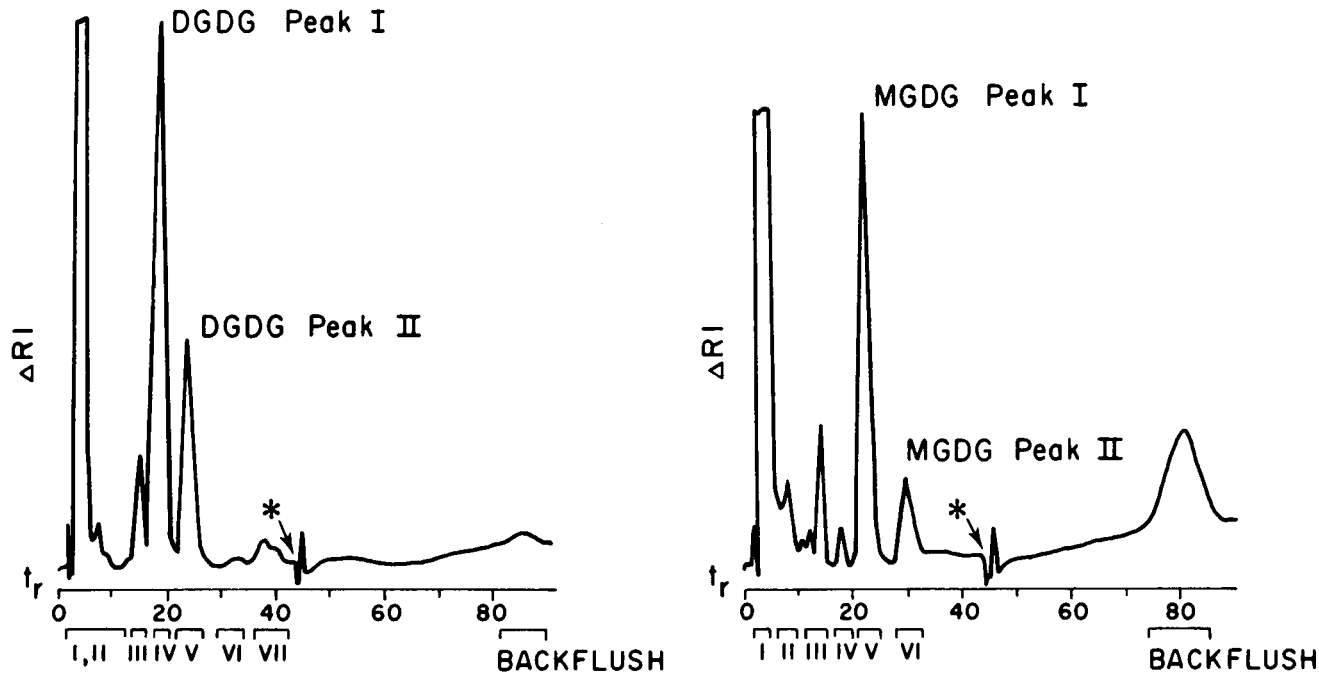


FIG. 3. HPLC fractionation of crude DGDG (left) and crude MGDG (right) separated on silicic acid open column from flour lipids extracted with Skellysolve B. HPLC conditions: Spherisorb ODS column (25 cm X 4.1 mm id), methanol/water (88:12), 1.4 ml/min for the forward flow and 1.6 ml/min for the reverse flow at (*), and RI detector.

Gas Chromatography and Thin Layer Chromatography (TLC)

A gas chromatograph (Model 5750, Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector was used. The separation was on 10% SP-2330 Chromosorb WAW 100/120 (Supelco, Inc., Bellefonte, PA) (1.8 m X 2 mm id stainless steel column) at a nitrogen flow rate of 20 ml/min and a column temperature of 185 C. Fatty acid methyl esters were prepared either by saponifying lipids with NaOH in methanol followed by boron trifluoride (in methanol, 14% w/v) catalyzed methylation of the fatty acids (26) or by acid hydrolysis followed by methanolysis (21). The results were integrated and displayed on the printer plotter of a 3385A laboratory automation system (Hewlett-Packard).

Glass TLC plates (20 X 20 X 0.38 cm) were coated with a 250-μm layer of Silica Gel G (E. Merck, A.G., Darmstadt, Germany) and activated for 2 hr at 130 C. Two solvent systems were used: chloroform/methanol/water (65:25:4) (6) and chloroform/methanol/ammonia (30% w/v)/water (60:35:5:2.5) (16). Plates were developed to 10 cm. Nonselective visualization of spots was done by heating with $K_2Cr_2O_7$ and H_2SO_4 (27). Phospholipids and glycolipids separated by TLC were selectively visualized by specific sprays and tentatively identified by comparing their R_f values to the literature (28-30) and to those of commercial plant lipid reference materials spotted on the same plates.

Spectrometric Analysis

Infrared spectra were recorded on an IR 457 (Perkin Elmer Corp., Norwalk, CT). Approximately 1-2 mg of glycolipid subfractions were placed on a NaCl salt disc. The sample was scanned in the double beam mode from 4,000 to 250 cm^{-1}.

The mass spectral analysis of lipids (5-10 μg) was done without prior derivatization on a Varian MAT 311A

TABLE III

Fatty Acid Composition of Shawnee Flour DGDG and MGDG[a]

Subfraction	Fatty acid[b] (%)				
	16:0	18:0	18:1	18:2	18:3
DGDG:					
IV (peak I)	2.3	0.2	0.4	94.1	2.9
V (peak II)	37.3	–	12.0	50.8	–
MGDG:					
V (peak I)	0.8	–	0.3	98.4	0.5
VI (peak II)	24.0	–	26.7	49.3	–

[a]Crude DGDG and MGDG were obtained by a silicic acid open column separation and subfractions were obtained by HPLC separation on Spherisorb ODS column.

[b]Average of 2 determinations.

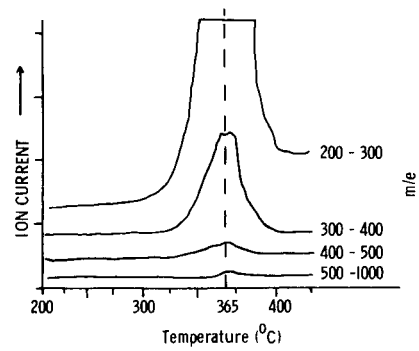


FIG. 4. Mass thermogram of DGDG peak I collected from the HPLC separation on Spherisorb ODS column of flour crude DGDG. Total ion current summed for the mass ranges indicated. Approximately 4-μg sample was introduced by direct sample inlet at 70 eV. Thermal ramp ca. 15 min.

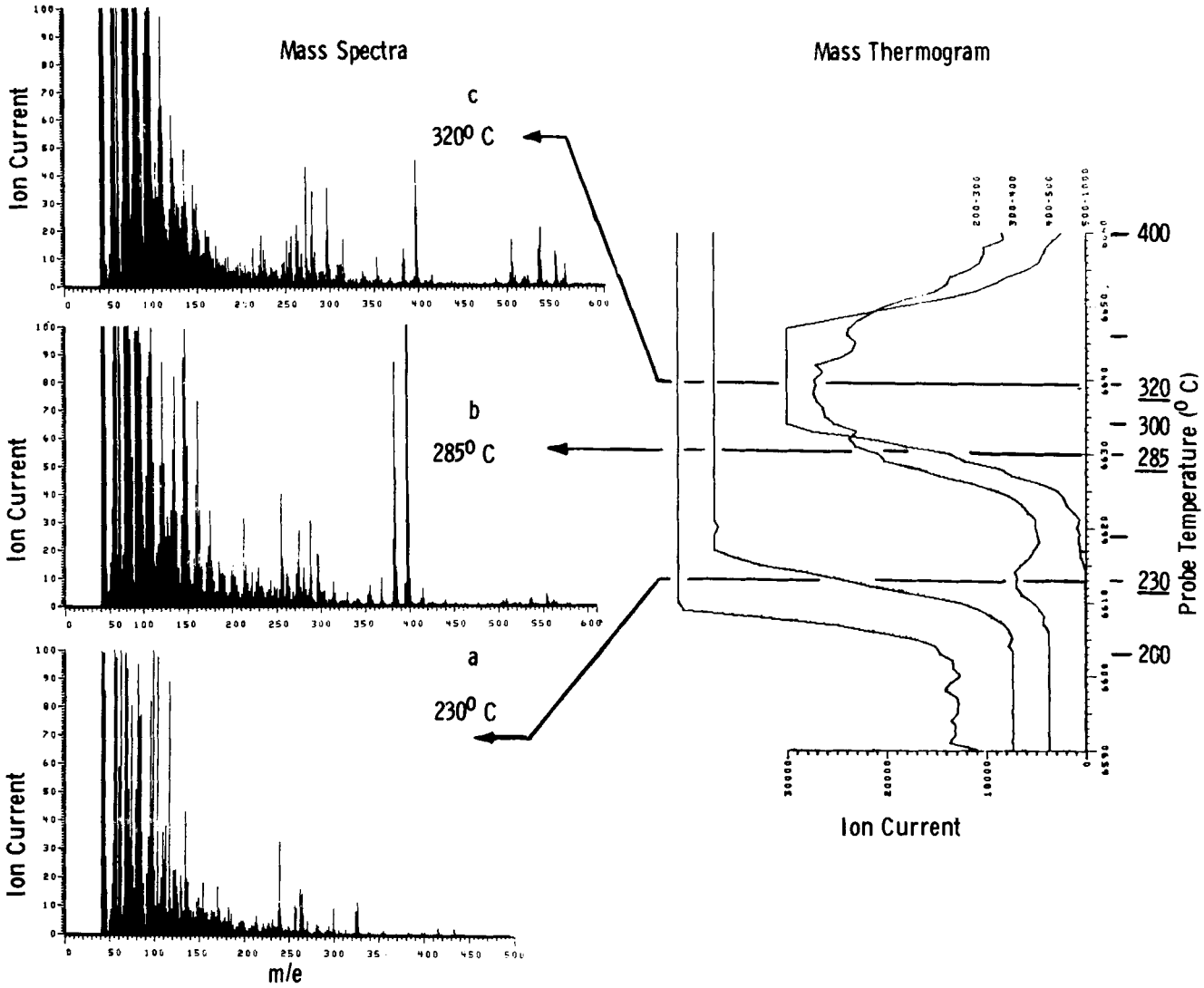


FIG. 5. Mass thermogram of RBS-75 flour polar lipid subfraction III collected from the HPLC separation on Spherisorb ODS column. Individual mass spectra shown for mass thermogram ionization peaks. Approximately 4-μg sample was introduced by direct sample inlet at 70 eV.

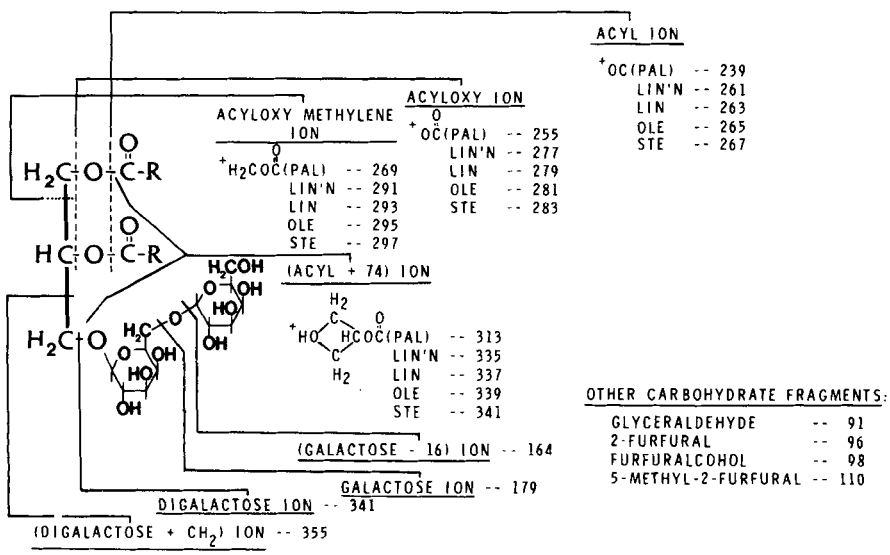
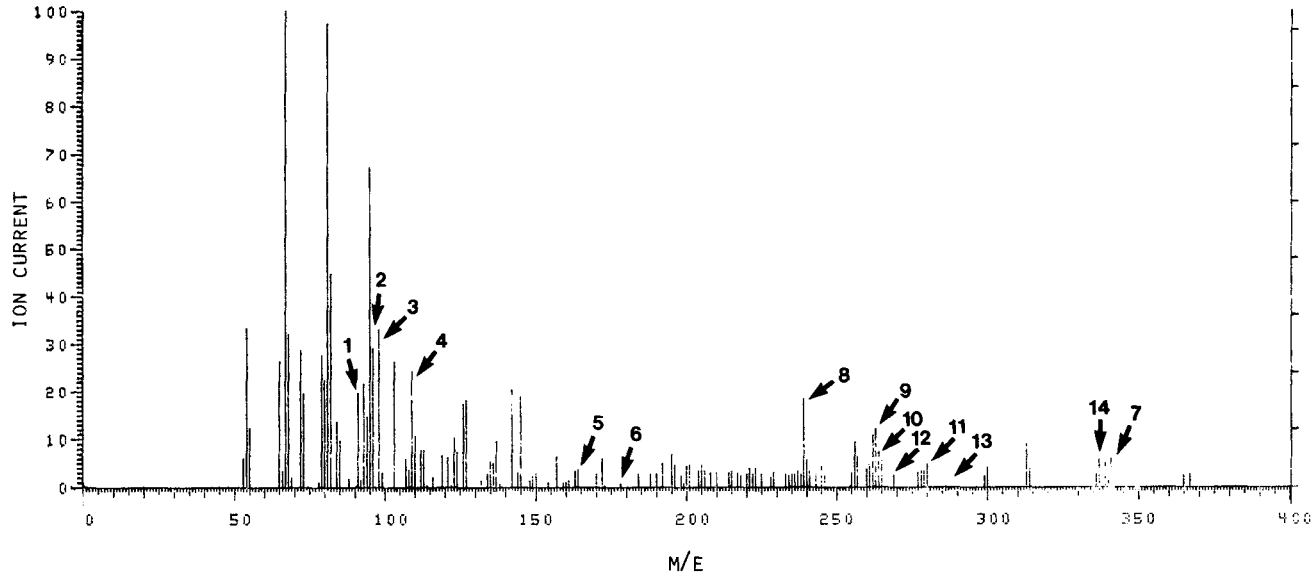


FIG. 6. Molecular ion fragments used to interpret the mass spectra of DGDG.



CARBOHYDRATE IONS	ACYL RCO+	ACYLOXY RCOO+	ACYLOXY METHYLENE $RCOOCH_2^+$	ACYL + 74 (RCO + 74)+
1. GLYCERALDEHYDE+	8. PALMITIC	11. LINOLEIC	12. PALMITIC	14. LINOLEIC
2. 2-FURFURAL+	9. LINOLEIC		13. OLEIC	
3. FURFURALCOHOL+	10. OLEIC			
4. 5-METHYL-2-FURFURALDEHYDE+				
5. (GALACTOSE - 16)+				
6. GALACTOSE+				
7. DIGALACTOSE+				

SN-1: PALMITIC SN-2: LINOLEIC SN-3: DIGALACTOSE
OLEIC

FIG. 7. Mass spectrum of DGDG peak II collected from the HPLC separation on Spherisorb ODS column and the interpretation of the ion fragment pattern. Sampling direct inlet at 70 eV.

equipped with a temperature-programmed sample probe and data handling system. The instrument was operated at 70 eV with an emission current of 1 mA. The multiplier voltage (M_V) was 2.1 kV and the multiplier gain (MG) was 5×10^6. The mass (m/e) range scanned was 40 to 850 at 5 sec/mass decade. The mass thermogram was a record of ion current response for one or more mass units over the temperature ramp imposed on the sample probe. Mass spectra were recorded at peak current responses for specific temperatures on the mass thermogram temperature ramp.

DISCUSSION

Characterization

Eight subfractions were collected from the semipreparative HPLC separation of polar lipids of RBS-75 flour (Fig. 2). Seven subfractions were collected in the forward direction of solvent flow; the eighth, by reversing the flow in the column to elute remaining material injected.

Phosphorus was not detected in subfractions III to VI of the polar lipids (Table I). Subfractions II and backflush subfractions contained substantial phosphorus indicating most phospholipid eluted early or late chromatographically. Carbohydrate analysis showed subfraction IV with a percentage of galactose close to the theoretical value calculated for DGDG (Table I), subfraction V intermediate for DGDG and MGDG, and subfraction VI close to that calculated for MGDG. Carbohydrate content of the backflush subfraction was low.

Linoleic acid was a major fatty acid and palmitic acid was a major saturated fatty acid for every subfraction of polar lipids and the unfractionated polar lipids (Table II). Subfraction II contained the highest percentage of saturated fatty acids. Saturated fatty acid content increased from subfraction IV to subfraction VII. A decrease in unsaturation stemmed primarily from linoleic acid.

Infrared spectra obtained for subfractions III to VII, glycolipids of concern, decreased successively in intensity (at 3010 wave numbers), the C-H alkene stretch band. These results supported the conclusion that unsaturated fatty acid content decreased from subfraction IV to VII as shown in Table II. Also, subfractions III and VII have less intense bands than subfractions IV to VI at 1740 wave numbers corresponding to the C = 0 stretch of the carboxyl group. It indicated that the glycolipids present in subfraction III or VII were not all glycerol-based glycolipids.

Thin layer chromatography of each polar lipid subfrac-

TABLE IV

Galactosyldiglyceride Structures Based on Mass Spectral Analyses[a]

Galactosyldiglycerides	Positional isomers: Fatty acid SN-1	Fatty acid SN-2	Carbohydrate SN-3
DGDG:			
peak I	Linoleic	Linoleic	Digalactose
peak II	Palmitic	Linoleic	Digalactose
	Oleic	Linoleic	Digalactose
MGDG:			
peak I	Linoleic	Linoleic	Monogalactose
peak II	Palmitic	Linoleic	Monogalactose
	Oleic	Linoleic	Monogalactose

[a]Mass spectrometer: direct inlet sampling, 70 eV.

TABLE V

Comparison of Detector Responses for the Determination of Digalactosyldiglycerides from Flour Polar Lipids

Detector	Working linear range (μg/inj.) Unfractionated polar lipids	Working linear range (μg/inj.) DGDG (peak I)	Sensitivity range (ΔRI full scale)	Detection limit (Attenuation)
Refractive index (deflection)	40.0 - 1920	20.0 - 800	5×10^{-6} - 3.2×10^{-4}	5.0 μg (X1)
Refractive index (interferometry)	25.0 - 375	1.0 - 50	5×10^{-7} - 1×10^{-5}	0.25 μg[a] (X5)

[a]Determined without water bath temperature control.

tion was used to obtain additional quantitative information. It showed that subfraction IV contained DGDG only, V contained a mixture of DGDG and MGDG, and VI contained mainly MGDG. Subfraction III contained sterylglycoside only and VII contained sterylglycoside mainly and a small amount of unidentified phospholipids. Subfraction I contained digalactosylmonoglycerides, monogalactosylmonoglycerides and lysophosphatidylcholines; II contained mainly N-acyl phosphatidylethanolamines and a small amount of sterylglycosides; and backflush contained a mixture of phosphatidylcholines, phosphatidylethanolamines and N-acyl phosphatidylethanolamines.

Results heretofore required the preparation of individual DGDG and MGDG quantitative standards from wheat lipid. The crude DGDG and MGDG fractions from silica gel open column chromatography were subfractionated by HPLC on the Spherisorb ODS column (Fig. 3). Retention time of each subfraction of crude DGDG or MGDG fractions was similar to that of polar lipids in Figure 2. Subfraction IV was the first and V was the second major fraction of the crude DGDG (left, Fig. 3), whereas subfraction V was the first and VI was the second major fraction of the crude MGDG (right, Fig. 3).

TLC indicated subfractions IV and V of crude DGDG contained only DGDG and subfractions V and VI of crude MGDG contained only MGDG. Consequently, wheat flour DGDG and MGDG showed two peaks each. We designated subfractions IV and V of crude DGDG as DGDG peaks I and II, respectively, and subfractions V and VI of crude MGDG as MGDG peaks I and II, respectively (Fig. 3). Retention time of DGDG peak II was same as retention time of MGDG peak I. Peaks I of both DGDG and MGDG were acylated with linoleic acid, whereas peaks II of DGDG and MGDG were comprised of mixture of fatty acids (Table

TABLE VI

Lipid, Polar Lipid, Digalactosyldiglyceride (DGDG) and Monogalactosylglyceride (MGDG) Contents in HRW Wheats and Milled Flours

Sample[a]/crop year	Wheat Lipid[b] (%)	Wheat Polar[c] (%)	Wheat DGDG[d] (%)	Wheat MGDG[d] (%)	Flour Lipid[b] (%)	Flour Polar[c] (%)	Flour DGDG[d] (%)	Flour MGDG[d] (%)
Parker/1975	1.959	9.45	1.12	0.69	0.875	19.84	6.69	3.04
Eagle/1075	1.885	11.35	1.42	0.48	0.925	21.58	7.53	3.39
Osage/1975	2.100	10.95	1.94	0.67	1.033	19.21	7.99	3.74
KS73159/1975	1.818	13.19	2.55	1.04	0.900	25.60	9.77	5.02
KS73199/1975	1.950	11.64	2.45	1.08	1.012	23.13	10.56	5.34
Plainsman V/1975	1.886	13.75	2.23	1.66	0.996	24.96	7.42	4.47
7303/1975	1.945	12.66	2.08	1.20	1.087	24.01	6.85	4.37
KS73H590/1975	1.855	11.24	1.81	1.31	0.927	20.50	8.20	4.90
KS73H593/1975	1.907	10.70	1.38	1.01	0.916	19.69	7.36	4.17
Lancota/1975	1.814	12.61	2.18	1.27	0.933	25.13	8.82	4.53
KS644/1974 & 1976	1.922	12.59	2.45	1.64	0.949	23.80	8.47	4.66
Shawnee/1975	1.897	13.67	2.53	1.72	0.895	28.83	9.82	3.79
Shawnee/1973	2.041	12.75	2.69	1.53	0.946	28.79	11.60	6.56
Ot. sel./1975	1.847	12.98	2.43	1.66	0.928	23.73	8.20	4.25
Ot. sel./1973	1.867	8.53	1.18	0.91	0.932	17.38	6.25	4.57
KS501097/1973	1.823	9.35	1.27	1.21	0.838	14.62	2.62	1.80
White RCh/1971	1.888	8.28	1.19	1.01	0.869	20.55	4.57	3.16
C.I. 12995/1972	2.303	13.64	2.59	1.74	1.045	26.15	3.45	5.03
KS501097 & KS501098	1.654	6.97	0.62	0.72	0.946	13.07	3.05	3.46
RBS/1973	–	–	–	–	0.910	24.30	7.94	5.50
RBS/1974	–	–	–	–	0.958	26.28	8.68	4.50
RBS/1975	1.969	11.01	2.34	1.52	0.998	25.04	9.75	5.38
Average	1.919	11.37	1.92	1.20	0.946	22.55	7.53	4.35

[a]Ot. sel. = Ottawa selections; RCh = Red Chief; KS501097 & KS501098 = a composite of 2 varieties harvested in 1974 and 1976; RBS = Regional Baking Standard; numbers are C.I. or selection numbers.

[b]Petroleum ether extracts, expressed as % sample weight on dry basis. Average of 4 extractions.

[c]Fractionated on silicic acid open column, expressed as % petroleum ether extracts. Average of 4 fractionations.

[d]DGDG and MGDG contents were determined by the HPLC method on Spherisorb ODS and are expressed as % petroleum ether extracts. Average of 2 determinations.

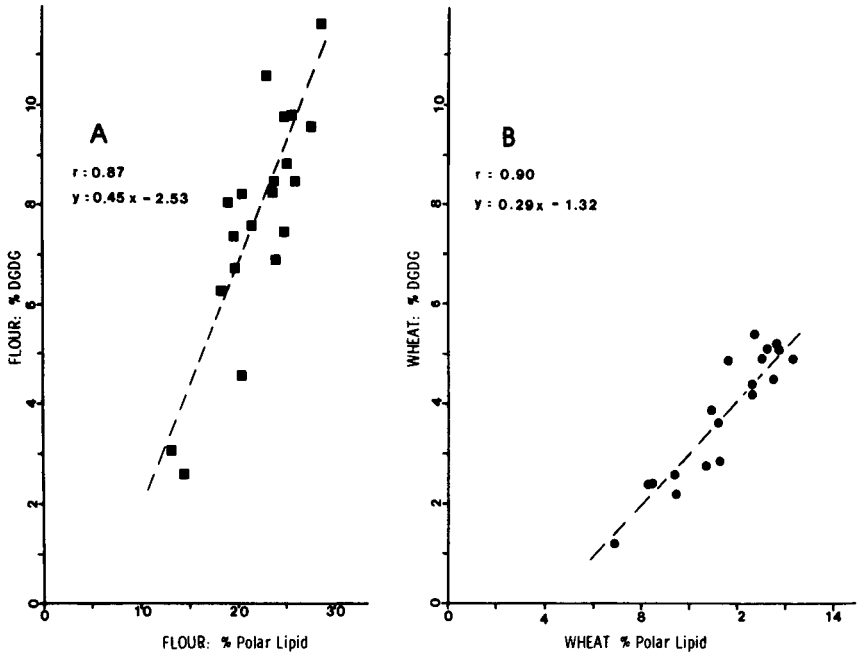


FIG. 8. Linear relation between polar lipid content and DGDG contents in lipids extracted with petroleum ether from 20 milled flours (A) and corresponding wheats (B).

III).

The DGDG, peaks I and II, MGDG, peaks I and II, along with subfractions III and VII (Fig. 3) were analyzed without prior derivatization by direct inlet electron impact mass spectrometer. A mass thermogram (Fig. 4), summation of ion current for a particular mass or combination of masses as a function of sample probe temperature, showed a single maximum for the mass ranges over the temperature ramp for subfraction IV indicating homogeneity. A similar thermogram of a single peak was obtained for each DGDG peak II or peaks I and II of MGDG (not shown). However, three separate ion current maxima were observed in the mass thermogram of subfraction III (Fig. 5) and two maxima were observed for subfraction VII (not shown) of flour polar lipids. Each mass thermogram maximum generates a mass spectrum with different fragment ion intensities. Consequently, it was concluded that polar lipid subfractions III and VII lacked homogeneity.

The mass spectrum of each subfraction provides information about both the glycolipid composition and structure. As no reports on using electron impact mass spectrometry to directly analyze glycolipids were found, we could make no direct comparison of mass fragments with literature data. However, mass spectral analyses of diglycerides and triglycerides were reported (31-34). The stereochemical numbering (SN) system is used when referring to glycerol structures.

Some ion fragments that would be observed for glycolipids appear in Figure 6 based on earlier information (31,32,34). The acyl ion (RCO^+) is a common fragment for the fatty acids. The acyloxy ion is formed in both SN-1 and SN-2 positions. In addition, the acyloxy-methylene ion and the (acyl + 74) ion are formed. The acyloxy-methylene ion is preferentially formed at the SN-1 position. The (acyl + 74) ion is formed from the fatty acid at the SN-2 position. Common in all the spectra are ion fragments differing by mass 14 or 28 due to loss of CH_2 or C_2H_4 units. Ion fragments due to the carbohydrate portion of the glycolipid are present. Ions formed from underivatized carbohydrates are galactose minus 16, galactose, digalactose, and digalactose plus methylene. Some other fragments commonly formed are: glyceraldehyde, 2-furfuraldehyde, 2-furfuraldehyde plus 1, furfuralcohol, 5-methyl-2-furfuraldehyde, galactose minus 1, and galactose-O-CH_2. We observed no parent molecule ion in the mass spectrum of each subfraction.

In the mass spectrum for DGDG peak II (Fig. 7), the carbohydrate fragments appear in the 50-200 mass range. The predominant fatty acids are palmitic, linoleic and oleic acids. The SN-1 position favors palmitic and oleic acid, based on the ion current responses for the acyloxy methylene ions. Linoleic acid is present at the SN-2 position, based on the ion current responses for the acyloxy ion and acyl plus 74 ion. The preferential location of fatty acids having greater unsaturation on the SN-2 position has been reported based on enzymatic techniques (35,36). A similar mass spectrum was obtained for MGDG peak II (not shown). Based on fatty acid composition and mass spectra of peak I or II of DGDG and MGDG, the proposed chemical structures of wheat flour galactosyldiglycerides are summarized in Table IV. The first peak of DGDG or MGDG was dilinoleate and the second peak was DGDG or MGDG containing palmitic or oleic acid acylated on SN-1 position and linoleic acid on SN-2 position.

Quantitation

Quantitative determinations of DGDG and MGDG contents were based on the wheat lipid standards prepared. Individual wheat lipid standards were required because of the improved resolution achieved by HPLC. DGDG and MGDG were resolved by HPLC based on both carbohydrate and fatty acid composition. Although deflection type of RI detectors were used during developing the analytical method, the interferometric type was preferred to the deflection type because of better sensitivity range and lower detection limit (Table V). As flour polar lipid subfraction V was a mixture of DGDG peak II and MGDG peak I (Fig. 2), it was necessary to establish the ratio for peak I to II of DGDG or MGDG based on a number of wheat and flour samples: the ratio of peak I to II was 1.96 ± 0.1 for DGDG and 3.65 ± 0.1 for MGDG (37). Based on the ratio, an empirical coefficient of 1.56 was determined

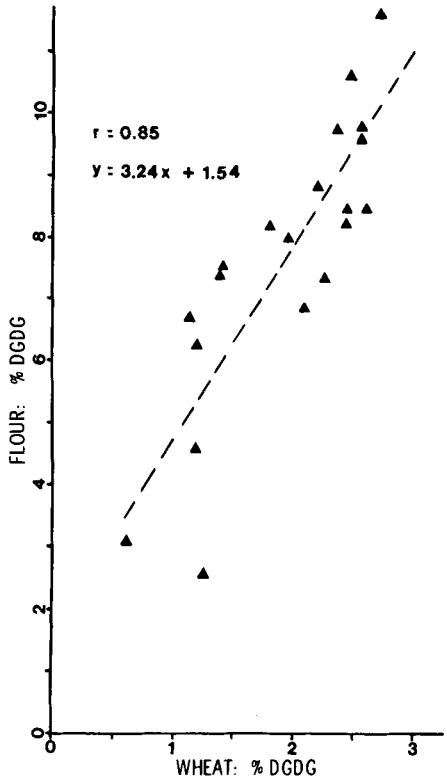


FIG. 9. Linear relation between wheat DGDG contents and flour DGDG contents in lipids extracted with petroleum ether from 20 wheats and flours.

for DGDG. Similarly, total MGDG content was obtained from using area of MGDG peak II (subfraction VI in Fig. 2), the response factor for standard MGDG peak II and empirical coefficient of 4.65.

A series of wheats and their corresponding flours with a wide range of baking quality were analyzed for DGDG and MGDG contents (Table VI). DGDG content in 20 flours ranged from 2.62 to 11.60% of the total PE lipid extract. The DGDG content in wheat PE lipid extract ranged from 0.62 to 2.59%. The MGDG ranged from 1.80 to 6.56% in flour and 0.48 to 1.74% in wheat lipid extract.

For both milled flours and whole wheats, DGDG contents were significantly linearly related to polar lipid contents (Fig. 8). Furthermore, DGDG contents in flour samples were also significantly linearly related to DGDG contents in wheat samples (Fig. 9). A highly significant correlation indicates a possibility that DGDG content in flour would be predicted from the known DGDG content in whole wheat.

The proposed HPLC method (Spherisorb ODS column, methanol/water as an eluting solvent, and a differential RI detector with interferometric optics) for quantitating DGDG and MGDG content in wheat or flour lipids have following advantages: better resolution and efficiency than conventional methods, a single solvent system, sensitivity and detection limit equal to or better than conventional methods, good reproducibility of laboratory packed analytical columns, and also separation of DGDG or MGDG into two peaks each based on structures differing in fatty acids and their acylating positions.

The DGDG content of the free lipid extract from a series of wheat flours correlated well with the DGDG content of the corresponding free polar lipid extract from whole wheat. Also, DGDG content of free flour lipid correlated well with the quantity of polar lipid in the wheat flour lipid extract. Thus, this HPLC procedure is adaptable for routine use and provides the cereal chemist with a direct means of analyzing for digalactosyldiglyceride. Glydolipids, while a minor component of wheat flour, are highly important in the bread-making quality of wheat.

ACKNOWLEDGMENTS

Wheat utilization funds supported analytical instrumentation as well as the graduate research of T.N. Tweeten. Lipid extraction and open column fractionation were done by B.G. Howard. For subfraction characterization, we used the mass spectrometer and data system of Midwest Research Institute, Kansas City, MO.

REFERENCES

1. Carter, H.E., R.A. Hendry and N.Z. Stanacev, J. Lipid Res. 2:223 (1961).
2. Carter, H.E., R.H. McCluer and E.D. Slifer, J. Am. Chem. Soc. 78:3735 (1956).
3. Carter, H.E., K. Ohno, S. Najima, C.L. Tipton and N.Z. Stanacev, J. Lipid Res. 2:215 (1961).
4. Lin, M.J.Y., V.L. Youngs and B.L. D'Appolonia, Cereal Chem. 51:17 (1974).
5. MacMurray, T.A., and W.R. Morrison, J. Sci. Food Agric. 21:520 (1970).
6. Pomeranz, Y., and O.K. Chung, J. Chromatogr. 19:540 (1965).
7. Chung, O.K., and C.C. Tsen, Cereal Chem. 52:533 (1975).
8. MacRitchie, F., J. Sci. Food Agric. 28:53 (1977).
9. Mecham, D.K., and N.E. Weinstein, Cereal Chem. 29:448 (1952).
10. Daftary, R.D., Y. Pomeranz, M.D. Shogren and K.F. Finney, Food Technol. 22:79 (1968).
11. Lin, M.J.Y., B.L. D'Appolonia and V.L. Youngs, Cereal Chem. 51:34 (1974).
12. Wehrli, H.P., and Y. Pomeranz, Chem. Phys. Lipids 3:357 (1969).
13. Chung, O.K., Y. Pomeranz, R.M. Jacobs and B.G. Howard, J. Food Sci. 45:1168 (1980).
14. Chung, O.K., Y. Pomeranz and K.F. Finney, Cereal Foods World 24:453 (1979).
15. Blank, M.L., and F. Snyder, "Analysis of Lipids and Lipoproteins," edited by E.G. Perkins, AOCS, Champaign, IL, 1975, p. 63.
16. Clayton, T.A., T.A. MacMurray and W.R. Morrison, J. Chromatogr. 47:277 (1970).
17. Graveland, A., JAOCS 45:834 (1968).
18. Erdahl, W.L., A. Stolyhwo and O.S. Privett, Ibid. 50:513 (1973).
19. Stolyhwo, A., and O.S. Privett, J. Chromatogr. Sci. 11:20 (1973).
20. Vogel, A.I., "A Textbook of Practical Organic Chemistry," 3rd Edition, Longmans, Green and Co., London, 1956, p. 169.
21. Kates, M., J. Lipid Res. 5:132 (1964).
22. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, Anal. Chem. 28:350 (1956).
23. Wetzel, D.L., Cereal Foods World 22:479, Abst. #155, Annual Meeting, American Assoc. Cereal Chem., San Francisco, CA, 1977.
24. Lowry, R.R., and I.J. Tinsley, Lipids 9:491 (1974).
25. "Approved Methods of the AACC," American Association of Cereal Chemists, St. Paul, MN, Methods 08-01, 44-15A, and 46-11.
26. "Official Methods of Analysis," 11th Edition, Association of Official Analytical Chemists, Washington, DC, 1970, p. 454.
27. Blank, M.L., J.A. Schmidt and O.S. Privett, JAOCS 41:371 (1964).
28. Dittmer, J.C., and R.L. Lester, J. Lipid Res. 5:126 (1964).
29. LePage, M., J. Chromatogr. 13:99 (1964).
30. Feldman, G.L., L.L. Feldman and G. Rouser, JAOCS 42:742 (1965).
31. Natale, N., Lipids 12:847 (1977).
32. Schulter, H.R., and W. Gortz, Anal. Chem. 50:428 (1978).
33. Selke, E., C.R. Scholfield, C.D. Evans and H.J. Dutton, J. Am. Chem. Soc. 38:614 (1961).
34. Sun, K.K., and R.T. Holman, JAOCS 45:810 (1968).
35. Arunga, R.O., and W.R. Morrison, Lipids 6:768 (1971).
36. Clayton, T.A., and W.R. Morrison, J. Sci. Food Agric. 23:721 (1972).
37. Tweeten, T.N., "Analytical High Performance Liquid Chromatography of Digalactosyldiglycerides in Wheat Flour Lipid Fractions," Ph.D. dissertation, Kansas State University, Manhattan, KS, 1979, pp. 30-32.

[Received August 12, 1980]