The Distribution of Lipids in Fractionated Wheat Flour[1]

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ABSTRACT

The free and bound lipids in four major fractions of Selkirk, a hard red spring, and Wells, a durum wheat, were analyzed by thin-layer chromatography. Gluten (high-protein fraction) contained 65% of the total extracted lipid; starch and sludge (low-protein fractions) together contained 32% of the total extracted lipid, and the water-solubles (high-protein fraction) the remaining 3%. Approximately 90% of the lipids was bound. The di- and triglycerides, mono- and diagalactosyl diglycerides, and phosphatidyl choline occurred primarily in the gluten. While the starch and sludge fractions contained the largest amounts of lysophosphatidyl choline, they contained very little phosphatidyl choline. Lysophosphatidyl ethanolamine and phosphatidyl serine, measured as a single spot, were most abundant in sludge and gluten. More free fatty acids were associated with the low-protein fractions, starch and sludge, than with gluten and water-solubles. Wells flour fractions were practically devoid of saturated sterol esters, but contained free sterols. The opposite was true for the Selkirk fractions.

To understand more fully the function of lipids in a dough, it is of interest to determine what specific lipid components are associated with different flour fractions. The two fractions that have received the most attention are starch and gluten. A review of starch lipids, published by Schmitz and Acker (1), reported that little is known about starch lipids, principally because of the difficulty in extraction. The major starch lipid components reported were fatty acids and phosphorus- and choline-containing compounds. Acker et al. (2) in another recent review of wheat lipids reported that wheat-starch lipids were composed principally of lysophosphatidyl choline, lysophosphatidyl ethanolamine, and free fatty acids. Other minor polar components also were listed. Data were presented on the molecular configuration of lysophosphatidyl choline. In this review 60% of the gluten lipids were reported to be polar, with galactolipids and phospholipids as the major components. Very little of the lysophosphatidyl compounds were present in gluten. Ponte et al. (3) studied the lipids in glutenin and gliadin extracted from gluten with 70% ethanol. They reported that gliadin lipids contained 75% polar material and 6% triglycerides, whereas glutenin had 24 and 58%, respectively, of these components. Chiu et al. (4) studied lipid binding in wheat flours of varying

[1]Presented in part at the 52nd AACC Annual Meeting, held at Los Angeles, Calif., April 1967. Co-operative investigations between the North Dakota Agricultural Experiment Station and the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota. Published with the approval of the Director of the Agricultural Experiment Station, North Dakota State University, Fargo, as Journal Series No. 221.

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breadmaking potential. They also reported on the lipid distribution in gluten, and in the combined starch and water-solubles.

In the present study, HRS and durum wheat flours were separated into four fractions, two high in protein (water-solubles and gluten), and two fractions low in protein (sludge and starch). The lipids were extracted from these fractions and analyzed by thin-layer chromatography (TLC).

MATERIALS AND METHODS

Selkirk, a HRS wheat, was milled into flour on a Buhler experimental mill, and Wells, a durum wheat, was milled into semolina with an Allis mill. The semolina then was reground to give a granulation similar to that of the Silkirk flour. The two wheat samples were grown in North Dakota in 1966.

Both flours were separated into four fractions; namely, water-solubles, sludge, gluten, and starch. The procedure followed was similar to that of Gilles et al. (5). Flour (200 g.) was slurried with 500 ml. of oxygen-free water in a Waring Blendor for 1 min. in an atmosphere of nitrogen, then centrifuged. The supernatant was removed, and the solids were reslurried as before and centrifuged. The two supernatants were combined and freeze-dried. These were designated as the water-solubles. The solids remaining in the centrifuge cups consisted of the gluten, sludge, and starch. The gluten was separated from the starch and sludge with a spatula. The gluten was kneaded and washed several times to remove most of the remaining starch, then freeze-dried. The starch and sludge were resuspended in water and centrifuged. The starch, being more dense, collected at the bottom of the centrifuge cup. Separation was made and the sludge was freeze-dried. The starch was air-dried for 2 to 3 days.

All fractions, including the original flours, were extracted for 8 hr. in a Soxhlet extractor, with petroleum ether (b.p. 30° to 60°C.); these extracts were called the free lipids. The extracted flour and flour fractions were air-dried and extracted again by the method of Tsen et al. (6) which uses chloroform, methanol, and water; these extracts were called the bound lipids. As a check on the distribution of polar lipids extracted from the four fractions by chloroform, methanol, and water, water-saturated butanol (WSB) also was used to a limited extent. These samples were placed in WSB and were shaken on a Burrell shaker for 0.5 hr.; the extract was evaporated to dryness in a rotoevaporator and the residue taken up in chloroform. The amount of lipid extracted from each fraction was determined, and all lipid extracts were diluted with petroleum ether or chloroform to give equal concentration for spotting on thin-layer plates.

Thin-layer chromatography was employed to determine the relative amounts of certain lipid constituents. Silica gel, with a binder of 10% calcium sulfate (Adsorbosil 1), was spread on glass plates to a thickness of 0.25 mm. An equal amount of lipid from each of the different flour fractions was spotted on these plates, and development was carried out in the solvent system required to separate the desired lipid constituents. The plates were sprayed with 50% aqueous sulfuric acid and heated to visualize the spots. Measurement of spot intensities was done with a Photovolt densitometer. Because spot intensities may vary with the lipid compounds, only those with similar structures were compared. Thus, the relative

amount of each class of lipid (triglycerides, sterols, etc.) was not determined, because this would have required density measurement of dissimilar compounds. If possible, all spots measured were near the center of the thin-layer plate. This was done by means of a variety of solvent systems having different polarities. The method used resulted in a standard deviation of 4.7%.

Lipid constitutents were identified by spotting commercial standards on plates and comparing R_f values, and by the color reactions of different spray reagents: sulfuric acid and 100°C. heat for free sterols and sterol esters, ninhydrin for alpha-amino groups, Dragendorff's reagent for choline-containing compounds, and diphenylamine for galactosyl diglycerides (7).

RESULTS AND DISCUSSION

Table I shows extraction data and protein composition for Wells and Selkirk flour. Of the four fractions from both flour samples, starch was the most abundant (46 and 56%, Selkirk and Wells, respectively), the sludge next (30 and 20%), gluten third (19%), and finally the water-solubles, which accounted for only 5% of the total flour weight. Yield percentages were based on total material recovered, which averaged 90 to 93% of the original flour. The two fractions highest in protein content were the gluten and water-solubles, respectively, followed by sludge and starch, the latter showing only 0.6% protein for Selkirk and 0.2% for Wells.

The fact that water and the mechanical action used in forming a dough have a binding effect on lipids is evident in Table I. While more free lipids (Soxhlet-petroleum ether-extracted) were extracted from the original flours than bound lipids (chloroform-methanol-water-extracted), the opposite was true when the lipids were extracted from the four flour fractions. Petroleum ether (b.p. 30° to 60°C.) extracted only about 6% of the lipids from Selkirk water-solubles, gluten, and starch. This compares favorably with the results of Wootton (8), who extracted

TABLE I. YIELD, PROTEIN, AND LIPID VALUES OF SELKIRK AND WELLS FLOUR AND FLOUR FRACTIONS

Flour and Flour Fraction (Sample)	Yield	Protein	Lipids in Flour or Flour Fractions[a]		Distribution of Total Lipids in Flour and Flour Fractions	
			Free	Bound	Free	Bound
	%	%	%	%	%	%
Selkirk flour		17.6	0.76	0.45	62.8	37.2
Selkirk fractions						
Water-solubles	5	38.0	0.07	0.85	0.2	2.9
Sludge	30	4.0	0.15	0.82	3.1	16.9
Gluten	19	68.5	0.28	4.64	3.7	60.8
Starch	46	0.6	0.02	0.26	0.7	11.7
Wells flour		14.0	0.90	0.45	66.7	33.3
Wells fractions						
Water-solubles	5	27.9	0.08	0.83	0.3	2.8
Sludge	20	2.1	0.20	0.94	2.7	13.0
Gluten	19	61.0	0.38	4.92	4.7	61.1
Starch	56	0.2	0.02	0.37	0.8	14.6

[a]Dry basis.

5% of the lipids from gluten by using light petroleum (b.p. 60° to 80°C.), and with those of Olcott and Mecham (9), who extracted only 6% of the lipids from dough that had been dried. The sludge fraction, however, contained more free lipids; 15.5% of the lipids was extracted with petroleum ether. In the Wells flour fractions, the water-solubles contained 9% free lipids, gluten 7% starch 5%, and sludge 17% free lipids. Over-all, approximately 90% of the lipids was bound. Although Selkirk and Wells gluten constituted only 19% of the total flour weight, they contained about 65% of the total lipids extracted by the methods described. Sludge and starch, both low-protein fractions, accounted for 32% of the total lipids, and the water-solubles contained the remaining 3%.

Analysis of the Lipids

The solvent systems used to produce lipid separations by TLC are shown in Table II.

Figures 1, 2, and 3 are photographs of typical thin-layer separations. On each plate equal amounts of lipid were spotted. If this had not been done, there would have been a vast difference in spot intensities, which could have affected the accuracy of the procedure.

To obtain the values shown in Table III, densitometer readings were corrected to give values based on the total amount of lipid present in the flour and flour fractions. Thus, the values in Table III show the relative distribution of each lipid component among the four flour fractions, and between the free and bound lipids extracted from the flour before mixing.

Free Sterols and Sterol Esters

The distribution of saturated sterol esters (principally sitosterol palmitate) and the free sterols in durum and common wheats has been reported (10,11). Basically, flour from U.S. durum varieties contains practically no saturated sterol esters, but

TABLE II. SOLVENT SYSTEMS AND MATERIAL MEASURED

Solvent System	Volume Ratio	Compounds Measured
Carbon tetrachloride	...	Sterol esters
Pet. ether:ethyl ether: acetic acid	90:10:1.5	Triglycerides; free fatty acids
Benzene:ethyl acetate	5:1	Free sterols; 1,3-diglycerides; 1,2-diglycerides
Pet. ether:ethyl ether:acetic acid	10:90:1.5	Monoglycerides
Chloroform:acetone:acetic acid[a]	76:14:1.5	1-Monoglycerides[b]; 2-monoglycerides[b]
Chloroform:methanol:water	80:5:1	Monogalactosyl glycerides
Chloroform:methanol:water	65:15:2	Phosphatidyl ethanolamine[b]; lysophosphatidyl ethanolamine and phosphatidyl serine[c]
Chloroform:methanol:water	65:20:2	Digalactosyl glycerides
Chloroform:methanol:water	65:25:4	Phosphatidyl choline; lysophosphatidyl choline

[a]Boric acid-silica gel plates.

[b]Relative amounts estimated.

[c]Measured as a single spot.

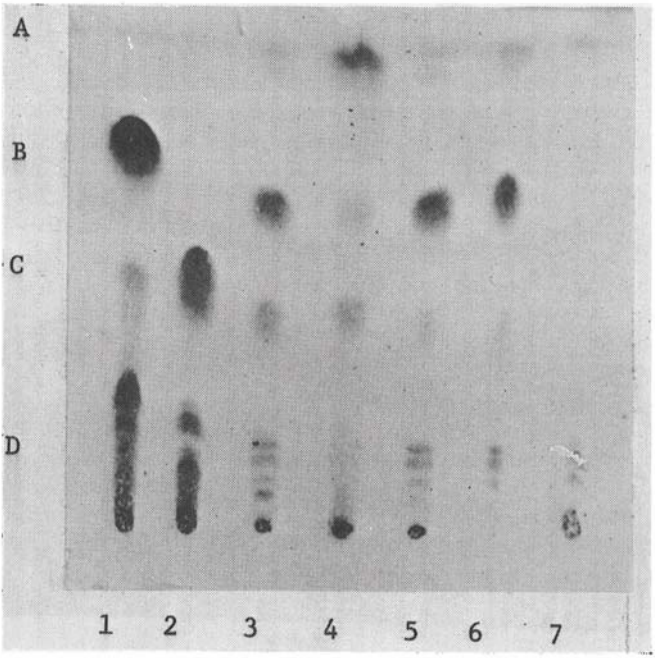

Fig. 1. Separation of triglycerides and free fatty acids in Wells flour and flour fractions. Solvent system: petroleum ether:ethyl ether:acetic acid, 90:10:1.5 (v.:v.:v.). A, solvent front; B, triglycerides; C, free fatty acids; D, diglycerides, free sterols, and polar lipids. 1, triolein; 2, linoleic acid; 3, sludge lipids; 4, starch lipids; 5, gluten lipids; 6, nonpolar lipids extracted from Wells flour; 7, standard containing monoolein on the origin, 1,2-diolein next, and finally 1,3-diolein.

does contain a considerable amount of free sterols. The opposite is true with flour from common wheats. A study of the distribution of these compounds in the flour fractions showed no measurable saturated sterol esters in the Wells flour fractions, and only very small amounts of free sterols in the Selkirk flour fractions. In the Selkirk fractions, 70% of the total saturated sterol esters was found in the gluten. Analysis of the Wells flour fractions showed 84% of the total free sterols also was associated with the gluten.

The Glycerides

The triglycerides were the largest single component present in wheat lipids. Wells flour contained 15% more of this component than Selkirk. In the fractions of both flours, gluten accounted for more than 90% of the total triglycerides, although Selkirk gluten contained more as free lipids than Wells. The starch fractions contained only a small amount of triglycerides.

The 1,3- and 1,2-diglycerides were also present in greater quantities in Wells flour lipids as compared with Selkirk. Again, the gluten fraction, as seen in Table III, contained most of the total amount of diglycerides. A comparison of the amounts of diglycerides showed nearly twice as much 1,3- as 1,2-diglyceride in each flour.

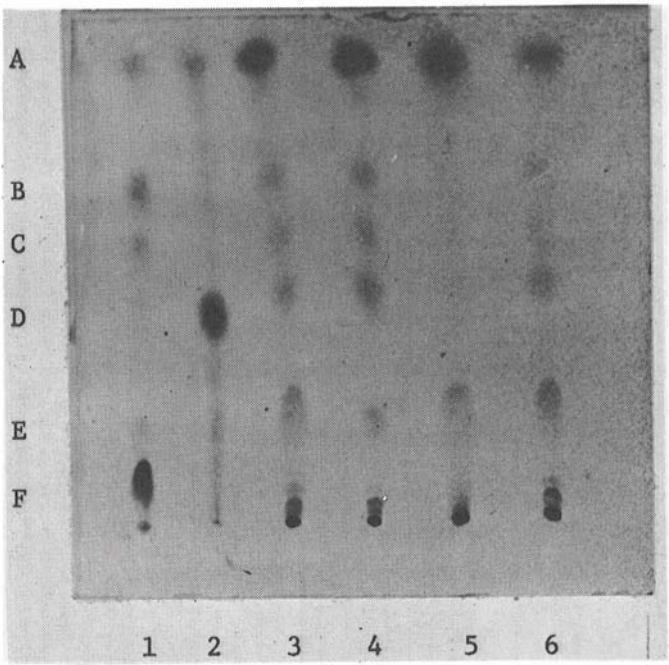

Fig. 2. Separation of diglycerides and free sterols in Wells flour and flour fractions. Solvent system: benzene:ethyl acetate, 5:1 (v.:v.). A, front (mostly triglycerides); B, 1,3-diglycerides; C, 1,2-diglycerides; D, free sterols; E, free fatty acids; F, monoglycerides. 1, standard containing monoolein, 1,2-diolein, and 1,3-diolein; 2, sitosterol; 3, sludge lipids; 4, gluten lipids; 5, starch lipids; 6, nonpolar lipids extracted from Wells flour.

The 1- and 2-monoglycerides of the Selkirk and Wells flour lipids were separated on boric acid-silica gel thin-layer plates (12). Although no densitometer measurements were taken, visual observations indicated that the 1-monoglycerides were present in excess of the 2-monoglycerides. It was estimated that the 1-monoglyceride spot intensities were one-half those of the 1,2-diglycerides.

The amounts of monogalactosyl and digalactosyl diglycerides were compared in the flour fractions by TLC and densitometry. They occurred primarily in the gluten fraction.

Free Fatty Acids

The amount of free fatty acids in the two flours was the same; however, more appeared in the sludge and starch (low-protein fractions) than in the gluten, as observed for sterol esters, sterols, and glycerides. In the variety Wells, 62% of the free fatty acids was associated with the low-protein fractions, and in Selkirk, 59%.

Phospholipids

The complexity of these compounds made separation difficult. Figure 3 shows separations obtained with chloroform, methanol, and water, 65:25:4 (v.:v.:v.) as a solvent system. Phosphatidyl inositol, phosphatidyl serine, and lysophosphatidyl

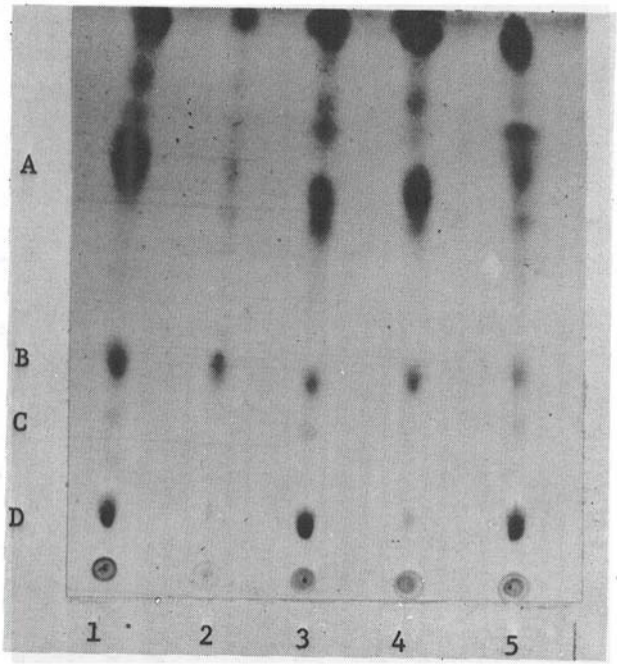

Fig. 3. Separation of polar lipids in Wells flour and flour fractions. Solvent system: chloroform:methanol:water, 65:25:4 (v.:v.:v.). A, digalactosyl diglycerides; B, phosphatidyl choline; C, combined phosphatidyl serine and lysophosphatidyl ethanolamine; D, lysophosphatidyl choline. 1, polar lipids extracted from Wells flour; 2, lipids from water-soluble fraction; 3, sludge lipids; 4, gluten lipids; 5, starch lipids.

ethanolamine had similar R_f values. To measure the two latter compounds without interference from the phosphatidyl inositol, the spots were sprayed with ninhydrin, heated to develop the color, and measured on the densitometer. This is reported in Table III as a single value for phosphatidyl serine and lysophosphatidyl ethanolamine. The greatest concentrations occurred in the sludge and gluten polar lipids. Phosphatidyl inositol could not be measured satisfactorily.

Phosphatidyl ethanolamine was identified by comparison with a standard and by its positive reaction to ninhydrin spray, but the red spots were too faint for densitometric analysis. Visually, it was estimated that most of this compound occurred in the gluten fraction. When the spot corresponding to phosphatidyl ethanolamine was sprayed with sulfuric acid instead of ninhydrin and charred, a much larger spot appeared. This indicated the presence of an unidentified compound having the same R_f value as phosphatidyl ethanolamine. Phosphatidyl choline was identified by comparing with standards and using Dragendorff's spray (orange color) (7). A comparison of these compounds among the flour fractions showed interesting differences. Very little lysophosphatidyl choline was present in the high-protein fractions (gluten and water-solubles); about 80% of it occurred in the starch and sludge. Conversely, over 86% of the lecithin occurred in high-protein rather than low-protein fractions. With the exception of phosphatidyl choline in

TABLE III. DISTRIBUTION OF LIPID COMPONENTS

Flour and Flour Fractions	Satd. Sterol Esters		Free Sterols		Triglycerides		1,3-Diglycerides		1,2-Diglycerides		Monoglycerides	
	Free %	Bound %	Free %	Bound %	Free %	Bound %	Free %	Bound %	Free %	Bound %	Free %	Bound %
Selkirk flour	78	22	trace	...	85	15	71	29	78	22	34	66
Selkirk fractions												
Water-solubles	1	13	4	...	1	2	trace	1	1	2	trace	4
Sludge	9	7	62	...	2	1	6	6	16	11	2	40
Gluten	27	43	29	...	25	69	5	74	11	51	6	32
Starch	...	...	5	...	trace	...	trace	8	trace	8	1	15
Wells flour	...	...	100	...	93	7	77	23	88	12	25	75
Wells fractions												
Water-solubles	...	...	trace	4	trace	2	trace	5	1	3	1	1
Sludge	...	...	2	10	2	3	8	14	9	13	5	21
Gluten	...	...	3	81	6	86	8	64	6	66	4	62
Starch	...	...	trace	...	trace	1	trace	1	1	1	2	4

Flour and Flour Fractions	Free Fatty Acids		Monogalactosyl Diglycerides	Digalactosyl Diglycerides	Phosphatidyl Choline	Lysophosphatidyl Choline	Lysophasphatidyl Ethanolamine and Phosphatidyl Serine
	Free %	Bound %	Bound %	Bound %	Bound %	Bound %	Bound %
Selkirk flour	78	22					
Selkirk fractions							
Water-solubles	1	2	1	3	3	3	2
Sludge	2	45	15	18	10	56	51
Gluten	2	36	83	78	86	14	39
Starch	1	11	1	1	1	27	8
Wells flour	77	23					
Wells fractions							
Water-solubles	1	5	2	3	2	4	1
Sludge	2	30	6	15	11	37	46
Gluten	3	29	85	70	84	20	36
Starch	2	28	7	12	3	39	17

Wells starch, all Selkirk flour fractions contained more phospholipids than Wells fractions.

A reduction of the phospholipid and free fatty acid content of gluten during the washing procedure by the use of tap water, pH 6, was reported by Wootton (8). Slight solubility of these compounds in water was suggested as a reason for part of the loss. To investigate this, 1,000 g. of flour was fractionated by slurrying with water in a Waring Blendor and centrifuged. The gluten and starch fractions were saved. The gluten was hand-washed and the wash water centrifuged to precipitate the starch and sludge. All of the gluten wash water was extracted with chloroform and the extract reduced in volume to 5 ml. The starch obtained by the first centrifugation and that precipitated from the wash water were extracted with the chloroform-methanol-water mixture. Equal amounts of the starch lipids and a portion of the gluten wash lipids were spotted on thin-layer plates and analyzed for fatty acids and lysophosphatidyl choline. The starch samples contained equal amounts of both compounds. The wash water produced no lysophosphatidyl choline, but some fatty acids were present. From these data it was concluded that the loss of phospholipids in gluten by further washing was due to removal of more starch and sludge from the gluten, with the phospholipids (particularly lysophosphatidyl choline) bound to the starch and sludge.

Several different methods of mixing the flour and water and extracting the four flour fractions were used to determine whether or not this had an effect on phospholipid distribution in the flour fractions. In addition to use of the Waring Blendor, flour and water were machine-mixed (100-g. dough mixer) and hand-mixed. Half of each sample was fractionated immediately and the other half freeze-dried, then fractionated. Also a baking formula including salt, dry milk, vegetable fat, yeast, sugar, and bromate was machine-mixed, then fractionated before and after freeze-drying. The only difference noted was slightly less than normal lysophosphatidyl choline in the baking-formula sludge.

The bound lipids of the four different flour fractions were also extracted with WSB. The chloroform-soluble material of these extractions contained all the polar lipids reported in Table III. However, two additional large spots appeared, particularly in the water-solubles and sludge fractions, and were tentatively identified as glucose and sucrose. The lipids extracted by the method of Tsen et al. (6) contained very few free sugars. However, when the water portion of this extracting system was concentrated and spotted on thin-layer plates, sugars were present.

Acknowledgment

The authors gratefully acknowledge the technical assistance of Mrs. D. Thompson.

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[Received September 26, 1969. Accepted April 6, 1970]