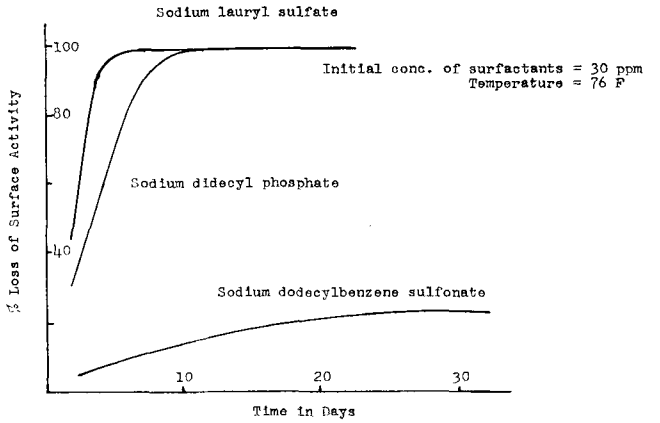

FIG. 5. Biodegradation of surfactants as determined by surface tension measurements.

when run in the so-called River Water Die-Away Test shows in Figure 5. Results indicate that at room temp sodium didecyl phosphates loses its surface active properties in approximately 10 days, sodium lauryl sulfate in 5 days and sodium dodecyl benzene sulfonate loses only 20% in 30 days.

Summary

A study of the salts of dialkyl hydrogen phosphates has shown that they possess some exceptional surface active properties particularly when the alkyl radicals are branched.

Laboratory evaluation of these surfactants for use in heavy duty detergents indicates that the branched chain didecyl ester performs exceptionally well. In fact, our laboratory evaluations show it to be considerably better than commercial surfactants being used in heavy duty detergents.

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The Endosperm Lipids of Three Canadian Wheats[1]

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Abstract

Flour samples were prepared from intact and degermed kernels of Hard Red Spring, Soft White Spring, and Amber Durum wheats. The "free" (hexane soluble) and "bound" (hexane resistant, water-saturated *n*-butanol extractable) lipids were extracted from the six flours and separated quantitatively by silicic acid column chromatography. Thin-layer chromatography (TLC) was used to monitor the column and to resolve the lipid classes into components. Gas-liquid chromatography (GLC) was used to obtain the fatty acid composition of the triglyceride, sterol ester, and phospholipid fractions, and also to determine the nature of the sterol components of the unesterified sterol and sterol ester classes.

Similar patterns of lipid classes were shown by all three varieties; the differences were in the degree of dominance. In fatty acid composition some varietal differences were found but the greatest difference was between lipid classes.

Introduction

LIPIDS COMPRISE only a very small portion of the wheat endosperm; yet they have been of continuing interest to cereal chemists. Evidence suggests that lipids are involved in the ageing of flour, the quality of bread, and in the staling process. Extensive descriptive work on the role of lipids in flour properties has, however, resulted in a vast accumulation of apparently contradictory data (1,2).

Recently, studies of the composition of wheat lipids have received increasing attention (1). Separation of lipid classes has been accomplished by solvent fractionation (3) and by chromatography (4). The phospholipids have been further investigated by Coulson (5) and Houston (6), and Stevens (7) has studied the lipid contribution of germ oil to flour. Carter (8,9,10) has made an extensive study of glycolipids and it has been estimated that phytoglycolipid might amount to 4% of wheat lipid (11). Sitosterols (2), sitosteryl glucoside (10), as well as other plant sterols (2), have been reported in wheat lipids.

The present work is intended as a comparative study of the endosperm lipids in very different types of wheat. The objective is to separate the free and bound lipids of wheat endosperm into their component classes and to study the differences within and between classes in three representative varieties.

Materials and Methods

The wheat varieties Thatcher, Lemhi, and Mindum were chosen to represent Hard Red Spring, Soft White Spring, and Amber Durum wheats, respectively. The embryos were removed through the courtesy of F. B. Johnston, Food Research Institute. Seven pounds each of intact and degermed grain were milled in a micromill in the laboratory of A.G.O. Whiteside, Genetics and Plant Breeding Research Institute. The grain was tempered at 15.5% moisture and the milling produced a straight run flour of 65–70% extraction. The free lipids of the wheat endosperm used in this investigation were extracted from the flour with hexane and the bound lipids released by a further extraction with water-saturated *n*-butanol (12). A rotary evaporator was used to remove hexane under vacuum at 20C and butanol at 30C. Vacuum was always broken with nitrogen and the lipid stored under nitrogen in the dark at —15C.

Silicic Acid Column Chromatography. Separation of free and bound lipids into their classes was accomplished in the apparatus described by Sahasrabudhe and Chapman (13) using 90 g silicic acid in a column 25 mm diam. The dehydrating procedure and the gradient from pure hexane to 60% diethyl ether in hexane were essentially those described by Hirsch and

[1] Contribution No. 5 of the Food Research Institute. Presented at the AOCS Meeting, Toronto, 1962.

Ahrens (14); the phospholipids were eluted with absolute methanol. A charge of 200–250 mg was placed on the column and 20-ml fractions were collected. The 150 fractions collected for each sample were each reduced in vol to 2 ml under nitrogen at temp below 35C. A 10-μl aliquot of each was then removed for thin-layer monitoring of the separation on the column. All fractions containing lipid were taken to dryness under nitrogen at temp below 35C.

TLC was used to monitor the column chromatography experiments: to determine the position of the peaks and the homogeneity of fractions eluted from the silicic acid column. The high sensitivity of iodine vapor to lipids permitted the detection of less than 1.0 μg lipid spot on the thin-layer plate, corresponding to 0.2 mg in the fraction.

The phospholipids were also separated batchwise on silicic acid by a modified Choudhury and Arnold procedure (15), using di-ethyl ether-hexane (1:1) to remove neutral lipids and methanol to extract the phospholipids.

TLC. The apparatus developed by Stahl (16) was used to spread 0.25 mm thick layers of Silica Gel G (Merck, Darmstadt) on plates of various sizes. To test fractions for homogeneity, the hexane-ethyl ether-acetic acid system of Mangold and Malins (17) was used. Unesterified sterols separated cleanly from phospholipids in diisobutyl ketone-acetic acid-water (8:5:1) (18) and the polar lipids were separated by Wagner's chloroform-methanol-water system (19). For two-dimensional TLC of polar lipids the chloroform-methanol-water system was used in the X-direction followed by diisobutyl ketone-acetic acid-water in the Y-direction (20).

Iodine vapor was used as a general detecting chemical for all lipids on thin-layer plates (21). Where only part of the plate was to be exposed, as in preparative work, 2-7 dichlorofluorescein (22) was sprayed on the unmasked portion of the plate. Ninhydrin (23), Dragendorff reagent (24), 20% perchloric acid (20) and molybdate (25) were used as sprays in the identification of phospholipids and glycolipids.

GLC. Methyl esters were prepared by heating at least 1 mg lipid in 0.5 ml hexane with 4 ml 5% hydrogen chloride in absolute methanol for 2 hr at 90C. After addition of water the methyl esters were extracted with hexane (26–27). Five μg of methyl esters from triglycerides, or 5–15 μg from phospholipids were fractionated on a 4 ft x $\frac{3}{16}$ in. column of 10% polyvinyl acetate (w/w) (Union Carbide, type AYAC 8285) on 60–80 mesh Gas Chrom-P (Applied Science Laboratories, State College, Pa.).

The trimethylsilyl ether derivatives of sterols were prepared according to the method described by RSCo (28) and Langer (29,30). Samples of 0.05–3.00 μg were fractionated on a 2 ft x $\frac{3}{16}$ in. column of Gas Chrom-P, 80–100 mesh (not siliconized) bearing 3% SE 30 (General Electric, Silicone Products Dept., Waterford, N.Y.) and conditioned for 4 hr at 250C.

These separations were effected on a Research Specialties series 600 gas chromatograph operating, for methyl esters, with its column at 205C, and a nitrogen flow of 40 ml/min; and, for sterol derivatives, with its column at 218C and a nitrogen flow of 83 ml/min. A hydrogen pressure of 7.5 lb/inch^2 and air pressure of 12 lb/inch^2 proved suitable for the operation of the flame ionization detector.

The quantitative nature of the gas chromatographic analysis of the methyl esters was confirmed by reference to Test Mixture D, distributed by the National Institutes of Health. The separation of the sterol derivatives was confirmed by reference to derivatives prepared from known sterols.

TABLE I
Yield of Flour and Lipid from 1000 Kernels of Wheat

	Thatcher		Lemhi		Mindum	
	Whole	Degermed	Whole	Degermed	Whole	Degermed
1000 Kernels	31.6	30.6	37.3	36.5	40.9	39.7
Flour g	24.5	25.4	28.4	28.3	30.8	32.5
Free lipid mg	217.7	200.7	323.7	248.9	298.6	305.4
Bound lipid mg	215.3	157.5	238.5	164.0	184.7	214.4

Experimental and Results

Milling Data. The weight of 1000 intact and degermed kernels was determined for each variety. The weight of 1000 embryos, and a percentage correction for the epidermis removed with the embryo, was subtracted from the weight of 1000 intact kernels to obtain a synthetic 1000 kernel weight for the broken degermed kernels. These broken kernels had a much greater surface area than intact kernels, and between tempering and milling lost more moisture due to evaporation. This was particularly noticeable in Mindum where water penetration was least. For this reason higher yields of flour were apparently obtained from degermed grain. This is further reflected in lipid yield. The percentage yields of flour were obtained in the milling process and lipid yields in the initial extraction (12). Both were calculated to a 1000 kernel basis. Results are given in Table I.

Quantitative Column Chromatography. Quantitative column chromatography showed that the general pattern of free lipid classes was similar in the three types of wheat but the proportions differed.

Variety Thatcher exemplified the general pattern (Fig. I). The shape of the free sterol peak varied slightly with variety, but the large triglyceride peak and the double phospholipid peak were characteristic of all three varieties. Triglycerides formed the major portion of the free lipid, from 60% in intact Lemhi to 72% in Mindum, and from 52–62% in the degermed material. In both Hard and Soft Spring wheats fraction E, the phospholipid and glycolipid fraction, was present in larger amounts than the free sterols, whereas in Durum these classes were present in approxi-

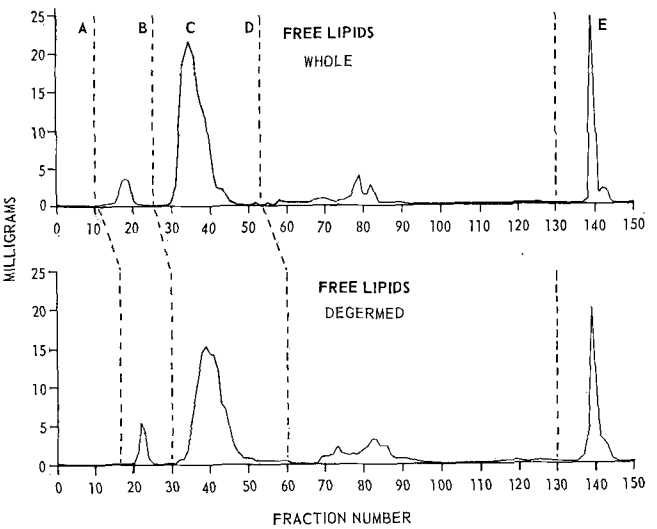

FIG. 1. Free lipid classes separated by silicic acid column chomatography. A=hydrocarbons, B=sterol esters, C=triglyceride, D=unesterified sterols, E=phospholipid plus glycolipid.

TABLE II
Lipid Classes of Free Lipid of Wheat Flour

Lipid classes	Thatcher		Lemhi		Mindum	
	Whole mg/1000	Degermed kernels	Whole mg/1000	Degermed kernels	Whole mg/1000	Degermed kernels
Hydrocarbons	0	0	0	0	4.4	1.9
Sterol esters	11.4	10.0	17.3	14.1	6.6	6.4
Triglycerides	138.0	108.9	194.2	129.5	214.8	199.8
U.E.[a] sterols	26.4	43.7	41.9	41.1	38.8	49.1
Phospholipids	41.9	39.1	70.4	64.2	34.0	48.1
Total free lipid	217.7	200.7	323.7	248.7	298.6	305.3
Lipid Classes of Bound Lipid of Wheat Flour						
Hydrocarbons	0.9	0	0	0	0	0
Sterol esters	0.4	0	0.5	0	0	0
Triglycerides	23.3	2.8	7.7	6.5	7.2	8.5
U.E.[a] sterols	15.5	12.4	27.1	20.8	21.5	22.7
Phospholipids	175.1	142.3	203.2	136.7	156.0	183.2
Total bound lipid	215.3	157.5	238.5	164.0	184.6	214.4

[a] Unesterified.

mately equal amounts. The data in Table II indicate the presence of some germ lipid in the flour milled from the intact Spring wheats, especially Lemhi. The contribution of germ lipid to the free lipid of the flour was predominantly triglyceride. The dominance of triglyceride in germ free-lipid was demonstrated by TLC separation of hexane-extracted wheat germ oil.

TLC confirmed the absence of hydrocarbons in the lipids of Thatcher and Lemhi and their presence in trace amounts in Mindum flour lipids. This method also showed that the free lipid sterol esters in all wheat varieties studied were highly homogeneous. Some fractionation of triglycerides occurred on thin-layer plates, a fraction of slightly lower Rf appearing in addition to the main triglyceride fraction.

The unesterified sterols were eluted from the silicic acid column over a considerable solvent gradient. The steroidal nature of this fraction was confirmed by its Rf on thin-layer plates. It was, however, accompanied by very small amounts of material of a higher Rf.

Components of fraction E obtained by silicic acid column chromatography were tentatively identified by comparison of their Rf values with those of known compounds on thin-layer plates. On this basis the large initial peak eluted from the column appeared to be almost entirely glycolipid in nature while the small peak was largely phosphatidyl choline. Further identification of these polar lipids was made by means of two dimensional TLC and the use of specific spray reagents (20). All three varieties of wheat had the same distribution pattern of polar lipids in free lipid and the pattern of the variety Thatcher (Fig. 2).

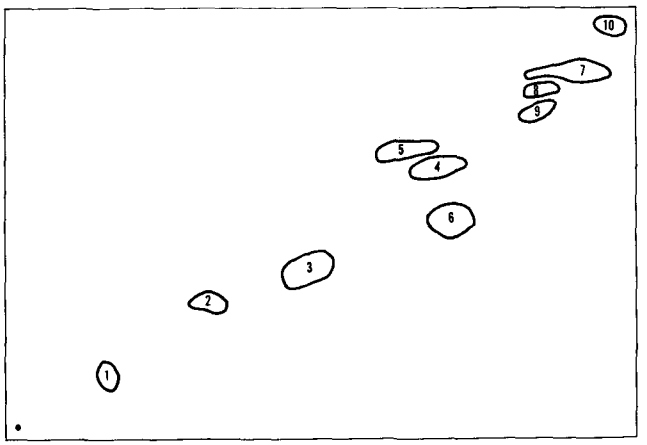

FIG. 2. TLC separation of free lipid polar lipids. Chloroform-methanol-water development 2 hr in X-axis and Diisobutyl ketone-acetic acid-water development 3 hr in Y-axis.

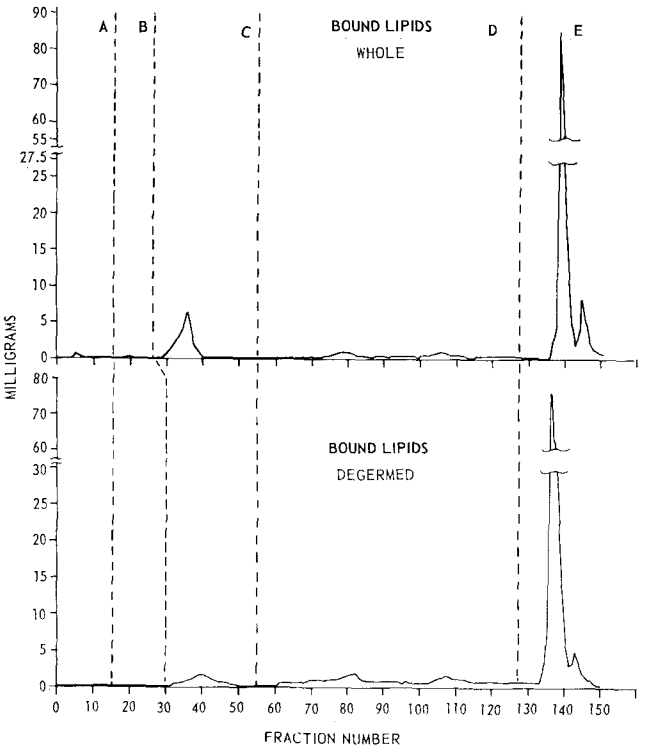

FIG. 3. Bound lipid classes separated by silicic acid column chromatography. A=hydrocarbons, B=sterol esters, C=triglyceride, D=unesterified sterols, E=phospholipid plus glycolipid.

can be considered representative.

Components 4 and 2 were ninhydrin positive and correspond to phosphatidyl ethanolamine and lysophosphatidyl ethanolamine, while components 3 and 1 were Dragendorff positive and corespond to phosphatidyl choline and lysophosphatidyl choline. Of these only 3 and 4 were present in sufficient quantity to give a positive reaction with molybdate spray, indeed, in Lemhi component 2 was not present in detectible quantity. Components 7 and 6 had the same Rf as the mono- and di-galactosyl glycerol lipids (20). The main components thus appeared to be galactolipid and phosphatidyl choline. Component 8, not positively identified, was present in all varieties and gave a distinctive blue color with perchloric acid spray. Component 9 was present in Thatcher and Mindum but not in Lemhi. A more detailed study of the polar lipids is under way.

The distribution of lipid classes in bound lipid differed from that in free lipid. However, the patterns obtained for each type of wheat resembled each other, and the distribution of classes in the bound lipid from Thatcher is used as an example (Fig. 3). The major component was fraction E comprising more than 80% of the bound lipid. The double polar lipid peak shows that in bound as in free lipid a fractionation of polar lipid took place on the silicic acid column. TLC monitoring of the eluant fractions showed that the large peak contained glycolipid, phosphatidyl ethanolamine, and inositol phosphatide while the second peak contained phosphatidyl choline. Unesterified sterols formed the bulk of the non-phospholipid portion of bound lipid (Table II).

As in the case of free lipid, some germ lipid was found in flour from intact Spring wheat. The bound lipid contributed by the germ contained fraction E and some sterol, and in the case of Thatcher, triglyceride also. TLC of bound lipid from Thatcher germ showed a preponderance of fraction E, some

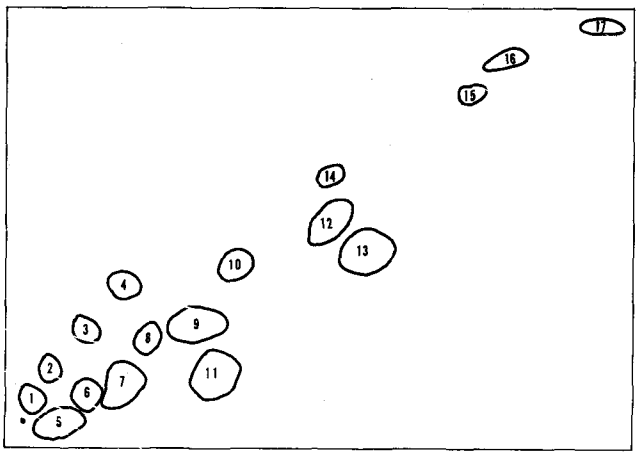

FIG. 4. TLC separation of bound lipid polar lipids. Chloroform-methanol-water development 1½ hr in X-axis and diisobutyl ketone-acetic acid-water development 2 hr in Y-axis.

triglyceride and smaller amounts of unesterified sterols and sterol esters.

TLC revealed only traces of hydrocarbon and sterol ester in whole Thatcher, only traces of sterol ester in whole Lemhi, and the complete absence of these components in the other flour samples.

The small triglyceride fraction from the bound lipid showed greater homogeneity than that from free lipid, while the pattern of free sterols was very similar, showing elution over a long solvent gradient and the presence of higher Rf material in very small quantity.

The pattern of the bound lipid fraction E was more complex than that of the free. Nevertheless, the galactolipids were still the major components, and of the glycerophosphoryl lipids, phosphatidyl choline was dominant. The pattern of the fraction E of bound lipid in the three varieties was similar and that of Thatcher is used as an example (Fig. 4). Phosphatidyl ethanolamine and lysophosphatidyl ethanolamine appeared as components 10 and 8, which were ninhydrin positive, whereas the Dragendorff positive components 9 and 6 were phosphatidyl choline and lysophosphatidyl choline. These four components all showed a positive reaction to the molybdate spray. Components 1,2,3, all ninhydrin positive, were free amino acids and component 7, molybdate positive, was probably an inositol phospholipid. Components 13 and 11 were identified as mono- and di-galactolipids. The di-galactolipid, component 11, showed some variation between varieties, with most present in Mindum and least in Lemhi. The polar lipids of wheat flour are being studied further.

GLC of Fatty Acids. To determine whether varietal differences existed between the fatty acid compositions of these wheats, the fatty acid-containing lipid classes were separated preparatively by TLC, methylated, and analyzed by gas chromatography. The data for the triglyceride fraction show in Table III.

The dominant fatty acid component of the free lipid triglycerides, linoleic acid, was present to the extent of over 50%. The other major fatty acids of this fraction were palmitic and oleic acids. The ratio of total saturated to total unsaturated fatty acid was nearly the same in all varieties. In the Spring wheats, Thatcher and Lemhi, the ratio of palmitic to linoleic was higher than that of oleic to linoleic, whereas in the Durum variety, Mindum, this relationship was reversed.

A much higher degree of saturation was exhibited by the fatty acids of the free lipid fraction E (Table IV) than by those of the triglycerides, particularly in the case of Lemhi and Mindum. In these polar lipids, linoleic and palmitic acids were dominant, linoleic being present in greater quantity than palmitic in Thatcher, whereas the reverse was true in Lemhi and Mindum. The other major fatty acid, oleic, was present in smaller amounts than in the triglycerides.

TABLE III
Fatty Acid Composition of Triglycerides In Free Lipid of Wheat Flour, Weight %

Fatty acid	Thatcher		Lemhi		Mindum	
	Whole	Degermed	Whole	Degermed	Whole	Degermed
16:0	24.3	22.4	25.5	24.3	20.7	21.4
16:1	1.3	0.5	0.3	0.5	0.4	0.3
18:0	0.9	0.8	0	0	1.9	1.8
18:1	21.0	17.9	17.6	17.5	22.1	23.4
18:2	50.6	56.2	55.0	53.4	53.2	52.0
18:3	1.9	2.2	1.6	2.4	1.6	1.0
In Bound Lipid of Wheat Flour						
16:0	35.5	32.5	26.9	32.2	30.9	28.1
16:1	1.6	2.1	1.4	1.3	1.9	0.6
18:0	2.2	3.0	2.4	1.8	3.0	3.2
18:1	15.7	13.2	12.0	8.6	16.7	16.2
18:2	45.1	48.2	55.6	54.0	47.5	50.3
18:3	0	1.0	1.7	2.0	0	1.6

The free lipid sterol esters (Table V) were more highly saturated than the corresponding triglycerides and fraction E except in the case of Mindum. In the Spring wheats, palmitic acid comprised 65% and linoleic acid 15–20% of the sterol ester fatty acids. In Mindum, palmitic and linoleic acids were each present to the extent of 40%. More oleic acid was found in Durum than in Spring wheats.

The fatty acid composition of the bound lipid fraction E (Table IV) showed a marked resemblance to this class of free lipids. Linoleic was the dominant fatty acid in Thatcher, palmitic and linoleic in Lemhi, and palmitic in Mindum. In all three varieties, there was a reduction of linoleic acid in the degermed flour, as compared with the flour from intact grain.

In the bound lipid triglycerides (Table III), the dominant fatty acid was again linoleic. Palmitic acid was present in larger amounts than in the free lipid triglycerides and the concentration of oleic acid was lower. Stearic acid, present in little more than trace quantities in free triglycerides, was present in bound triglycerides to the extent of 2–3%. The appearance of stearic and the higher content of palmitic acid were reflected in the higher ratio of total saturated to total unsaturated acid; the ratio of palmitic to linoleic was more than double that of oleic to linoleic.

GLC of Sterols. A preliminary survey was made of the sterol composition of the sterol ester and unesteri-

TABLE IV
Fatty Acid Composition of Free Lipid Fraction E
Weieght %

Fatty acid	Thatcher		Lemhi		Mindum	
	Whole	Degermed	Whole	Degermed	Whole	Degermed
15.5	1.3	0	0	0	0	0
16:0	24.8	21.8	45.5	39.1	43.9	39.2
16:1	1.0	1.0	1.9	1.2	0.8	0.8
18:0	1.9	1.1	2.6	1.9	4.7	4.2
18:1	14.1	11.7	14.7	16.0	20.3	17.8
18:2	55.1	63.8	32.6	39.1	28.8	35.5
18:3	1.9	0.7	0	0	0	0.4
20:3	0	0	2.8	1.1	0.7	0
23:0	0	0	0	1.6	0.8	2.0
Fatty Acid Composition of Bound Lipid Fraction E						
16:0	22.0	24.6	39.5	41.5	43.5	49.9
16:1	1.0	0.4	1.1	1.7	1.4	1.4
18:0	1.2	1.5	3.4	2.6	3.5	5.0
18:1	11.4	15.0	8.9	12.7	13.4	11.9
18:2	58.6	55.6	45.4	38.4	38.2	31.9
18:3	2.2	1.3	0.6	0	0	0
20:1	3.8	1.8	0	0	0	0
20:3	0	0	1.2	0.5	0	0

TABLE V
Fatty Acid Composition of Free Lipid Sterol Esters
Weight %

Fatty acid	Thatcher		Lemhi		Mindum	
	Whole	Degermed	Whole	Degermed	Whole	Degermed
14:0	0.6	0.6	0	0.6	0	1.8
16:0	74.6	70.9	75.8	75.6	42.0	42.9
16:1	1.4	1.1	1.8	0.5	2.9	1.0
18:0	2.2	2.0	2.1	0.7	3.1	1.8
18:1	5.7	4.9	4.2	2.8	13.1	12.2
18:2	16.1	20.6	15.1	19.8	36.5	40.3

fied sterol classes of free lipid and of the unesterified sterols of bound lipid. The sitosterols accounted for almost all of the material recovered from the column, and of this β sitosterol made up ca. 75%. Little variation in sterol composition was found between varieties or between the esterified and unesterified sterols from the same wheat. A more detailed study of plant sterols is currently under way.

Discussion

In this type of investigation, attention must be given to possible sources of artifacts. To eliminate autoxidation and adventitious hydrolyses, the extracted lipid was stored under nitrogen at —15C and oxygen-free solvents were used. Success in this attempt was indicated by the absence of free fatty acids and partial glycerides, as shown by column and TLC. Some lysophospholipids were indeed present, but these were possibly due to the action of lipolytic enzymes within the wheat kernel.

In the comparison of intact and degermed wheat, an uncertainty was introduced by the breaking of the kernels in the degerming process. Between conditioning and milling, more evaporation took place from the broken kernels than from intact kernels due to the greater surface area of the broken kernels. This was more noticeable in the case of Mindum where the water penetration was least. This differential moisture content accounts for the greater amount of flour obtained from degermed wheat, especially Mindum, than from intact wheat. Thus the contribution of germ lipid to flour may well have been greater in quantity than appeared in this study. Removal of the germ before milling and the consequent elimination of any traces of germ oil in the flour reduced the triglyceride content of the free lipid of all flours. The degerming operation had a different effect on the bound lipid. With the Spring wheats all lipid classes were affected, whereas with Mindum little if any effect was observed.

In general, these three very different varieties of wheat showed similar lipid patterns. In the free lipid, the same class, triglyceride, was dominant in all varieties and varied only in the degree of dominance. Similarly, only a slight difference was apparent in the relationship of the other two major classes of free lipid, phospholipid plus glycolipid, and unesterified sterols. Slightly more sterol ester was found in the Spring wheats than in the Durum, but only Durum flour contained hydrocarbon. A different class, fraction E, the phospholipids plus glycolipids formed the bulk of the bound lipid, but the degree of dominance varied very little between varieties. Traces of sterol esters in Spring wheat bound lipid were contributed by the germ and were not found in degermed flours.

The recovery of "unesterified" sterol from the silicic acid column was noteworthy. In free lipid, and to an even greater extent in bound lipid, this class was eluted over a greatly extended solvent gradient. The complexity suggested by this extended peak and by the very low yield in a preliminary GLC survey showed the necessity for a more detailed study of these unesterified sterols.

The three wheat varieties exhibited similarity in fatty acid composition. The free lipid triglycerides of the Spring wheats, however, were slightly more saturated than those of Durum wheat. In the phospholipid plus glycolipid class, both free and bound, Hard Red Spring wheat showed greater unsaturation than either Soft White Spring or Amber Durum wheats. The highest degree of unsaturation occurred in the free lipid triglycerides and, in general, the free lipid was more unsaturated than the bound lipid.

A varietal difference, not apparent from Tables and Figures, was the much greater complexity of both free and bound lipid from Hard Red Spring wheat. In GLC and in preparative separations, Thatcher lipids always presented more difficulty of separation. For example, additional separations on thin-layer plates were required to bring Thatcher lipids to an equivalent degree of purity and in GLC, the yield (mm^2 chart area/μg injected) was lower than that encountered with equivalent material from the other two wheats. Another varietal difference was the presence of triglyceride in the bound lipid of whole Thatcher wheat; the lipid from the corresponding degermed material, however, contained very little triglyceride.

It would have been desirable to compare the present data with those obtained by other researchers using different wheat varieties. No fruitful comparison could be made, however, because in all other cases where lipid classes were studied *per se*, separation was less complete than was effected here. Sub-fractionation of the lipid classes separated in this study will be described in a subsequent paper.

ACKNOWLEDGMENTS

GLC analysis by J. C. Mes; TLC separations by J. A. G. Larose; separation of the germ and estimation of the epidermis lost in the degerming process by F. B. Johnston.

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