THE FATTY ACIDS OF WHEAT AND ITS MILLED PRODUCTS[1]

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ABSTRACT

The fatty acids of the free and the bound lipids of whole wheat and its Buhler-milled fractions were analyzed by gas-liquid chromatography. The total fatty acid content of the free lipids was approximately twice that of the bound lipids and ranged from 77 to 92% in the former and from 41 to 53% in the latter. In all fractions analyzed, linoleic acid predominated, followed by palmitic, oleic, linolenic, and stearic acid in decreasing order. Straight-grade flour contained considerably more linoleic and palmitic acids and less oleic and linolenic acids than did the bran and the shorts. Calculated iodine values were virtually identical for the lipids of all three fractions.

The fatty acids of wheat and wheat products have been studied by many investigators. As early as 1932 Jamieson and Baughman (1) had established that the principal fatty acids of wheat germ were linoleic, oleic, palmitic, linolenic, and stearic acids. Differences in the fatty acids of bran, germ, and endosperm as indicated by iodine values were reported by several workers (1,2,3). They concluded that germ lipids were more unsaturated and flour lipids less unsaturated than those of bran. Sullivan and her co-workers (4,5,6) greatly extended the range of information concerning the chemical nature of wheat

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lipids. Bailey (2) has reviewed much of this work in detail through 1943. Fisher (7) has reviewed wheat lipids and fatty acids through 1961 and more recently Mecham (8) has presented a very thorough review through 1963.

The advent of gas-liquid chromatography has greatly renewed interest in this area. Fisher (7) and Fabriani (9) analyzed the fatty acids of a number of wheats. Fisher and Boughton (10) and Daniels (11) determined the fatty acid composition of the petroleum ether-soluble (free) lipids of wheat flours and obtained results which were in good agreement. Daniels also examined the fatty acids of the bound lipids (acetone-soluble) and noted a higher linoleic acid content than in the free lipids. McKillican and Sims (12) compared the fatty acids of the various lipid classes of the endosperm of three Canadian wheat varieties. Some varietal differences were noted, but these were relatively minor as compared to differences in the fatty acid composition of the lipid classes. Nelson *et al.* (13) investigated the distribution of fatty acids between bran, germ, and endosperm, and although differences were noted, their conclusions were admittedly tentative, since the bran and germ were obtained from commercial mill streams and the "endosperm" was actually first middlings, also obtained commercially.

No successful attempt, apparently, has yet been made to account for all of the fatty acids of wheat in terms of their distribution between bran, germ, and endosperm and between those fatty acids contained in the more readily extractable lipids and those in the more tightly bound lipids. The present paper summarizes the results of such a survey of the fatty acids of a single variety of wheat.

Materials and Methods

Wheat. A specimen of the variety Selkirk, a hard red spring wheat grown at Crookston, Minnesota, in 1959, was used. It had been stored, unground, at –15°C. since then.

Milling. The wheat was tempered to 15% moisture and milled on a Buhler experimental laboratory mill (Type MCK, Buhler Brothers Inc., Uzwil, Switzerland). Eight streams were obtained: three break streams, three reduction streams, a bran stream collected from the tailover of the 3rd break, and a shorts stream collected from the tailovers of the three reduction streams. Straight-grade flour was obtained by combining all six of the flour streams. Ground whole wheat was prepared using a Brabender sample grinder and the fine setting.

Moisture, protein, and ash of wheat and mill fractions were determined by AACC methods (14).

Lipids. The lipids were obtained by successive extractions of the

material with diethyl ether and water-saturated 1-butanol to give the free and the bound lipids respectively. Fifty grams of material were slurried at room temperature with 150 ml. of diethyl ether (ether) and allowed to stand, with occasional stirring, for 30 min. The clear supernatant was removed by decantation through an ether-washed fluted filter paper following centrifugation at 2,000 r.p.m., and the process was repeated twice, omitting the waiting period. The ether was removed from the combined extracts at room temperature under reduced pressure in a rotary flash evaporator. The lipids were dissolved in redistilled petroleum ether (b.p. 60°–70°C.) and stored in an atmosphere of nitrogen at –15°C. until used for analysis.

The bound lipids were obtained by further extraction of the ether-extracted residue with water-saturated 1-butanol (butanol) in a completely analogous manner. The solvent was removed from the combined extracts under reduced pressure in a rotary flash evaporator at 45°C. The residue was dissolved in petroleum ether and washed, to remove free sugars and other water-soluble material, with an aqueous 10% potassium chloride solution until the washings gave a negative Molisch test. The washed petroleum ether-soluble material, considered the bound lipid, was flushed with nitrogen and stored at –15°C. The entire process, when carried out on four samples simultaneously with no interruptions, required 3 hr.

Fatty Acid Content. The total fatty acid content of a lipid fraction was obtained by refluxing a known amount of lipid with three times its weight of potassium hydroxide (dissolved in 95% ethanol) for 3 hr. The solvent was removed at 40°C. in a rotary flash evaporator and the residue dissolved in distilled water. The solution was acidified by the addition of 3*N* sulfuric acid, and the fatty acids were obtained by extracting the acidified material three times with equal volumes of petroleum ether. The combined petroleum ether extracts were washed once with distilled water, dried over anhydrous sodium sulfate, and then made to volume. The quantity of fatty acid present in the extracts was determined gravimetrically after removal of solvent from a suitable aliquot in tared aluminum dishes. The fatty acid preparations so obtained were subsequently used for gas-chromatographic analysis.

The above procedure was developed using pure triglycerides and monitoring the process by thin-layer chromatography. It was ascertained that hydrolysis was complete in the 3-hr. period and that quantitative recoveries of the fatty acids were obtained in the final petroleum ether extract. The increase in diene conjugation as measured by the ultraviolet absorption method of the American Oil

Chemists' Society (15) was less than 1%. Sterols and other nonsaponifiables which appear in the final fatty acid preparation were estimated, from the data of Nelson *et al.* (16) and from thin-layer chromatography, to constitute less than 2% of the total.

Fatty acid methyl esters for gas-liquid chromatography were prepared by the technique described by Schlenk and Gellerman (17). Diazomethane ("Diazald"), generated by the action of alkali on N-methyl-N-nitroso-*p*-toluene sulfonamide, was bubbled through an ether solution of the fatty acids using the apparatus described by these authors. The resulting solutions of fatty acid methyl esters were analyzed directly after an assay for completeness of methylation by thin-layer chromatography.

Gas-liquid chromatography was carried out using a F&M Model 609 Flame Ionization Gas Chromatograph. Aluminum tubing (¼ in. o.d., 5-ft. lengths) was packed with 80- to 100-mesh acid-washed Chromosorb W containing 25% (w/w) diethylene glycol succinate and 2% (w/w) orthophosphoric acid. Analysis was generally carried out on a 5-μl. sample of a 10% methyl ester solution in diethyl ether, with column temperature of 200°C., and helium flow rate of 90 cc./min.

The methods of Miwa *et al.* (18) and of Woodford and Van Gent (19) were used for presumptive identification of the fatty acid methyl esters. Plots were made of the logarithm of the retention time of normal, saturated, monocarboxylic acid methyl esters *vs.* carbon number. For a particular column packing and helium flow rate there was a linear relationship between the molecular weights of the reference compounds (Hormel Foundation, Austin, Minnesota) and the logarithms of their retention time.

Peak areas were measured using a planimeter, and the ratios of the areas of individual peaks to the sum of the areas of all component peaks gave the percent fatty acid composition. Linear detector response to fatty acid methyl esters over the range of molecular weights encountered was established using fatty acid standards A–D of the U.S. Public Health Service, Metabolism Study Section, National Institutes of Health. The compositions of the standards, when analyzed under the described experimental conditions, checked within 1% of the known percentage composition by weight. This is within the tolerance limits for checks between laboratories suggested by the Lipids Advisory Committee of the National Institutes of Health.

Results and Discussion

The degree of fractionation achieved by the Buhler mill on Selkirk wheat as assessed by protein, ash, and lipid content is given in Table I.

The bran, collected from the tailover of the 3rd break stream, contained the highest ash content, and its lipid and protein content was intermediate between the straight-grade flour and the shorts. The shorts, collected from the tailovers of the three reduction streams, had much the highest lipid content, and although constituting only 3.1% of the wheat, it contained 10% of the wheat lipid. The shorts were visibly high in germ particles, and the composition of this stream may probably be considered as quite characteristic of germ.

TABLE I

COMPOSITION OF SELKIRK WHEAT AND ITS MILLED[a] FRACTIONS

COMPONENTS	PERCENT OF WHEAT	PROTEIN	ASH	LIPID			FREE LIPID	PERCENT OF WHEAT LIPID
				Free	Bound	Total		
	%	(% N × 5.7)	%	%	%	%	% of total	%
Bran	21.5	15.9	7.60	3.38	0.87	4.25	79.4	39.5
Shorts	3.1	16.7	4.00	5.89	1.62	7.51	78.4	10.0
Flour stream								
1st break	7.5	15.3	0.67	0.85	0.59	1.44	59.2	4.6
2nd break	12.9	17.5	0.69	0.97	0.55	1.52	62.6	8.4
3rd break	2.3	18.1	0.97	1.42	0.85	2.27	60.0	2.2
1st reduc.	45.4	13.3	0.58	0.88	0.60	1.48	59.4	28.9
2nd reduc.	5.1	13.5	0.55	1.13	0.63	1.76	64.2	3.9
3rd reduc.	2.2	13.4	0.79	1.61	0.94	2.55	63.2	2.4
Straight-grade flour								
Experimental	75.4	13.7	0.59	0.89	0.66	1.55	57.6	50.5
Calculated[b]	75.4	14.4	0.62	0.95	0.61	1.55	60.5	50.3
Whole wheat								
Experimental	100.0	14.8	2.24	1.48	0.85	2.33	63.7	100.0
Calculated[c]	100.0	14.3	2.20	1.58	0.74	2.32	68.3	100.0

[a] Milled on a Buhler laboratory mill.
[b] Calculated from the weighted contribution made by each of the six flour streams.
[c] Calculated from the weighted contribution made by the total flour, bran, and shorts.

Among the six flour streams there was a rather wide variation in both yield and composition. The high ash and lipid contents of the 3rd break and reduction streams were undoubtedly indicative of contamination by bran and germ respectively. However, the contribution of these two streams to the straight-grade flour, obtained by pooling all six flour streams, was quite small. The composition of the straight-grade flour was largely a reflection of that of the 1st reduction and the 2nd break streams which together contributed 77.3% of the total flour.

The total lipid content was quite variable, not only between bran, shorts, and straight-grade flour, but also between the six flour streams. The range (1.44–7.51%) was quite comparable to that observed for ash (0.55–7.60%) and for protein (13.3–18.1%). The distribution of lipid into free and bound material was also quite variable. While

almost 80% of the bran and shorts lipids were extractable by diethyl ether (free lipids), less than 60% of the flour lipids were so extracted. Within the six flour streams the lipids were more nearly comparable with respect to relative amounts of free and bound material.

Included in Table I are values for the composition of straight-grade flour and for whole wheat obtained by calculation from the weighted contribution of the component parts. Because of the large number of manipulations involved in progressing from the original wheat to the analysis of the fatty acid methyl esters, it is felt that such calculations give a good indication of the over-all recoveries and of the degree of confidence that may be placed upon the results. Variations in the components of replicate chromatograms did not exceed 1%. It is felt that the data calculated and presented in Table I are in very good agreement with the experimental values for whole wheat and for straight-grade flour.

The total fatty acid content of wheat and its three major milled components was determined (Table II) so that subsequent analyses for individual fatty acids could be discussed in terms of the absolute quantities of each present. The results are expressed in the three left-hand columns of Table II as g. of fatty acid obtained from the hydrolysis of 100 g. of lipid. Rather large differences in the total fatty acid content of the three wheat fractions were observed with straight-grade flour (67.0%), the lowest, and bran (82.0%), the highest. The distribution of fatty acids between the free and bound lipids was quite varied. In flour the free lipids contained 77.0% fatty acids as compared to 53.0% for the bound lipids. Similar ranges in fatty acid content were observed in the bran and the shorts. The higher fatty acid contents of the free lipids as compared to bound lipids indicate that glycerides are the major constituent of the diethyl ether-extractable material, as was observed by Nelson *et al.* (16). These workers reported that the bound lipids consist of more than 75% phospho- and glycolipids and they would, therefore, be expected to have a lower fatty acid content than the free lipids. This was found here to be the case. Further reflection upon these observations shows that the surprisingly high values of fatty acid content given in Table II are entirely plausible. For example, calculations show pure trilinolein to consist of 95.6% fatty acid, lecithin 72.7%, and digalactosyl glyceride (containing two linoleic acid residues) 58.5%. It would appear from the data in Table II that the bound lipids of all of the wheat fractions contained, in addition to digalactosyl glycerides, other compounds whose fatty acid contents are considerably lower than 58.5%. Further work is needed to establish the presence and nature of such compounds.

TABLE II

FATTY ACID CONTENT[a] OF WHEAT AND ITS MILLED FRACTIONS

	Fatty Acids					
	As Percent of Lipids			As Percent of Wheat Component		
	Free	Bound	Total	Free	Bound	Total
	%	%	%	%	%	%
Bran	92.0	41.0	82.0	3.10	0.36	3.46
Shorts	87.0	54.0	80.0	5.13	0.87	6.00
Flour[b]	77.0	53.0	67.0	0.69	0.35	1.04
Whole wheat						
Experimental	87.0	47.0	72.0	1.29	0.40	1.69
Calculated	85.0	50.0	72.0	1.34	0.37	1.71

[a] Expressed as g. of fatty acid obtained from 100 g. of lipid.
[b] Straight grade, obtained by combining the six flour streams.

The three right-hand columns of Table II present the same data recalculated to show the fatty acid content of the original material rather than of the lipids. From Table II, then, it can be seen that while the total flour lipids contained 67.0% fatty acids, the flour itself contained only 1.04% fatty acids, approximately two-thirds of which were extractable by diethyl ether.

The distribution of individual fatty acids in the lipids of whole wheat, bran, shorts, and straight-grade flour is given in Table III. Linoleic, palmitic, oleic, and linolenic acids constituted more than 97% of the fatty acids of all fractions. Considering first the total lipids, the fatty acids of the bran and the shorts were virtually identical. The flour lipids, however, contained considerably more palmitic

TABLE III

FATTY ACID COMPOSITION OF THE LIPIDS OF WHEAT AND ITS MILLED PARTS

	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Others	Total
	%	%	%	%	%	%	%
Total lipids[a]							
Bran	17.9	1.0	16.8	57.3	5.6	1.3	99.9
Shorts	17.7	1.3	15.7	58.2	5.9	1.3	100.1
Flour	20.0	1.2	11.2	62.7	3.9	0.9	99.9
Wheat	17.4	1.0	13.9	61.1	5.0	1.6	100.0
Free lipids							
Bran	17.6	1.0	18.1	56.1	6.1	1.1	100.0
Shorts	16.1	1.4	18.1	57.1	6.1	1.3	100.1
Flour	19.6	1.2	12.8	61.5	3.7	1.1	99.9
Wheat	16.3	1.0	15.4	60.2	5.3	1.9	100.1
Bound lipids							
Bran	18.5	1.0	14.0	60.1	4.6	1.8	100.0
Shorts	20.2	1.0	12.0	60.0	5.5	1.4	100.1
Flour	20.6	1.2	8.8	64.4	4.3	0.7	100.0
Wheat	19.5	1.1	11.1	62.7	4.5	1.2	100.1

[a] Calculated iodine value: bran, 135; shorts, 136; flour, 135; wheat, 137.

and linoleic acids, with a corresponding decrease in the content of oleic and linolenic acids. Calculated iodine values indicate that all fractions had virtually identical degrees of unsaturation. This is at variance with other published reports (1,2,3) in which iodine values indicated that germ lipids have a considerably higher degree of unsaturation than the rest of the wheat kernel.

Some differences in the distribution of fatty acids between the free and the bound lipids may also be noted. In all fractions the free lipids had a higher content of oleic and linolenic acids and a lower content of palmitic and linoleic acids. That these differences are a reflection of a considerable degree of specificity in the incorporation of fatty acids into different groups of lipids will be the topic of a future publication.

Table IV presents the data of Table III recalculated to show the total fatty acid content of the various wheat fractions rather than of the lipids. A more useful account of the fatty acid distribution is thereby obtained, one which should enable at least approximate estimations of the fatty acid content of wheat products to be made.

TABLE IV

INDIVIDUAL FATTY ACIDS OF WHEAT AND ITS MILLED PARTS

	Wheat	Flour		Bran		Shorts	
		Percent of Flour	Percent of Wheat	Percent of Bran	Percent of Wheat	Percent of Shorts	Percent of Wheat
	%	%	%	%	%	%	%
Total lipids	2.32	1.55	1.17	4.25	0.92	7.51	0.23
Total fatty acids[a]	1.69	1.04	0.79	3.46	0.75	6.01	0.18
Palmitic acid	0.30	0.21	0.16	0.62	0.13	1.08	0.03
Stearic acid	0.02	0.01	0.01	0.03	0.01	0.06	0.00
Oleic acid	0.24	0.12	0.09	0.59	0.13	1.01	0.03
Linoleic acid	1.05	0.65	0.49	2.02	0.44	3.44	0.11
Linolenic acid	0.09	0.05	0.04	0.19	0.04	0.34	0.01
Other	0.03	0.01	0.01	0.04	0.01	0.01	0.00
Total	1.73	1.05	0.80	3.49	0.76	5.92	0.18

[a] Determined directly (see Table II).

Table V gives the fatty acid content of the six flour streams. The calculated value for straight-grade flour was very close to the experimental value. As would be expected, the composition of the flour is largely a reflection of the composition of the 1st reduction and 2nd break streams which together constituted 77% of the straight-grade flour. The higher oleic acid and lower linoleic acid content of the 3rd break and reduction streams as compared to the flour is considered indicative of contamination of these streams by bran and germ.

The presence in wheat and flour of fatty acids with odd-numbered carbon chains has been reported by Coppock *et al.* (20) and others

TABLE V

FATTY ACID COMPOSITION OF THE LIPIDS OF BREAK AND REDUCTION FLOURS

FATTY ACID	BREAK FLOURS			REDUCTION FLOURS			STRAIGHT-GRADE FLOUR	
	1st	2nd	3rd	1st	2nd	3rd	Calc.	Det.
	%	%	%	%	%	%	%	%
Palmitic	19.6	18.6	20.6	20.3	19.2	18.9	19.9	20.0
Stearic	1.6	1.4	1.2	1.2	1.0	1.3	1.3	1.2
Oleic	10.6	11.1	13.2	10.7	12.4	14.9	11.1	11.2
Linoleic	62.2	63.4	59.7	63.0	62.3	58.8	62.7	62.7
Linolenic	5.0	3.9	4.3	4.0	3.8	4.7	4.1	3.9
Other	1.1	1.6	1.0	0.8	1.3	1.4	1.0	0.9
Total	100.1	100.0	100.0	100.0	100.0	100.0	100.1	99.9

(13,21). Table VI lists those acids observed during the course of this investigation. Identifications were made by enrichment of the mixtures with authentic specimens when available and from plots of log-retention time *vs.* carbon number. Stearic acid is included in Table VI as a reference.

All of the odd-numbered straight-chain fatty acids between C_{11} and C_{21} were detected and in quite widely differing amounts within each fraction. Peaks corresponding to lauric, myristic, myristoleic, palmitoleic, arachidic, arachidonic, and behenic acids were also observed.

TABLE VI

MINOR FATTY ACIDS OF WHEAT AND ITS MILLED FRACTIONS

FATTY ACID[a]	FLOUR	BRAN	SHORTS	WHOLE WHEAT
	% × 100	% × 100	% × 100	% × 100
$C_{11:0}$	1	7	4	8
$C_{12:0}$	5	7	9	5
$C_{13:0}$	2	3	2	2
$C_{14:0}$	13	25	22	20
$C_{14:1}$	1	1	3	2
$C_{15:0}$	8	12	13	11
$C_{16:1}$	13	20	17	12
$C_{17:0}$	12	12	13	9
$C_{18:0}$	120	100	130	100
$C_{19:0}$[b]	p	p	p	p
$C_{20:0}$	23	32	30	20
$C_{20:4}$	4	14	8	27
$C_{21:0}$	7	15	10	16
$C_{22:0}$	15	21	21	33

[a] Listed by carbon number and by number of double bonds.
[b] Indicated as being present only. In the original methyl ester preparation, the C_{19} acid was obscured by the presence of linoleic acid and detection required hydrogenation prior to gas chromatography.

The quantities of these acids present were extremely small, and the instrument had to be operated at almost maximum sensitivity to obtain peaks large enough for accurate measurement. This in turn resulted in considerable "noise" on the chromatograms. The chroma-

tograms were, however, quite reproducible, and it is felt that within each fraction, although not necessarily between fractions, a good estimation of the relative amounts of the various trace acids was obtained.

Summary

The above represents what is believed to be a rather comprehensive survey of the fatty acids contained in a pure variety of wheat, Selkirk. The total fatty acid content of whole wheat, bran, shorts, and straight-grade flour as obtained from a Buhler laboratory mill was determined directly and found to vary over a considerable range. A distinction was made between free lipids and bound lipids. It was found that the bound lipids of bran contained 41% fatty acids, defined as the grams of fatty acid obtained from alkaline hydrolysis of 100 g. of lipid, and that the free lipids of bran contained 92% fatty acids. These represented the extremes observed. The total fatty acid content of straight-grade flour lipids was 67% and of whole-wheat lipids 72%.

Differences of as much as 5% were observed in the individual fatty acid content between mill fractions and between the free and bound lipids of an individual mill fraction. The component mill streams that comprised the straight-grade flour were more nearly alike, as would be expected.

Although the distribution of fatty acids in bran, shorts, and flour was somewhat different, calculated iodine values for the lipids of these fractions were identical. It would thus appear that any differences observed in the lipids of various wheat fractions are a result of preferential iodine absorption of components other than fatty acids.

The presentation of results as a percentage of the parent material rather than of the lipids is somewhat of a departure from custom in the current literature and should be of interest from a nutritional standpoint, particularly in view of the current interest in intake of saturated fatty acids.

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