THE TRIGLYCERIDES AND FATTY ACIDS OF WHEAT[1]

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ABSTRACT

The triglycerides of wheat, the fatty acids obtained from them, and the total fatty acids of wheat, bran, germ, and endosperm were analyzed by countercurrent distribution and gas-liquid chromatography. A relatively few types of triglycerides were found to be present. The total as well as triglyceride fatty acids present in bran, germ, and endosperm are quite similar although differences do exist.

The fatty acids in the glycerides of wheat and in the total lipid material of wheat or wheat products have been studied by many investigators using various methods. Palmitic, stearic, oleic, linoleic, and linolenic acids have been reported by several different workers. Jamieson and Baughman (8) and Sullivan and Bailey (12) reported the

[1]Manuscript received June 22, 1962. Presented at the 47th annual meeting, St. Louis, Mo. Contribution from the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn. Paper No. 5038, Scientific Journal Series, Minnesota Agricultural Experiment Station. The data presented are taken from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy by J. H. Nelson, July 1961. Present address: Quality Control Department, General Mills, Inc., Minneapolis 14, Minn.

presence of lignoceric acid in wheat germ. Palmitoleic acid in wheat was reported by Barton-Wright (1). Coppock *et al.* (2), using gas-liquid chromatography, found unsaturated C_6 and C_7 acids, all of the normal acids from C_8 to C_{13}, a branched-chain C_{12} acid, and margaric acid (C_{17}) in addition to those previously reported in wheat flour. Lee and Tkachuk (9), also using gas-liquid chromatography, found palmitoleic, pentadecanoic, and a C_{21} unsaturated fatty acid in wheat-flour lipids.

Mason and Johnston (10) applied countercurrent distribution methods to a comparative study of the phosphatides of flour milled from two different varieties of wheat and showed them to be quite complex mixtures.

This paper presents the results obtained by countercurrent distribution and gas-liquid chromatographic analysis of whole-wheat lipids, whole-wheat triglycerides, and the lipids obtained from bran, germ, and "endosperm" (first middlings).

Materials and Methods

Wheat. The variety Selkirk, a hard red spring wheat grown at Crookston, Minnesota, in 1959, was used.

Wheat Fractions. Samples of bran, germ, and "endosperm" were obtained from a commercial milling company. These fractions were components of the same mill mix. The bran contained traces of endosperm adhering to the coat, the germ was of good quality (fresh germ), and the "endosperm" was a pure first-middlings stream.

The lipids were extracted from ground whole wheat and from the various fractions by water-saturated n-butanol at room temperature, and the triglycerides were isolated by chromatography on silicic acid columns exactly as described in a previous paper (11).

Countercurrent Distribution Analyses. These were carried out on a Craig-Post apparatus with 40-ml. lower-phase capacity (3). The solvent system used was that described by Dutton and Cannon (6); i.e., nitroethane-furfural-Skellysolve F, 4:4:10 (v/v/v). A total of 4.3 g. of triglyceride were analyzed by the withdrawal method described by Craig and Craig (4); a total of 927 transfers. Gravimetric analysis of a portion of the contents of each tube was made after removal of solvent under vacuum.

Gas Chromatography. The methyl esters of the saponified fatty acids were prepared, by the methylation technique of Gellerman and Schlenk (7). Diazomethane was generated from N-methyl-N-nitroso-*p*-toluene sulfonamide and passed through a diethyl ether solution of the fatty acid until excess diazomethane was present, causing the solution

to turn yellow.

Gas-liquid chromatograhpic analyses were made with a Beckman GC-2 instrument employing a thermal conductivity detector and a Brown recorder. A 10-ft. aluminum column packed with Craig ester (butanediol succinate polyester) as the liquid phase supported on chromosorb, was used. All analyses were carried out with a column temperature of 209°–210°C., a flow rate of 40–45 ml. helium per min., a current of 250 ma., and maximum attenuation of the detector signal. Quantitative data were obtained with a planimeter from the curves. Standards of authentic specimens were run to determine correction factors for converting peak areas to percentage values.

Results

The results of a countercurrent distribution fractionation of 4.3 g. of whole-wheat triglycerides, shown in Fig. 1, indicate that there are three main classes of triglycerides in wheat. The rate of migration of

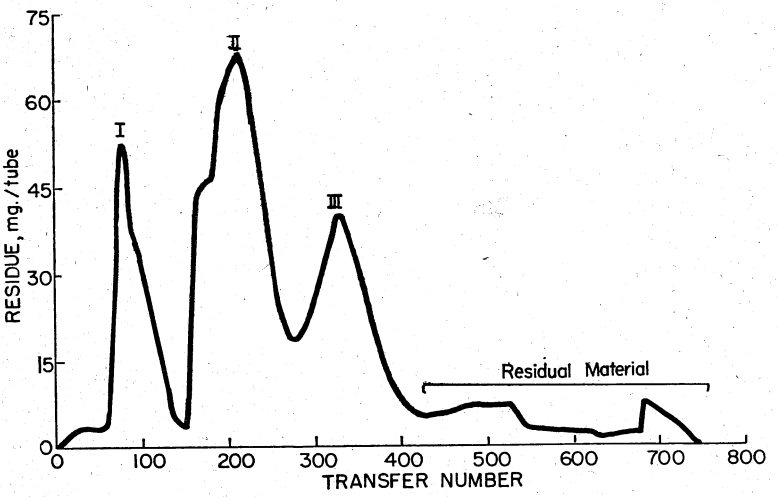

Fig. 1. Countercurrent distribution of whole-wheat triglycerides.

triglycerides is governed by two factors, the chain length and the degree of unsaturation present in the component fatty acids. The more highly unsaturated triglycerides and those containing shorter fatty acids will migrate more slowly, since the stationary phase, in this system, was the more polar phase. It has been observed (5) that the addition of a double bond affects the rate of migration of a compound to approximately the same extent and in the same direction as does the removal of two carbons. Triolein, for instance, migrates

at virtually the same rate as tripalmitin.

The various wheat triglycerides are thus comprised of rather markedly different component fatty acids. These various solubility classes can be expected to differ from each other either in fatty acid chain length or degree of unsaturation, or both.

The methyl esters obtained by alkaline hydrolysis of the triglycerides from the central portion of each of the three major peaks shown in Fig. 1, a pooled portion of all of the slowly migrating material, termed residual material, as well as unfractionated whole-wheat triglycerides, were analyzed by gas-liquid chromatography with the results shown in Table I. The various methyl esters were identified by comparison with the retention time of authentic specimens.

TABLE I

THE FATTY ACID COMPOSITION OF WHOLE-WHEAT TRIGLYGERIDES — ALKALINE HYDROLYSIS

FATTY ACID METHYL ESTER	WHOLE-WHEAT TRIGLYCERIDE	PEAK I	PEAK II	PEAK III	RESIDUAL
	%	%	%	%	%
Myristate ($C_{14:0}$)	trace	0.6	trace	0.0	trace
Palmitate ($C_{16:0}$)	16.7	34.6	20.6	3.5	3.0
Palmitoleate ($C_{16:1}$)	0.7	2.2	1.9	0.7	0.5
"Margarate" ($C_{17:0}$)	trace	0.0	0.0	0.0	18.7
Stearate ($C_{18:0}$)	0.3	1.3	0.6	0.0	1.0
Oleate ($C_{18:1}$)	16.5	28.9	10.9	3.5	2.0
Linoleate ($C_{18:2}$)	59.0	32.4	59.8	84.8	50.3
Linolenate ($C_{18:3}$)	4.3	0.0	6.0	7.6	23.2
Arachidate ($C_{20:0}$)	1.9	0.0	0.0	0.0	0.0
Others	0.7	0.0	0.0	0.0	1.2
Total	100.1	100.0	99.8	100.1	99.9

The unfractionated whole-wheat triglycerides contained 59.0% linoleic acid, and lesser and virtually equal amounts of palmitic and oleic acids (16.7 and 16.5% respectively). Much smaller amounts of linolenic, arachidic, palmitoleic, and stearic acids were also observed. The fatty acids thus identified comprised 99.4% of those recovered. The remaining material emerged from the column as a number of small indistinct peaks which were not identified.

As is consistent with the known behavior of triglycerides in the countercurrent distribution system, the fatty acids contained in peak I (Fig. 1) had the least amount of unsaturation of the four groups of triglycerides obtained by countercurrent distribution. Linoleic and palmitic acids in approximately equal amounts and oleic acid in only slightly lesser quantity comprised 95% of the total. Assuming a random distribution of the fatty acids, it would appear that the trigly-

cerides of peak I were essentially oleoylpalmitoylinolein. Not given in Table I are the results of analyses made on material at the leading and trailing edges of the peaks. In all cases the results were virtually identical with those obtained from the central portions.

The triglycerides of peak II contained more highly unsaturated fatty acids than those in peak I, with linoleic acid constituting 60% of the total. Considering that a C_{16} saturated fatty acid is equivalent, in the countercurrent system, to a C_{18} fatty acid with one double bond, the triglycerides in peak I may be said to have a relative unsaturation of four double bonds (linoleic = 2, oleic = 1, palmitic = oleic in this system) and those in peak II a relative unsaturation of five double bonds (two linoleic residues plus one palmitic or oleic acid residue $= 2 + 2 + 1 = 5$). The mixed triglycerides in this peak appear to be of two types, palmitoyldilinolein and oleoyldilinolein.

The fatty acids contained in the triglycerides of peak III consisted primarily of linoleic acid (84.8%). This indicates that more than 80% of peak consisted of trilinolein, with a relative unsaturation of 6. On the basis of their migration rates the remaining triglycerides must contain one linolenic residue, one linoleic residue and one residue of either palmitic or oleic acid to give, again, a relative unsaturation of six double bonds.

The residual material, consisting of all of the material that migrated more slowly than peak III, was quite highly unsaturated, as would be expected. Figure 1 indicates that this is a very heterogeneous fraction, and speculation as to its probable triglyceride composition would be valueless. Linolenic and linoleic acid residues comprised 73.5% of the total.

A fraction appeared on the chromatogram which was tentatively ascribed to the C_{17} saturated fatty acid margaric acid. Although positive identification awaits its isolation, further presumptive evidence for the presence of margaric acid, in surprisingly large amounts, was obtained by comparison with the authentic material. Figure 2 is a chromatogram of a mixture of the methyl esters of authentic saturated straight-chain C_{14}, C_{16}, C_{17}, and C_{18} fatty acids and an isomeric saturated C_{17} fatty acid. Figure 3 is a chromatogram obtained from the same material to which had been added the methyl esters of a hydrogenated sample of the residual material shown in Fig. 1. Only the C_{16}, C_{18}, and straight-chain C_{17} peaks were enhanced.

The fatty acid distribution within the wheat kernel, considering both those present in the triglycerides and in the total lipid of bran, germ, and "endosperm" (first middlings), was determined, by the

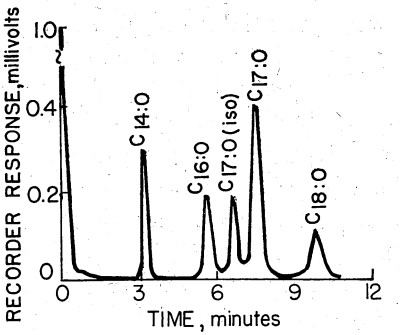

Fig. 2. Chromatogram of the methyl esters of an authentic mixture of saturated fatty acids.

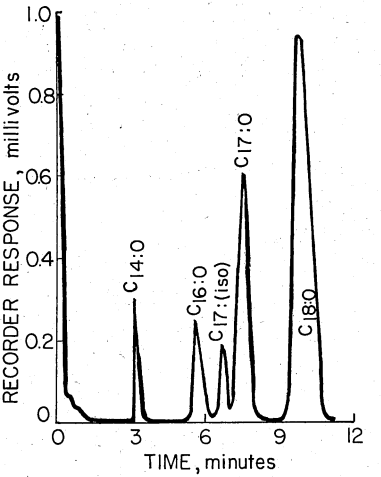

Fig. 3. Chromatogram of material shown in Fig. 2, to which had been added the methyl esters of a hydrogenated sample of fatty acids obtained from wheat.

methods previously outlined. The results, shown in Table II, indicate that a considerable similarity exists in the fatty acid content of the three major parts of the wheat kernel, both in the triglyceride fatty acids and the total fatty acids. The greatest differences observed were in the germ, which had a higher content of linolenic acid and a lower content of oleic acid than did the other components. These differences, however, were slight.

Presumably the apparent difference in the fatty acid content of the whole kernel as compared to that of its constituent parts is the result of using a first-middling stream for the endosperm portion. This would quite obviously not be representative of the entire endosperm.

The four major fractions of whole-wheat lipids were isolated from a

TABLE II

FATTY ACID COMPOSITION OF THE TOTAL LIPID AND TRIGLYCERIDES FROM WHEAT, BRAN, GERM, AND ENDOSPERM

FATTY ACID METHYL ESTERS	TOTAL LIPID				TRIGLYCERIDES			
	From Whole Wheat	From Bran	From Germ	From "Endosperm"	From Whole Wheat	From Bran	From Germ	From "Endosperm"
	%	%	%	%	%	%	%	%
Myristate ($C_{14:0}$)	0.1	trace	trace	trace	trace	trace	trace	trace
Palmitate ($C_{16:0}$)	24.5	18.3	18.5	18.0	16.7	17.9	19.4	12.9
Palmitoleate ($C_{16:1}$)	0.8	0.9	0.7	1.0	0.7	0.7	0.8	1.1
Stearate ($C_{18:0}$)	1.0	1.1	0.4	1.2	0.3	0.8	0.5	0.7
Oleate ($C_{18:1}$)	11.5	20.9	17.3	19.4	16.5	20.3	19.6	15.1
Linoleate ($C_{18:2}$)	56.3	57.7	57.0	56.2	59.0	56.2	52.5	65.1
Linolenate ($C_{18:3}$)	3.7	1.3	5.2	3.1	4.3	2.9	4.5	3.5
Arachidate ($C_{20:0}$)	0.8	trace	trace	trace	1.9	0.7	0.5	0.0
Others	1.1	trace	0.8	1.1	0.7	0.8	2.4	1.5
Total	100.0	100.2	99.9	100.0	100.1	100.3	100.2	99.9

single silicic acid column run (11). These were each saponified and the fatty acid methyl esters so obtained were chromatographed.

The results, Table III, indicate that rather large differences occur in the fatty acid composition of the different types of wheat lipids. Fraction I, which presumably contained the stearyl esters from wheat, showed a relatively simple fatty acid pattern, high in palmitic, palmitoleic, stearic, and oleic acids, when compared to the fatty acid composition of whole-wheat lipid. Relatively little linoleic acid (compared to whole wheat) and only a trace of linolenic acid were present.

TABLE III

FATTY ACID COMPOSITION OF THE TOTAL AND THE FOUR FRACTIONS OF WHOLE-WHEAT LIPIDS

FATTY ACID METHYL ESTERS	WHOLE WHEAT	FRACTION I	FRACTION II	FRACTION III	FRACTION IV
	%	%	%	%	%
Myristate ($C_{14:0}$)	0.1	0.0	trace	0.4	0.0
Palmitate ($C_{16:0}$)	24.5	30.4	16.7	24.8	17.5
Palmitoleate ($C_{16:1}$)	0.8	4.7	0.7	2.4	0.6
"Margarate" ($C_{17:0}$)	0.2	0.0	0.0	0.0	0.0
Stearate ($C_{18:0}$)	1.0	4.7	0.3	1.1	0.6
Oleate ($C_{18:1}$)	11.5	30.4	16.5	10.0	8.5
Linoleate ($C_{18:2}$)	56.3	29.7	59.0	54.8	67.2
Linolenate ($C_{18:3}$)	3.7	trace	4.3	4.9	3.7
Arachidate ($C_{20:0}$)	0.8	0.0	1.9	0.9	1.5
Others	1.1	0.0	0.7	0.5	0.1
Total	100.0	99.9	100.1	99.8	99.7

Fraction II, which was the triglyceride fraction, had a fatty acid composition similar to that of whole wheat except that it was somewhat lower in palmitic acid and higher in oleic acid.

Fraction III, which probably contains xanthophyll esters and tocopheryl esters, besides esters of unknown chemical nature, and possibly traces of diglycerides and monoglycerides, was also similar to whole wheat in its fatty acid with only very minor differences to be noted.

Fraction IV had a considerably greater amount of linoleic acid than did the other fractions but was otherwise quite comparable in fatty acid composition.

Discussion

These results indicate that the triglycerides of wheat are relatively simple in their fatty acid composition. Gas-liquid chromatography of the fatty acids obtained by countercurrent distribution permits them to be divided into three major groups on the basis of unsaturation and chain length. These had relative unsaturations of 4, 5, and 6 respectively. In this classification the introduction of a double bond is considered equivalent to the removal of two carbon atoms.

A selective esterification of the various fatty acid-containing compounds was indicated by the differences, although slight, found in the fatty acid content of bran, germ, and "endosperm" and, more strikingly, in those observed in the four groups of compounds obtained by silicic acid column chromatography.

Obviously much work remains to be done in the chemical identification of the many compounds observed during the course of this work.

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