SILICIC ACID CHROMATOGRAPHY OF WHEAT LIPIDS[1]

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ABSTRACT

Lipid material extracted from whole-wheat meal by water-saturated 1-butanol was chromatographed on silicic acid columns. With a gradient elution solvent system of increasing concentrations of ethyl ether in petroleum ether followed by absolute methanol, wheat lipids were separated reproducibly into four fractions of increasing polarity. Fraction I presumably contained hydrocarbons and steryl esters; fraction II consisted almost entirely of triglycerides; fraction III was very heterogeneous and contained, among other compounds, the bulk of the wheat pigments; and fraction IV, which contained phosphorus, nitrogen, and sugars, was presumed to consist largely of phospholipids and glycolipids. Wheat lipids extractable by water-saturated 1-butanol but not by ethyl ether consist almost entirely of polar material as distinguished from the predominantly nonpolar nature of the ethyl ether-extractable substances. Marked differences in the lipids of bran, germ, and "endosperm" were observed.

Lipid chemistry has recently been marked by the development of a number of techniques suitable for the investigation of this complex group of substances. These include silicic acid chromatography, countercurrent distribution, thin-layer chromatography, and gas-liquid chromatography. By the use of these methods it is now possible to make separations cleanly, quickly, and reproducibly.

Several authors (1,3,5,6) have emphasized the need for a detailed knowledge of the lipids present in wheat and flour prior to understanding the role which lipids may play in the processing and the storage behavior of wheat and wheat products. Although progress has been made, it has been rather slow because of the great number of individual compounds present in the lipid fraction of wheat, and the difficulty with which these compounds can be separated from each other.

Fisher and Broughton (2) and Wren and Elliston (10) have applied the silicic acid column technique of Hirsch and Ahrens (4) to a study of wheat and flour lipids, respectively, and obtained good resolution of a great number of individual components.

This paper presents data on the silicic acid column fractionation of whole-wheat lipids and of the lipids obtained from bran, germ, and "endosperm."

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Materials and Methods

Wheat and Wheat Fractions. The variety Selkirk, a hard red spring wheat grown at Crookston, Minnesota, in 1959 was used. Samples of germ, bran, and "endosperm" (1st middlings) were obtained from a commercial mill. These fractions were from the same mill mix. The wheat from the mix was not obtained and all comparisons are between the three fractions and Selkirk. It is felt that, since in this work rather coarse lipid fractions are being considered, the comparisons will be essentially valid.

Reagents. Silicic acid, specially prepared for the chromatography of lipids, from Bio-Rad Laboratories, Richmond, California.

"Silica Gel G" for thin-layer chromatography was purchased from E. Merck Ag., Darmstadt, Germany.

Water-saturated 1-butanol (butanol), technical grade, was redistilled in the presence of zinc granules and potassium hydroxide. The redistilled alcohol was mixed with an excess of water, heated to approximately 100°C., and then cooled to room temperature while a stream of nitrogen was bubbled through the mixture.

Preparation of Lipid Extracts. The wheat to be extracted was ground in a Brabender sample grinder using the fine setting. The ground grain or other material (60 g.) was immediately filled into a sintered glass Büchner funnel which was fitted to a suction flask. The apparatus was contained in a chamber under an atmosphere of nitrogen. The material was slurried with 120 ml. of butanol, allowed to stand 15 min., and filtered under reduced pressure. The ground material was further extracted three times with 50-ml. aliquots of fresh solvent and the combined extracts were evaporated to dryness, under reduced pressure, in a rotary flask evaporator at 40°C. The residue was extracted with petroleum ether (b.p. 60°–70°C.). The petroleum ether-soluble material was considered to be the total lipid extract. The entire process required 1.5 hr. from grinding and was always performed without interruptions. The extracts were stored in petroleum ether solution, in the dark, under nitrogen, at −10°C.

Column Chromatography. Essentially, the procedure of Hirsch and Ahrens (4) was used. To aid in the uniform packing of the column a vibrator (Vibro-Graver, Burgess Vibrografters, Chicago, Ill.) was applied to the side of the column as the dry silicic acid powder was dusted into the column. The final column was 15 mm. in diameter and 180 mm. high. It had a flow rate of about 0.5 ml. per min.

Approximately 500 mg. of the material to be chromatographed were dissolved in 10–15 ml. petroleum ether and placed on the column.

The column was then developed, using a gradient solvent system of increasing concentrations of ethyl ether in petroleum ether such that the initial solvent was 100% petroleum ether and the final concentration was 70% ethyl ether in petroleum ether. Material remaining on the column at this point was eluted with absolute methanol.

Thirty-minute fractions (about 15 ml.) were collected; aliquots were removed, the solvent evaporated, and weight distribution curves obtained.

Thin-Layer Chromatography. Glass plates (20 × 20 cm.) covered with a thin layer (0.25 mm.) of silica gel were prepared by applying a slurry of Silica Gel G (45 g. gel per 100 ml. water) to clean glass plates using the equipment described by Stahl (8,9). The plates were dried for 3 hr. at 130°C.

Material to be chromatographed was dissolved in ethyl ether and applied to the silica gel film with a capillary pipet. One hundred lambda of a 1% solution were routinely used. The plates were subjected to ascending chromatography using ethyl ether in petroleum ether, or methanol in chloroform, in various proportions, as solvents. After drying, the plates were sprayed with sulfuric acid and heated. The organic components appeared as black, brown, or red spots on a white background.

Paper Chromatography. This was carried out in the usual manner using the descending technique, the solvent system 1-butanol-pyridine-water (6:4:3, v/v), and Whatman No. 1 paper. The sugars were located, after development of the chromatogram, by spraying with ammoniacal silver nitrate and heating.

Results and Discussion

A comparison of the amounts of lipid extracted from wheat and from its various parts by butanol is given in Table I. A rather large amount of nonlipid material was extracted from whole wheat, bran, and germ, but all of the material extracted from "endosperm" was, after removal of the butanol, soluble in petroleum ether and hence

TABLE I

BUTANOL EXTRACTION OF WHEAT AND ITS COMPONENTS

MATERIAL	PERCENT EXTRACTED WITH BUTANOL	PERCENT SOLUBLE IN PET. ETHER	PERCENT LIPID
Whole wheat	2.8	83	2.3
Bran	6.7	46	3.1
Germ	12.9	90	11.6
"Endosperm"	1.4	100	1.4

was considered to be lipid.

Silicic acid column chromatography of the whole-wheat lipids gave the results shown in Fig. 1 and Table II. For convenience the material eluted from the column was pooled into four groups as indicated in

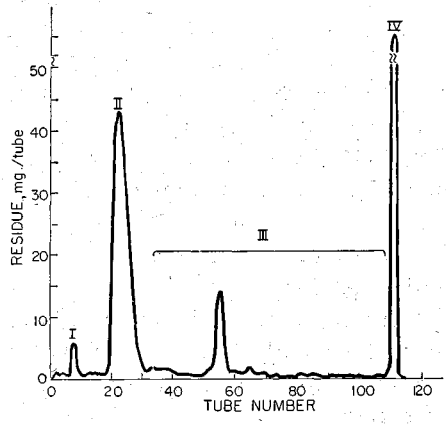

Fig. 1. Gradient elution chromatography of whole-wheat lipids.

TABLE II

SILICIC ACID CHROMATOGRAPHY OF WHOLE-WHEAT LIPIDS

FRACTION	ETHER EXTRACT	BUTANOL EXTRACT	PERCENT OF TOTAL IN ETHER EXTRACT
	%	%	
I	4.0	1.5	72.7
II	70.0	9.0	88.6
III	14.5	12.0	54.7
IV	12.5	77.5	13.9

Fig. 1. The pattern obtained was very reproducible, and the method is quite suitable for obtaining sufficient amounts of the various groups of compounds to make isolation and identification feasible. The classes of compounds most likely present in the four groups are, according to Hirsch and Ahrens (4), as follows:

Fraction	*Lipid-Class*
I	Hydrocarbons and steryl esters
II	Triglycerides
III	Mono- and diglycerides, fatty acids, and sterols
IV	Phospho- and glycolipids

It is realized that this classification is of a very presumptive nature. That it is generally true, however, is indicated by the following.

Fraction I contained no nitrogen or phosphorus and gave a negative Molisch test. Its migration rate on the column indicated that it was the least polar of the four groups. From it was obtained a white crystalline material, m.p. 92°–94°C., which gave a positive Liebermann-Burchard test for sterols. On this basis it is presumed to be sitosterol palmitate isolated by Spielman (7). Fraction I was shown to be very heterogeneous by thin-layer chromatography on silicic acid.

Fraction II contained no nitrogen or phosphorus and gave a negative Molisch test. Thin-layer chromatography showed it to consist of triglycerides in an estimated excess of 99% purity. From 35 runs on the column 12 g. of triglycerides were obtained in which no contaminants could be detected. The fatty acid composition of this fraction will be reported in a subsequent publication.

Fraction III was extremely heterogeneous and contained virtually all of the wheat pigments. Only trace amounts of nitrogen and phosphorus were present; they gave a negative Molisch test. The presence of sterols was indicated by a very strongly positive Liebermann-Burchard test.

Fraction IV contained the more polar lipid components. It was eluted from the column by methanol. It contained virtually all of the nitrogen and phosphorus present in the original wheat extract. Bound galactose and glucose were present in this fraction as identified by paper chromatography following hydrolysis of the material with 0.1*N* hydrochloric acid.

A comparison was made, on silicic acid columns, of the lipids extracted from ground whole wheat by ether with those subsequently extracted by butanol. The results, Figs. 2 and 3 and Table II, illustrate the marked difference between the "free" and the "bound" lipids of wheat. Ether extracted approximately 73% of the total fraction I lipids, 89% of the fraction II lipids, 55% of the fraction III lipids, but only 14% of the polar fraction IV lipids. The use of ether followed by butanol constitutes a quite efficient method for making a preliminary fractionation of wheat lipids into classes based on polarity.

In Table III are given the results obtained when butanol extracts of bran, germ, and endosperm were chromatographed on silicic acid in the manner described for whole-wheat lipids. The data obtained on the latter are included in Table III for comparison.

Quite marked differences in the distribution of the four lipid classes within the kernel are apparent. In excess of 50% of the bran

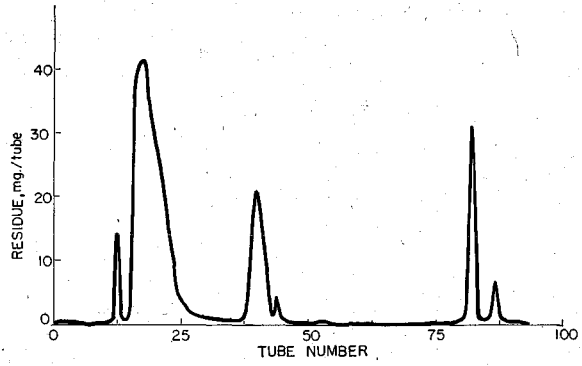

Fig. 2. Gradient elution chromatography of the diethyl ether extract of ground whole wheat.

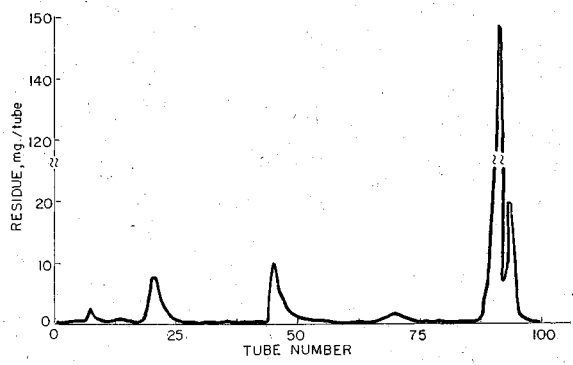

Fig. 3. Gradient elution chromatography of the water-saturated 1-butanol extract of ground whole wheat previously extracted with diethyl ether.

TABLE III

SILICIC ACID CHROMATOGRAPHY OF WHEAT BRAN, GERM, AND ENDOSPERM LIPIDS

FRACTION	BRAN	GERM	"ENDOSPERM"	WHOLE WHEAT
	%	%	%	%
I	0.5	3.7	trace	1.9
II	56.1	57.0	29.4	45.9
III	25.1	17.8	17.1	16.4
IV	22.5	16.5	52.4	32.0
Total	104.2	95.0	98.9	96.2

and germ lipids consisted of triglycerides and less than 25% consisted of the polar lipid material of class IV. Almost the reverse was true of "endosperm" lipids. In this case the polar lipids comprised 52% of the total and triglycerides only 29%.

It is recognized that the fractions used in these analyses were each

contaminated to some extent with the other. Also the 1st middlings undoubtedly cannot be considered entirely representative of endosperm. Nevertheless, the results obtained are believed to indicate real differences which future work will refine without markedly changing.

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