roform, and water-washed to remove soluble materials. The samples were dried and the chloroform was stripped. Portions were titrated for acid. Other portions of the samples were treated with diazomethane to zero acid value and molecular weights measured by using the Mechrolab vapor pressure osmometer. Previous experience had shown that gelation would occur in 17 hr. The resin produced was characterized by: molecular weight 1,420, equivalent weight 645, iodine value 112.3, and lack of alcohol absorption in the infrared. The resin was soluble in toluene. When the resin was treated with dimethylaminoethanol equivalent to the acid present, the resin was soluble in isopropyl alcohol-water from 23–55% resin plus amine (wt/wt). Dilution of these samples with water to the point of phase separation showed that the higher the resin content the greater the resin tolerance for water.

Film Properties

Films were cast from 20% solutions of the polymers and from two commercial samples at 5 mil wet-film thicknesses by using a doctor blade on 6-in. diameter steel discs. The drying data in Table I were determined with a Sanderson drying time meter.

ACKNOWLEDGMENT

Preparation of resins, J. E. Kaufman; infrared spectra, G. E. McManis.

REFERENCES

1. Commercial Solvents Corp., Technical Bulletin No. 12, Nitroparaffins Dept., p. 18.
2. Frey, E. M., J. Org. Chem. *15*, 806–808 (1950).
3. Hill, J. W., and W. E. McEwen, Org. Syn., Collective Vol. *II*, 53 (1943).
4. Kienle, R. H., P. A. van der Meulen and F. E. Petke, J. Am. Chem. Soc. *61*, 2258–2268 (1939).
5. Schildknect, C. E., "Vinyl and Related Polymers," John Wiley Sons, New York, 1952, p. 308.
6. Standard Oil Company (Ohio), British 846,231 (1960).
7. Wenker, H., J. Am. Chem. Soc. *57*, 1079–1080 (1935).

[Received August 20, 1965—Accepted October 27, 1965]

The Lipid Composition of Wheat Flours Varying Widely in Bread-Making Potentialities[1,2]

Y. POMERANZ, OKKYUNG CHUNG and R. J. ROBINSON, Crops Research Division, Agricultural Research Service, US Department of Agriculture and Department of Flour and Feed Milling Industries, Kansas State University, Manhattan, Kansas

Abstract

Lipids were extracted with petroleum ether (average 0.93%) and with water-saturated n-butanol (average 1.20%) from flours milled from composite 8 hard red winter, 5 hard red spring, and each from red soft, durum, and club wheat varieties. The butanol-extracted lipids were fractionated into nonpolar and polar lipids by silicic acid column chromatography, and the two major fractions were subfractionated by thin-layer chromatography. The extracted, washed, lipids contained about 52% nonpolar, and 48% polar lipids. Flours milled from durum wheat contained substantially less polar lipids, than flours milled from hard red winter or hard red spring wheats. The triglycerides constituted about 50% of the nonpolar lipids. Among the polar lipids, digalactosyl glyceride was the major component (about 40%); an unidentified compound, and a mixture of monogalactosyl glyceride with phosphatidic acid were about 20% each; and phosphatidyl ethanol amine, phosphatidyl choline and phosphatidyl serine comprised about 4, 7, and 4.5% of the polar lipids, respectively.

Introduction

LIPIDS COMPRISE only a small portion of most cereals. They form about 2% of the barley, rice, rye and wheat kernel, about 3.5% of millet, and around 5% of corn. Nevertheless, lipids have engaged the attention of cereal chemists because research over many years has suggested that lipids are involved in processing and storage, as a source of fat soluble vitamins and essential fatty acids, and in complex transformations during plant development and germination. A number of workers (1–4) have reviewed the role of wheat flour lipids in bread-making and nutrition. Reviews of the complex cereal lipids, and methods of their isolation and fractionation, were presented by Fisher (5) and Mecham (6). A number of reports dealing with the neutral lipids and fatty acids of wheat flour have been published, but publications on cereal polar lipids are of a rather limited scope (7–13). This report presents data on fractionation of wheat flour lipids from 16 wheat varieties (from a number of locations and varying widely in bread-making potentialities) by silicic acid column and quantitative thin-layer chromatography (TLC).

Materials and Methods

Flours

Untreated flour, used for extraction of lipids and preparation of standards for quantitative TLC, was milled on an Allis experimental mill from a composite grist of several hard-red winter-wheat varieties grown at a number of locations throughout the Great Plains in 1963 (14). In addition 16 flour samples from the 1963 crop were milled on a Miag "Multimat." The flours were milled from wheat composites of single varieties grown at an average of 8 locations. The chemical composition and baking quality of these flours, summarized in Table I, were determined as described elsewhere (14).

Lipid Extraction

Lipids were extracted exhaustively with Skellysolve B in a Goldfish extractor. In addition, lipids were extracted from 15 g flour with water-saturated n-butanol by the following procedure. The lipids were extracted in a Stein Mill with 100, 50, and 50

[1] Contribution No. 514. Kansas Agricultural Experiment Station, Manhattan.

[2] A report of work done in part under contract with the US Department of Agriculture and authorized by the Research and Marketing Act 1946. This contract was supervised by the W. Res. and Dev. Div., ARS, USDA.

TABLE I

Chemical and Bread-Making Characteristics of Flours

Sample No.	Class and variety	Extraction %	Moisture %	Ash %	Protein (N × 5.7) %	Farinograph valorimeter	Bromate requirement mg%	Loaf volume cc
	Hard Red Winter							
1	Pawnee	71.5	12.7	0.56	12.4	44.0	4.5	902
2	Comanche	69.3	12.0	0.48	13.2	72.0	3.0	979
3	Qv-Tm × Mql-Oro	66.9	12.6	0.48	12.2	68.0	2.0	939
4	501097	73.9	12.1	0.58	12.7	40.0	5.0	877
5	501099	72.4	11.9	0.53	13.4	34.0	5.0	832
6	Yogo	72.7	12.2	0.45	10.6	47.0	3.5	851
7	Warrior	74.3	11.7	0.45	12.7	58.0	2.5	950
8	Karmont	71.7	10.8	0.49	14.0	52.0	5.0	1000
	Hard Red Spring							
9	Thatcher	69.9	13.0	0.46	13.7	68.0	2.0	1000
10	Selkirk	69.9	12.9	0.46	13.6	68.0	3.0	1059
11	Marquis	65.1	13.0	0.46	12.9	60.0	2.0	1055
12	Lee	67.7	12.6	0.45	14.2	68.0	1.5	1078
13	Pilot	65.0	12.9	0.44	12.8	60.0	1.5	1053
	Soft Red Winter							
14	Seneca	52.5	13.3	0.36	11.3	47.0	2.0	924
	Durum							
15	Wells	71.2	12.2	0.74	11.6	22.0	2.0	336
	Soft White (Club)							
16	Omar	71.5	12.2	0.40	6.6	20.0	2.0	543

ml of water-saturated n-butanol, for 4, 2, and 2 min, respectively. The combined extracts were decanted, filtered, and evaporated almost to dryness under vacuum in a glass apparatus at about 45C. The extracts were kept under vacuum in a desiccator for 40 hr over P_2O_5 at 4C, extracted three times with Skellysolve B and the combined extracts evaporated under vacuum. The lipids were dissolved in 80 ml of a chloroform-methanol mixture (2:1), washed with 17.5 ml of 0.04% aqueous calcium chloride solution, followed by two washings with 10 ml each of 0.02% calcium chloride solution. The volume of the washed lipids was made to 50 ml with chloroform. Total lipids were determined by drying two 5 ml portions to constant weight; the remaining 40 ml were concentrated under vacuum to about 2 ml for separation on silicic acid columns.

Silicic Acid Column Chromatography

Silicic acid columns were 15 cm long and 2 cm in diameter. Lots of 20g of silicic acid for chromatography of lipids from Mallinkrodt, New York, were washed with distilled water and dried at 120C for 4 hr. The silicic acid was washed twice with 60 ml of a 7:1, and once with 60 ml of a 15:1 chloroform-methanol mixture, and finally with 80 ml chloroform.

TABLE II

Original and Silicic Acid Column Fractionated Wheat Flour Lipids

Sample No.	Petroleum-ether soluble lipids %	Butanol-soluble lipids			
		Total %	Non-polar [a,b] %	Polar [a,b] %	Recovery from column
1	1.07	1.35	57.3	42.7	94.7
2	0.88	1.19	54.8	45.2	102.5
3	0.86	1.20	55.0	45.0	99.5
4	0.86	1.12	50.9	49.1	96.5
5	0.87	1.18	54.7	45.3	98.7
6	1.04	1.22	54.4	45.6	102.0
7	1.10	1.37	55.8	44.2	100.4
8	0.86	1.20	50.2	49.8	97.0
9	0.89	1.16	49.2	50.8	98.1
10	0.83	1.11	49.4	50.6	96.2
11	0.95	1.19	45.7	54.3	100.3
12	0.88	1.16	48.0	52.0	95.6
13	0.81	1.15	52.0	48.0	105.8
14	0.92	0.98	47.0	53.0	103.6
15	1.19	1.54	61.0	39.0	101.3
16	0.79	1.10	50.2	49.8	102.6

[a] As percent of recovered lipids.
[b] Average of four determinations.

The slurry was transferred to columns; the neutral lipids were eluted with 120–150 ml chloroform and the polar lipids with 120–150 ml methanol. Completion of elution was checked by thin-layer chromatography (TLC). Each of the fractions was concentrated to 100 ml under vacuum, and two 10 ml aliquots were drawn from each, to determine neutral and polar-lipid content, respectively. The remaining fractions were freed of solvent under reduced pressure, for use in TLC. The ratios of nonpolar to polar lipids as determined by the above method are empirical. However, the satisfactory reproducibility, and reasonable intra- and inter-laboratory (26) agreement allow useful comparison.

Lipid fractions for reference use in quantitative TLC were prepared from a washed, water-saturated n-butanol extract from the composite hard red winter wheat flour from a number of varieties. The lipids were separated into 5 nonpolar and 5 polar fractions by elution from silicic acid (Bio-Rad Lab., Richmond, Calif.) columns by employing the apparatus described by Hirsch and Ahrens (15). The neutral lipids (52.7% of total) were eluted by the solvents given by Barron and Hanahan (16), followed by elution of polar lipids (47.3% of total), according to Hanahan et al., (17).

TLC

Glass plates (20 × 20 cm) were coated with a 250 μ layer of Silica Gel G (Merck, G. A., Darmstadt, Germany) in a conventional manner by using a commercial spreader (C. A. Brinkman and Co., Great Neck, N.Y.). The plates were dried for 3 hr at 130C. The most useful solvent for one-dimensional, ascending development of 25g spots were: chloroform; a mixture of petroleum ether-ethyl ether-acetic acid (80:20:1) (ether mixture); or a mixture of chloroform-methanol-water (65:25:4) (chloroform mixture). All solvents were of analytical grade, redistilled from glass; ether was redistilled from above metallic sodium. The spots were located and visualized by exposure to iodine vapor; by heating the plates for 25 min at 180C after spraying them lightly with a saturated solution of $K_2Cr_2O_7$ in 70% volume of aqueous sulfuric acid (18); by spraying with a 0.2% solution of ninhydrin in butanol containing 1% pyridine (19) as a spray specific for free amino acids;

with a modified Dragendorff reagent (20) for choline phosphatides and glycolipids; or with a molybdenum spray (21) for the detection of phospholipids. Lipids separated by TLC were tentatively identified by comparing R_f values with literature data (11,19,20), use of specific sprays, and comparing R_f values with those of pure compounds. Among the neutral lipids, fatty acids, mono- di- and triglycerides were used. Among the polar lipids, plant phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine were used (from Applied Science Labs., Inc., State College, Pa.). Plates were viewed under ordinary light. In addition, plates were observed under ultraviolet light (long wave, 3660Å) prior to and after the plates were sprayed with sulfuric acid. Lipid fractions from silicic acid columns were measured, after TLC-separation, by photodensitometry of spots charred with sulfuric acid (18). Color intensity was determined with a densitometer with scanning stage and varicord recorder.

Results and Discussion

The total lipid content of the 16 flours, and the proportions of nonpolar and polar lipids in the water-saturated n-butanol soluble lipids, are summarized in Table II. The lipid content of the flour from durum wheat was highest. Petroleum ether extracted consistently less lipids (average 0.93%) than the more polar butanol solvent (average 1.20%). The amount of lipids extracted with the butanol solvent is lower than reported in literature (10). The lower figure seems to have resulted from washing of the crude lipid extract and is in agreement with the results of Fisher et al. (11) who observed that Folch-washed lipids were lower than the unwashed lipid extracts. The recovery of lipids placed on the column averaged 99.7%. The nonpolar lipids of the 16 wheat flours averaged 52.2% of the total washed, water-saturated n-butanol extract, and this value compared well with 52.7% for nonpolar lipids from the wheat flour composite of a number of hard red winter varieties. The average polar-lipid content of the 8 hard red winter flours was 45.9% and of the 5 hard red spring wheat flours was 51.1%. The polar-lipid content of durum wheat flour of very poor bread-making quality was much lower than that of any of the other flours. These results must be interpreted, however, with caution for two reasons. There was no consistent difference in the polar:nonpolar ratio in lipids of flours milled from high- and poor bread-making quality, hard red winter flours. Lipids from the wheat endosperm contain more polar lipids than do lipids extracted from the germ or the bran (24). Consequently, the ratio of polar-nonpolar lipids is most likely affected by the germ or bran content of the flour. The ash content of the flours seems to indicate that the separation of bran and germ was much more efficient in the soft red winter than in the durum flour.

Fractionation of nonpolar lipids by TLC (Table III and Fig. 1) shows similar patterns for the tested flours. Thin-layer chromatograms are shown for 8 of the tested flours, as qualitative results indicated the same number and similar relative intensities of components in all tested flours. The amount of hydrocarbons and sterol esters would seem higher than expected, probably due to their movement to the solvent line and interference from rapidly moving components. It has not been possible to separate clearly, for quantitative determinations, the slow-moving components. Consequently, mono- and di-glycerides are reported together. In addition, the amount of mono- and di-glycerides might have been affected by the presence of unresolved components at the line of origin.

TABLE III

Fractionation of Nonpolar Wheat Flour Lipids by Thin-Layer Chromatography

Sample No.	Hydrocarbons and sterol esters [a] %	Triglycerides [a] %	Mono- and di-glycerides [a] %
1	7.5	52.2	21.6
2	8.6	41.6	25.1
3	7.5	48.1	24.8
4	7.5	49.1	27.4
5	8.1	54.3	26.7
6	8.5	48.0	24.2
7	10.3	57.4	21.5
8	12.4	53.1	22.3
9	10.0	45.6	34.0
10	10.2	44.2	27.2
11	6.8	46.3	30.6
12	9.1	47.4	33.5
13	7.7	48.6	33.2
14	10.3	48.9	28.7
15	5.1	49.7	29.4
16	6.9	54.5	29.1

[a] As % of total nonpolar lipids; averages of at least four determinations.

The R_f values and tentative identification of polar lipids separated by TLC with the chloroform mixture were: a (0.96) mixture of unknown components; b (0.88) mixture of monogalactosyl glyceride and phosphatidic acid; c (0.79) digalactosylglyceride; d (0.73) phosphatidyl ethanolamine; e (0.54) phosphatidyl choline; f (0.31) lysolecithin; and g (0.07–0.13) phosphatidyl serine.

It is realized that the results of fractionation of polar lipids, shown in Table IV and Figure 2, are an oversimplification, and are of a tentative and comparative nature. Fractionation of polar lipids by TLC indicates that digalactosyl glyceride is the major component (average 40.4%). Mason and Johnston (7) reported on the basis of countercurrent distribution that digalactosyl glycerides comprised about 40% of the total polar lipids. Carter et al. (22) had found that the ratio of mono- to digalactosyl glyc-

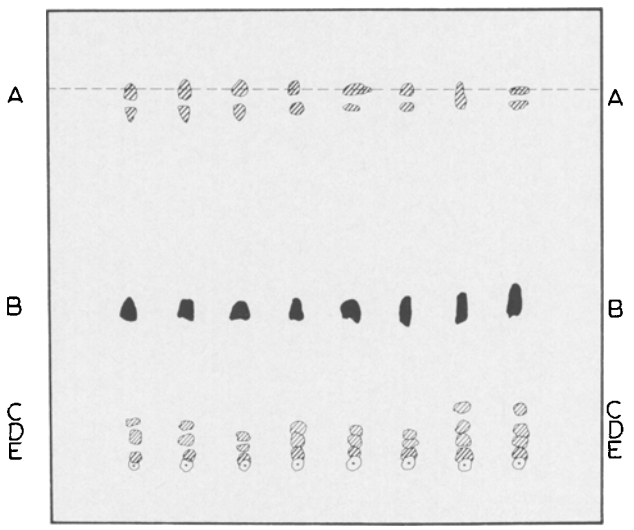

FIG. 1. TLC of nonpolar lipids. From left to right, lipids from 5 hard red spring, soft red winter, durum, and soft white (club) flours. Developed with chloroform; spots visualized by charring with sulfuric acid. Increasing intensity of shading indicates increasing concentrations of resolved components. Tentatively identified as: *A*, hydrocarbons and sterol esters; *B*, triglycerides; *C*, diglycerides; *D*, monoglycerides; and *E*, unresolved polar lipids.

TABLE IV

Fractionation of Polar Lipids by Thin-Layer Chromatography [a, b, c]

Sample No.	A %	B %	C %	D %	E %	F %	G %	Origin %	Recovery %
1	23.8	20.4	40.3	4.4	6.5	traces	3.2	1.5	91.1
2	22.2	22.3	39.2	3.7	7.6	traces	4.0	1.1	109.7
3	22.4	21.0	42.3	3.2	7.0	traces	2.9	1.2	103.3
4	23.2	18.5	40.9	3.4	7.0	traces	5.4	1.5	97.8
5	23.5	19.9	39.9	3.9	7.1	traces	4.0	1.6	103.1
6	21.5	20.7	39.8	4.0	7.3	traces	5.0	1.8	97.5
7	21.6	19.2	41.0	4.0	8.3	traces	4.1	1.9	99.3
8	17.3	19.3	41.0	4.5	8.3	traces	6.1	1.7	101.8
9	22.1	21.5	38.4	4.1	7.0	traces	4.9	2.2	90.6
10	18.1	22.4	40.1	3.8	7.3	traces	5.8	2.5	88.7
11	19.3	22.8	40.2	3.5	7.9	traces	4.4	1.9	89.8
12	27.4	18.9	36.3	3.2	7.3	traces	5.3	1.7	91.5
13	22.5	22.5	40.6	3.4	6.1	traces	3.1	1.9	92.6
14	21.2	19.4	43.3	5.4	4.0	traces	4.7	2.2	93.2
15	21.2	20.8	38.0	4.2	8.1	traces	5.6	2.2	89.7
16	21.6	20.3	45.3	3.9	4.7	traces	2.6	1.6	100.6

[a] As % of total polar lipids.
[b] Averages of at least 4 determinations.
[c] Tentatively identified as: *A*, unknown; *B*, mixture of monogalactosyl glyceride with phosphatidic acid; *C*, digalactosyl glyceride; *D*, phosphatidyl ethanolamine; *E*, phosphatidyl choline; *F*, lysolecithin; *G*, phosphatidyl serine.

erides was roughly 3:7. Barton-Wright (23) examined petroleum ether extracts of germ, bran, and low grade and patent flours, and reported that phosphatidic acids were the predominant type of polar lipids in germ and flour but not in bran. The occurrence of a large proportion of phosphatidic acids in flour polar lipids has been questioned by Mason and Johnston (7). According to Mecham (6), the amount of phosphatidic acid in wheat flour might be affected by the extent of enzymatic breakdown of polar lipids during extraction and isolation. The R_f values of monogalactosyl glyceride and phosphatidic acid in the solvent system used for one-dimensional TLC of polar lipids were not sufficiently different to permit their separation. The sum of the two compounds averaged 20.6% of the polar lipids. From the intensity of the molybdate- and Dragendorff-reagent reactions, and from two-dimensional TLC (25), it appears, however, that flour lipids contain only small amounts of phosphatidic acid and substantial amounts of monogalactosyl glycerides. In view of the structural similarities between the flour glycolipids and bread softeners, the galactolipids may be of importance in bread-making (7). The average levels of the other components (as percent of polar lipids) were: phosphatidyl ethanolamine 3.9%, phosphatidyl choline 7.0%, and phosphatidyl serine 4.4%. The unidentified component a, which contains a mixture of Dragendorff- and molybdate-reagent positive compounds, averaged 21.8% of the polar lipids. No consistent correlations between the levels of individual polar lipids and wheat class or bread-making potentialities were found. Polar lipids resolved by one-dimensional TLC accounted for 96.3% of the total lipids applied to the plate; the amount of unresolved lipids at the point of origin was small. It would seem, therefore, that no major components of flour polar lipids are likely to have been unaccounted for. It is possible, however, that some components have not been separated by the solvent system employed, and that some artifacts have occurred during extraction and fractionation of the lipids.

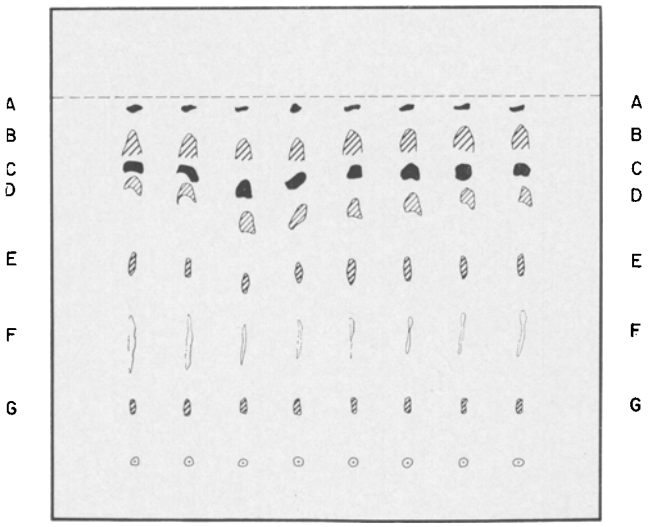

FIG. 2. TLC of polar lipids. From left to right, lipids from 5 hard red spring, soft red winter, durum, and soft white (club) flours. Developed with chloroform mixture; spots visualized by charring with sulfuric acid. Increasing intensity of shading indicates increasing intensity of resolved components. Tentatively identified as: *A*, unknown; *B*, mixture of monogalactosyl glyceride and phosphatidic acid; *C*, digalactosyl glyceride; *D*, phosphatidyl ethanolamine; *E*, phosphatidyl choline; *F*, lysolecithin; and *G*, phosphatidyl serine.

Flour lipids are related to quality, baking behavior, bread improvement by oxidizing treatments and shortening fats, and to storage stability of wheat flour (2). Many workers have stressed the importance of fractionating cereal lipids in elucidating their role in processing and in storage behavior. The present report indicates that quantitative TLC, combined with silicic acid column chromatography, is a useful tool in simple and rapid fractionation of cereal lipids. The use of additional fractionation technics, in combination with the methods used in this study, is being investigated.

ACKNOWLEDGMENTS

G. D. Miller for determination of part of the analytical data, A. B. Ward for milling the flour, and D. Miller for the baking tests.

REFERENCES

1. Cookson, M. A., and J. B. M. Coppock, J. Sci. Food Agr. *7*, 72 (1956).
2. Mecham, D. K., and J. W. Pence, Bakers' Dig. *31*, 40 (1957).
3. Glass, R. L., Cereal Sci. *5*, 60 (1960).
4. Coppock, J. B. M., and N. W. R. Daniels, "Recent Advances in Processing Cereals," Soc. Chem. Ind. London, Mongr. No. 16, 1962, p. 113–127.
5. Fisher, N., "Recent Advances in Food Science," Vol. 1, Ed. J. Hawthorn and J. M. Leitch, Butterworth's, London, 1962, p. 226–245.
6. Mecham, D. K., "Wheat Chemistry and Technology," Ed. I. Hlynka, A.A.C.C., St. Paul, Minn., 1964, p. 353–396.
7. Mason, L. H., and A. E. Johnston, Cereal Chem. *35*, 435 (1958).
8. Houston, D. F., Cereal Sci. *6*, 288 (1961).
9. Fisher, N., and M. E. Broughton, Chem Ind. (London) 1962, 869.
10. Nelson, J. H., R. L. Glass and W. F. Geddes, Cereal Chem. *40*, 337 (1963).
11. Fisher, N., M. E. Broughton, D. J. Peel and R. Bennett, J. Sci. Food Agric. *15*, 325 (1964).
12. McKillican, M. E., and R. P. A. Sims, JAOCS *41*, 340 (1964).
13. McKillican, M. E., *Ibid.* *41*, 54 (1964).
14. Jankiewicz, M., and Y. Pomeranz, Cereal Chem. *42*, 39 (1965).
15. Hirsch, J., and E. H. Ahrens, Jr., J. Biol. Chem. *233*, 311 (1958).
16. Barron, E. J., and D. J. Hanahan, *Ibid.* 231, 493 (1958).
17. Hanahan, D. J., J. C. Dittmer and E. Warashina, *Ibid.* 228, 685 (1957).
18. Blank, M. L., J. A. Schmit and O. S. Privett, JAOCS *41*, 371 (1964).
19. Lepage, M., J. Chromatog. *13*, 99 (1964).
20. Mangold, H. K., JAOCS *38*, 708 (1961).
21. Dittmer, J. C., and R. L. Lester, J. Lipid Res. *5*, 126 (1964).
22. Carter, H. E., R. H. McCluer and E. D. Slifer, J. Am. Chem. Soc. *78*, 3735 (1956).
23. Barton-Wright, E. C. Cereal Chem. *15*, 723 (1938).
24. Pomeranz, Y., and O. Chung, J. Chromatog., in press.
25. Abramson, D., and M. Blecher, J. Lipid Res. *5*, 628 (1964).
26. Houston, D. F., private communication.

[Received April 14, 1965—Accepted October 5, 1965]