Lipids in Wheat from Various Classes and Varieties[1,2]

Y. POMERANZ, OKKYUNG CHUNG and R. J. ROBINSON, Department of Flour and Feed Milling Industries, Kansas State University, and Crops Research Division, ARS, USDA, Manhattan, Kansas

Abstract

Lipids were extracted with petroleum ether and with water-saturated n-butanol from 8 hard red winter, 5 hard red spring, and one each from soft red, durum, and club wheat varieties from 2 harvests. 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. Durum wheats contained the highest lipid contents, and the highest concentration of nonpolar lipids. The breadmaking wheat varieties had a lipid content which was consistent for the 2 years examined. The total and nonpolar lipid contents of hard red spring wheats were higher than of hard red winter wheats. The polar lipid contents of wheats from the two classes were essentially equal. Total lipid contents were substantially higher in wheats than in flours milled from the wheats. Nonpolar lipids constituted about one-half of the flour lipids and two-thirds of the wheat lipids. Concentrations of triglycerides were higher in wheat than in flour nonpolar lipids. Glycolipids were present in comparable concentrations in wheat and in flour polar lipids; concentration in polar lipids of phosphatidyl choline was higher and of other phospholipids was lower in wheat than in flour polar lipids.

Introduction

PREVIOUS STUDIES in our laboratories have been concerned with the lipid composition of wheat flours which vary widely in bread-making potentialities (1.) A flour milled from durum wheat contained substantially more total lipids and a lower concentration of polar lipids, than flours milled from hard red winter or hard red spring wheats. There were, however, no significant or consistent differences in the levels and composition of lipids from hard red winter and spring wheat flours. Shollenberger and coworkers (2) found that the oil content of whole hard red spring wheats was appreciably higher than that of hard red winter wheats. Recently, Stevan and Houston (3) reported that flours milled from hard red spring wheat contained more total lipids and a higher nonpolar:polar lipid ratio than hard red winter flours.

In wheat, lipids form 1–2% of the endosperm, 8–15% of the germ, and about 6% of the bran with an average of 2–4% of the whole kernel (4). There is also a large difference in the distribution of polar and nonpolar lipids in the various grain tissues (5–7). Consequently, small differences in the highly empirical milling flowsheet and in extraction rate can affect considerably the amounts and kinds of lipids present in the flour. Such differences are especially pronounced when flours are milled from various wheat classes and varieties (1). Information was, therefore, sought on the composition of lipids in the whole wheat kernel to eliminate variations resulting from differences in the flour milling process. To study the varietal nad eliminate the environmental effects, lipid analyses were conducted for 2 years on samples composited by variety from a number of locations.

[1] No. 547, Kansas Agricultural Experiment Station, Manhattan.
[2] Done in part under contract with the U.S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. Supervised by the Western Utilization Research and Development Div., ARS.

Materials and Methods

Wheat Samples

Eight hard red winter and five hard red spring wheat samples were composited by variety from equal portions of wheat, as described previously (8). Composite samples were prepared separately for the 1963 and 1964 crop.

Certain chemical analyses and indirect parameters of bread-making quality of the wheat samples are given in Table I. All results are expressed on an "as is" basis. Potential yield was calculated by sieving through U.S. standard sieves.

Lipid Analysis

Lipid extraction, silicic acid column and thin-layer chromatography, were made as described previously

TABLE I
Chemical and Bread-Making Characteristics of Wheat Samples

Sample No.	Class and variety	Test wt. lb/bu		1000 Kernel wt.		Potential flour yield[a]		Moisture %		Ash %		Protein (N × 5.7) %		Sedimentation value	
		1963	1964	1963	1964	1963	1964	1963	1964	1963	1964	1963	1964	1963	1964
	Hard Red Winter														
1	Pawnee	61.5	59.6	25.8	24.6	74.8	74.5	11.1	11.0	1.5	1.6	13.1	13.6	38.5	38.0
2	Comanche	61.1	60.0	27.9	26.0	74.8	74.9	11.6	11.0	1.7	1.7	14.1	14.2	62.4	61.0
3	Qv-Tm × Mql-Oro	62.0	59.7	26.5	23.0	74.6	74.0	11.5	10.8	1.6	1.6	13.1	14.0	53.5	59.8
4	501097	63.3	61.0	29.5	26.2	75.2	74.5	11.5	10.7	1.6	1.7	13.3	14.2	33.6	37.9
5	501099	63.4	60.7	29.3	28.1	75.2	74.9	11.2	10.7	1.7	1.7	14.3	14.8	27.8	30.8
6	Yogo	62.8	60.6	27.9	23.2	73.9	73.1	9.9	10.1	1.8	1.5	11.4	13.8	25.3	54.9
7	Warrior	63.9	62.1	27.2	29.7	74.7	76.1	11.7	9.2	1.6	1.5	13.1	12.2	59.6	66.5
8	Karmont	61.4	62.7	28.7	26.1	74.6	74.5	11.1	10.0	1.7	1.6	15.0	10.7	66.0	37.8
	Hard Red Spring														
9	Thatcher	58.8	57.5	24.8	22.2	74.5	75.0	10.9	10.6	1.8	1.9	15.1	14.8	63.5	53.5
10	Selkirk	58.5	56.4	30.5	26.0	76.1	75.2	10.9	10.3	1.8	1.9	15.3	15.0	64.0	52.1
11	Marquis	59.3	55.8	24.7	19.9	74.9	73.8	10.4	10.4	2.0	2.1	14.4	15.2	59.3	52.9
12	Lee	58.8	57.9	31.0	24.7	76.7	75.3	11.0	10.0	1.8	1.9	15.1	15.0	62.0	45.0
13	Pilot	58.5	57.2	26.2	22.2	75.1	74.1	10.4	10.0	1.8	1.9	14.2	15.0	57.2	56.1
	Soft Red Winter														
14	Seneca	60.1	54.2	34.6	30.9	77.1	76.8	9.9	12.5	1.5	1.8	14.3	11.1	40.0	34.0
	Durum														
15	Wells	62.5	57.0	32.4	31.2	75.4	75.0	12.0	14.3	1.6	1.6	12.5	13.5	12.1	14.0
	Soft White (Club)														
16	Omar	60.1	60.2	27.8	29.1	76.5	75.9	11.1	12.0	1.3	1.3	7.9	9.1	6.6	8.5

[a] Calculated as cumulative index of wheat % passing through wire sieves no. 7, 9, and 12 multiplied by factors 0.78, 0.73, and 0,67, respectively.

TABLE II
Petroleum Ether Soluble Lipids of Wheat [a]

Sample No.	Petroleum-Ether-Soluble Lipids %	
	1963	1964
1	1.67	1.72
2	1.35	1.43
3	1.55	1.65
4	1.23	1.32
5	1.33	1.41
6	1.59	1.56
7	1.60	1.56
8	1.41	1.42
Average HRW	1.47	1.51
9	1.64	1.75
10	1.57	1.63
11	1.81	1.92
12	1.71	1.72
13	1.93	1.91
Average HRS	1.73	1.79
14	1.58	1.65
15	2.05	2.18
16	1.71	1.59

[a] On dry matter basis.

(1). Determinations of total lipid contents and polar:nonpolar ratios were made at least in duplicate. Results of determinations of lipids separated by TLC are averages of at least 4 determinations. Lipids were fractionated by TLC after separation into nonpolar and polar lipids by silicic acid column chromatography. The solvents used for one-dimensional, ascending development of 25 γ spots were: chloroform for nonpolar, and a mixture of chloroform-methanol-water (65:25:4) for polar lipids. Results of fractionation of nonpolar lipids by TLC do not include unfractionated components at the point of application and small amounts of free fatty acids.

The spots were visualized by exposure to iodine vapor, or by spraying with a saturated solution of $K_2Cr_2O_7$ in 70% volume of aqueous sulfuric acid. More specific spraying methods included ninhydrin, modified Dragendorff reagent, and molybdenum spray. In addition, TLC plates were sprayed with α-naphtol (9) for identification of glycolipids. For identification of polar lipids separated by TLC, phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine (from Applied Science Labs., Inc., State College, Pa.) and mono- and digalactosyl glyceride (gift from Dr. D. H. Hughes, Procter and Gamble, Co., Cincinnati, Ohio) were used as standards.

Results and Discussion

Tables II and III summarize the lipid contents of the 16 wheat samples. As expected, both the petroleum ether and water-saturated butanol extracts of the wheat samples are substantially higher than of the previously tested flour samples. Petroleum ether extracted consistently less wheat lipids (average 1.61 and 1.65%) than the more polar water-saturated butanol solvent (average 2.46 and 2.58% in 1963 and 1964 wheat samples, respectively). As in flours, the lipid content of durum wheat was the highest. Comparison of results for 2 years shows a high degree of consistency in lipid contents of samples from various classes and varieties. The average lipid content of the hard red winter wheats in both years was lower than of the hard red spring wheats. For both classes of bread-wheats, the lipid contents were negatively correlated with kernel size ($r = -0.59$), and positively correlated with ash content ($r = 0.54$). Both correlation coefficients are significant at the 1% level. As a class, the lipid-rich spring wheats were characterized by higher ash, protein, and sedimentation values than the hard red winter wheats.

The nonpolar:polar lipid ratio in the wheat samples is much higher than in the previously studied flours (1). Nonpolar lipids constituted almost two-thirds of wheat lipids, but only one-half of wheat flour lipids. These results are explained by the relatively high concentration of polar lipids in endosperm, and the predominance of nonpolar lipids in the germ and aleurone (6). The nonpolar:polar lipid ratio was highest in durum wheat, and higher in the hard red spring wheats than in the hard red winter wheats. The higher levels of total lipids in hard red spring wheat were a result of increase in nonpolar lipid contents. The amounts of polar lipids were essentially the same in the two classes of the two breadmaking wheats.

Data on nonpolar and polar components separated by TLC from lipids from the 1963 wheat crop, are given in Tables IV, V, and VI and in Figure 1. These results are of a comparative nature, only. Carter et al. (10) pointed out that estimation of lipids by reflectance scanning of charred spots is only semiquantitative if varying fatty acid composition is involved. The nonpolar lipids of wheat contained comparable amounts of hydrocarbons and sterol esters, but higher concentrations of triglycerides than wheat flour lipids. The ratios of polar:nonpolar lipids and concentrations of triglycerides in wheat lipids are in good agreement with the results of Nelson et al. (11) based on separations on silicic acid columns.

Concentrations in polar lipids of glycolipids, were similar in wheat and in flour. Polar wheat lipids contained higher concentrations of phosphatidyl choline, and lower concentrations of phosphatidyl serine than wheat flour polar lipids. No consistently significant differences were found in concentrations of single

TABLE III
Folch-Washed Water-Saturated Butanol Soluble Lipids of Wheat [a]

Sample No.	Total %		Nonpolar %		Polar %	
	1963	1964	1963	1964	1963	1964
1	2.50	2.51	65.7	64.4	32.5	31.3
2	2.17	2.24	62.6	62.8	34.4	35.1
3	2.36	2.48	67.2	61.8	32.9	29.6
4	2.27	2.17	65.2	68.2	33.0	30.9
5	2.24	2.47	64.8	63.8	29.4	33.3
6	2.46	2.77	66.7	67.2	28.7	27.7
7	2.57	2.50	65.0	69.3	30.2	30.0
8	2.27	2.32	64.2	67.3	33.4	30.7
Av. HRW	2.36	2.43	65.2	65.6	31.8	31.1
9	2.46	2.80	66.5	71.2	33.4	29.0
10	2.23	2.60	63.6	66.1	29.8	31.2
11	2.49	3.07	69.9	70.3	29.6	27.9
12	2.61	2.77	66.4	69.2	30.2	30.2
13	2.90	2.89	71.5	69.5	27.5	28.5
Av. HRS	2.54	2.83	67.6	69.3	30.1	29.4
14	2.13	2.21	70.0	67.9	31.8	31.0
15	3.26	3.28	70.7	74.4	24.7	23.9
16	2.40	2.18	68.2	66.4	31.4	31.2

[a] On dry matter basis.

TABLE IV
Fractionation of Nonpolar Wheat Lipids by Thin-Layer Chromatography

Sample No.	Hydrocarbons and sterol esters %	Triglycerides %	Mono- and diglycerides %
Rf	0.79	0.43	0.39
1	9.5	69.2	11.2
2	11.8	61.8	11.3
3	9.1	67.8	9.5
4	9.7	66.8	10.2
5	11.0	66.3	8.6
6	8.8	69.4	10.0
7	6.6	71.0	11.7
8	7.0	68.6	11.4
Average HRW	9.2	67.6	10.5
9	8.0	69.8	7.1
10	9.2	68.4	7.8
11	6.8	72.2	8.2
12	8.1	72.6	8.0
13	6.6	68.9	10.2
Average HRS	7.8	70.4	8.3
14	8.5	67.2	9.3
15	7.6	70.0	11.3
16	8.0	72.8	8.1

TABLE V

Tentative Identification of Polar Wheat Lipids Fractionated by Thin-Layer Chromatography

Fraction No.	Reagent						Tentative identification
	Sulfuric acid	Iodine vapor	Molyb-denum	Nin-hydrin	Dragen-dorff	α-naphthol	
1	+	+	−	−	+	+[a]	Unknown
2	+	+	−	−	+	+[b]	Monogalactosyl glyceride
3	+	+	+	−	+	+[a]	Phosphatidic acid and unknown glycolipid
4	+	+	−	−	+	+[b]	Digalactosyl glyceride
5	+	+	+	+	−	−[c]	Phosphatidyl ethanolamine
6	+	+	+	−	+	−[c]	Phosphatidyl choline
7	+	+	+	+	−	−[c]	Unknown
8	+	+	+	+	−	−[c]	Phosphatidyl serine
9	+	+	+	−	+	−[c]	Lysophosphatidyl choline
10	+	+	+	−	−	−[c]	Unknown

[a] Bright violet. [b] Dark-gray purple. [c] Various shades of brown.

nonpolar or polar components in wheats from various classes or varieties. It is realized that the results of fractionation of the polar lipids are an oversimplification. Difficulties involved in separation by TLC and identification of components in complex lipid mixtures were discussed by Rouser et al. (12). Some of the difficulties are illustrated in Figure 1. Thus, for example, phosphatidyl serine showed considerable streaking which affected the precision of determinations of lipids with low R_f values. In addition, day to day variations in R_f values were encountered.

The results of this study pose the question whether total lipid content and nonpolar:polar ratio are related in a causative or casual manner to bread-making potentialities. The present data and that from our other recent findings seem to favor the concept that no direct correlation exists between wheat lipid contents and bread-making strength. Biosynthesis of gluten proteins in maturing wheat was related to improvement of bread-making potentialities (13), but, no change in the nonpolar:polar lipid ratio was found in the developing wheat kernel (14).

In the present study, the total lipid content and nonpolar:polar ratio were highest both in the high bread-making quality, hard red spring wheats and in the durum wheats which are suitable for manufacture of alimentary pastes but totally unsuited for bread manufacture. The hard red winter wheats were selected to represent a wide range of protein contents and quality, and overall bread-making potentialities. These differences could not be correlated with lipid contents or kinds of lipids present in those wheats.

The average bread-making quality of the hard red spring wheats was substantially higher than that of the hard red winter wheats. With the higher proportion of nonpolar lipids in hard red spring than in hard red winter wheats, one would expect the nonpolar lipids to improve bread quality more than the polar lipids. The opposite is true. It was shown recently (15–17) that loaf volume of bread baked without shortening was increased strikingly by adding polar flour lipids; nonpolar lipids had little effect. The improving effect was independent of wheat class or variety, and polar lipids from durum improved bread quality as much as polar lipids from hard red spring wheat flours.

The high total and nonpolar lipid content is a varietal characteristic and is typical of the spring wheats (hard red and durum). Unlike protein content and quality, the lipids contribute to but do not seem to govern the inherent bread-making properties. This is borne out by a highly positive correlation between a wide range of protein contents and loaf volume. With lipids the loaf volume increase is effected

TABLE VI

Fractionation of Polar Wheat Lipids by Thin-Layer Chromatography

Sample No.	Fraction No. (% of total)									
	1	2	3	4	5	6	7	8	9	10
1	5.2	9.3	25.6	39.0	2.1	12.2	1.6	1.7	2.0	1.3
2	5.6	9.2	26.8	37.5	2.1	10.9	1.6	2.0	2.9	1.5
3	5.1	9.3	24.6	39.2	2.5	11.2	1.5	2.0	2.8	1.5
4	5.2	11.2	23.6	38.7	2.0	10.8	2.0	2.4	2.5	1.6
5	5.9	11.1	21.0	38.6	1.9	12.6	2.0	2.1	3.2	1.5
6	6.1	12.8	20.9	38.3	2.1	12.2	1.8	1.9	2.8	1.2
7	5.9	11.3	23.0	37.8	2.4	11.6	1.4	2.2	2.9	1.5
8	6.6	9.9	21.5	39.0	2.7	12.2	2.0	2.0	2.6	1.6
Average HRW	5.7	10.5	23.4	38.5	2.2	11.7	1.7	2.0	2.7	1.5
9	5.6	12.1	23.3	36.7	2.6	11.6	1.9	1.9	2.8	1.5
10	5.3	12.8	24.6	35.8	2.7	11.2	1.8	1.8	2.5	1.7
11	5.1	13.1	23.2	37.2	2.5	11.4	1.7	2.0	2.5	1.5
12	6.6	11.1	22.5	36.1	2.6	12.5	2.0	2.4	2.6	1.6
13	6.5	11.2	23.4	35.9	2.4	11.5	1.9	2.4	2.3	1.5
Average HRS	5.8	12.1	23.4	36.3	2.6	11.7	1.9	2.1	2.7	1.5
14	6.6	7.3	26.5	36.8	3.2	10.6	1.8	2.3	3.4	1.7
15	6.9	8.4	23.4	36.7	2.6	11.9	1.9	2.2	4.7	1.2
16	7.6	10.8	21.2	39.7	2.8	9.5	1.8	2.5	3.0	1.6

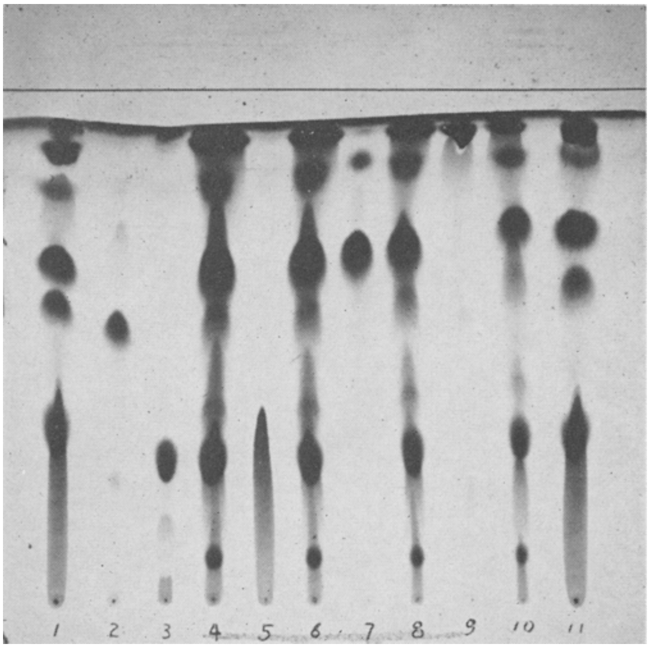

FIG. 1. TLC of polar lipids: 1) mixture of all standard lipids, 2) phosphatidyl ethanolamine, 3) phosphatidyl choline, 4) polar wheat lipids, 5) phosphatidyl serine, 6) polar wheat lipids, 7) digalactosyl glyceride, 8) polar wheat lipids, 9) monogalactosyl glyceride, 10) polar wheat lipids, 11) mixture of all standard lipids. Standard and mixture of all standard lipids contained 25 γ of each lipid. Polar lipids from hard red winter wheat were applied at 100, 75, 50, and 25 γ levels in samples 4, 6, 8, and 10, respectively. Developed with chloroform mixture; spots visualized by charring with sulfuric acid.

by small additions; larger amounts have no additional improving effect. The lipids seem, therefore, to satisfy certain functional properties. Once those requirements are met, no additional benefit can be derived by adding more lipids.

The present data do not preclude the possibility that certain relationships between lipid contents or composition and bread-making quality may yet be established. Separations on silicic acid columns used in this study are highly empirical; the fractions are crude mixtures. Substantial losses were encountered during washing of lipids. More refined and more meaningful fractionation procedures could likely show the presence of additional, unidentified components and reveal significant differences. Differences in bread-making quality may be related (to some extent at least) to levels of individual components, to degree of unsaturation of fatty acids, or to the interaction between lipids and other wheat components, and stability of the complex lipids.

REFERENCES

1. Pomeranz, Y., O. Chung and R. J. Robinson, JAOCS *43*, 45 (1966).
2. Shollenberger, J. H., J. J. Curtis, C. M. Jaeger, R. R. Earle and B. B. Bayles, U.S. Dept. Agr. Tech. Bull. 995 (1949).
3. Stevan, M. N., and D. F. Houston, Cereal Chem., *43*, 353 (1966).
4. Fisher, N., "Recent Advances in Food Science," Vol. 1, Ed. J. Hawthorn and J. M. Leitch, Butterworth's, London, 1962, p 226–245.
5. Mecham, D. K., "Wheat Chemistry and Technology," Ed. I. Hlynka, AACC, St. Paul, Minn. 1964, p 353–396.
6. Pomeranz, Y., and O. Chung, J. Chromatog. *19*, 540 (1965).
7. Pomeranz, Y., Qualitas Plantarum et Materiae Vegetabilis *12*, 321 (1965).
8. Shoup, F. K., Y. Pomeranz and C. W. Deyoe, J. Food Sci. *31*, 94 (1966).
9. Feldman, G. L., Feldman, L. S. and G. Rouser, JAOCS *42*, 742 (1965).
10. Carter, H. E., P. Johnson and E. J. Weber, Ann. Rev. Biochem. *34*, 110 (1965).
11. Nelson, J. H., R. L. Glass and W. F. Geddes, Cereal Chem. *40*, 337 (1963).
12. Rouser, G. G. Kritchevsky, D. Heller and E. Lieber, JAOCS *40*, 425 (1963).
13. Hoseney, R. C., Y. Pomeranz and K. F. Finney, J. Sci. Food Agr. *17*, 273 (1966).
14. Daftary, R. D., and Y. Pomeranz, J. Food Sci. *30*, 577 (1965).
15. Pomeranz, Y., G. L. Rubenthaler and K. F. Finney, Food Technol. *19*, 1724 (1965).
16. Pomeranz, Y., G. L. Rubenthaler, R. D. Daftary and K. F. Finney, Food Techol., in press.
17. Pomeranz, Y., G. L. Rubenthaler and K. F. Finney, Food Technol., in press.

[Received March 1, 1966]