Influence of Germination on Wheat Quality. III. Modification of Flour Lipid[1]

O. M. LUKOW,[2] F. BEKES,[3] and W. BUSHUK

ABSTRACT

Cereal Chem. 62(6):419-422

Controlled laboratory germination of two wheat cultivars, Glenlea and Neepawa, produced significant changes in lipid content and composition of flour and modified Osborne solubility fractions. Total lipid content of the flours decreased at different rates for the two cultivars. Free lipids, particularly triglycerides, decreased more rapidly than bound lipids. Qualitative and quantitative changes in the phospholipids were noted. Both Glenlea and Neepawa flours milled from wheat germinated for 18 h contained the lowest level of phospholipid. Substantial differences in the phospholipid composition were found between the two cultivars. Total lipid content of the modified Osborne fractions decreased with germination for both cultivars. Rates of decrease were, in descending order: albumin-globulin, gliadin, glutenin, and residue. The ratio of nonpolar to polar lipid was not negatively correlated with loaf volume. Maximum loaf volumes for Glenlea and Neepawa occurred at similar nonpolar to polar lipid values.

The lipid content and composition of the wheat kernel undergoes continual change during germination (Morrison 1978). Although lipids contribute to the baking quality of sound flour (Békés et al 1983b, Chung et al 1978, MacRitchie 1981), the impact of changes in lipid content and composition during germination on flour quality has not been documented. In this study, lipids of two wheat cultivars that differ widely in breadmaking quality and that had been subjected to various germination treatments were investigated to provide further insight into the known changes in quality that occur as a result of postharvest sprouting (Lukow and Bushuk 1984a,b).

MATERIALS AND METHODS

Germination Procedure

Samples of wheat (3 kg) were surface-sterilized by soaking in a solution of 2% aqueous sodium hypochlorite for 15 min at room temperature (about 20° C) and then rinsed well with distilled water for at least 20 min. Before germination, the wheat was soaked overnight (about 16 h) in excess distilled water at 4° C with one water change. After rinsing with distilled water, the steeped wheat was spread on wet cellulose pads and germinated at 21° C, 67% rh. At intervals of 18, 35, and 54 h, the samples were withdrawn and frozen at −30° C and then freeze-dried. Roots and coleoptiles were removed, and the freeze-dried grain was stored at −4° C. Sound (control) samples were surface-sterilized and freeze-dried immediately.

Milling

Wheat samples were tempered to 15.5% moisture content and milled into straight-grade flour on a Bühler pneumatic laboratory mill (MLV 202).

Analytical

Flour proteins were fractionated by the modified Osborne procedure of Chen and Bushuk (1970) as described in Lukow and Bushuk 1984b.

Flours (5 g) and the protein fractions (0.5 g) (albumin-globulin, gliadin, glutenin, and residue) from sound, soaked, and two germinated (18 and 54 h) wheat samples were analyzed for lipid content and composition. Lipids were extracted sequentially by *n*-hexane and water-saturated *n*-butanol yielding free (FL) and bound (BL) lipids, respectively. Total lipid (TL) was calculated as the sum of FL and BL. FL was fractionated on a silicic acid column into three fractions: nonpolar lipid (NL), glycolipid (GL), and phospholipid (PhL). Polar lipid (PL) was defined as the sum of GL and PhL. Lipids extracted from flour samples were examined by thin-layer chromatography (TLC). Methods for lipid extraction, column chromatography, and TLC were as described by Békés et al (1983a). Lipid data are expressed in milligram per 100 g of sample on a dry basis. The average total recovery of lipids from silicic acid column chromatography was 93.6%.

Statistical Analysis

Lipid extractions and column chromatography of flour samples and protein fractions were performed in triplicate and duplicate, respectively, and averaged. Analysis of variance was calculated, and least significant differences were computed at the 5% level of significance.

RESULTS AND DISCUSSION

Lipid Content and Composition of Flour Samples

TL contents of Glenlea and Neepawa sound flours (1.59 and 1.52%, respectively; from Table I) are in general agreement with published data (Hoseney et al 1970). The values decreased substantially as germination time increased for both cultivars (Table I). These data agree with those of Bolling and El Baya (1982), who reported that TL decreased until 72 h of germination and then increased until 168 h of germination. The rate of decrease for Glenlea was greater than for Neepawa. After 54 h of germination, Glenlea flour contained 64% of the original TL content, whereas the corresponding Neepawa sample contained 70%. Similar trends were found for FL. The rate of decrease for FL was greater than BL for both cultivars. For example, Glenlea FL decreased to 51.1% and BL decreased to 78.3% of the sound levels after 54 h of germination. The decrease of BL was greater for Glenlea than Neepawa in the first 18 h of germination.

The change in the NL/PL value of the total lipid was different for the two cultivars. NL/PL decreased monotonically for Glenlea (approximately 2.02, 1.92, 1.75, 1.60), whereas the value for Neepawa increased during the first 18 h of germination and then decreased (approximately 1.88, 1.96, 2.04, 1.41).

The major effect of germination on the flour lipid composition of the two cultivars was the decrease of free triglycerides (Fig. 1). The level of bound triglycerides remained fairly constant. Tavener and Laidman (1972) reported that the breakdown of triglycerides commences 12 h after imbibition of water and proceeds rapidly until the second day of germination. Bahl et al (1983) noted rapid degradation of triglycerides after 8 h of germination of three wheat

[1]Presented at the AACC 68th Annual Meeting, Kansas City, MO, October, 1983. Publication 685, Department of Plant Science, University of Manitoba, Winnipeg R3T 2N2 with financial assistance of the Natural Sciences and Engineering Research Council of Canada, and publication 1154, Agriculture Canada, Research Station, Winnipeg, Manitoba.

[2]Present address: Agriculture Canada, Research Station, 195 Dafoe Road, Winnipeg, Manitoba R3T 2M9.

[3]Present address: Department of Biochemistry and Food Technology, Technical University of Budapest, 1111 Budapest, Hungary.

cultivars. In contrast, the present study showed a slight loss of triglycerides during the soaking stage, particularly for Glenlea. GL decreased during germination at similar rates for both cultivars. Disappearance of GL was proportional to that of TL.

Qualitative and quantitative changes during germination were found in the total PhL (Fig. 2). Both 18-h samples contained the lowest amounts of PhL (Fig. 2 and Table I). Because of this observation, the composition of PhL subfractions was investigated by TLC. PhL was calculated as the sum of N-acyl phosphatidyl ethanolamine, phosphatidyl ethanolamine, phosphatidyl choline, and phosphatidyl serine. The lysophospholipids were calculated as the sum of N-acyl lysophosphatidyl ethanolamine, lysophosphatidyl ethanolamine, and lysophosphatidyl choline. Substantial differences in the composition of the PhL subfractions were found for the two cultivars (Fig. 2). In Neepawa, the amount of PhL decreased (162, 155, 132, 118), whereas the amount of lysophospholipids doubled (38, 38, 46, 81) during germination. In Glenlea, PhL and lysophospholipids decreased and then increased in a parallel manner (148, 146, 94, 114, and 50, 52, 40, 52, respectively) during germination. In contrast, Varty and Laidman

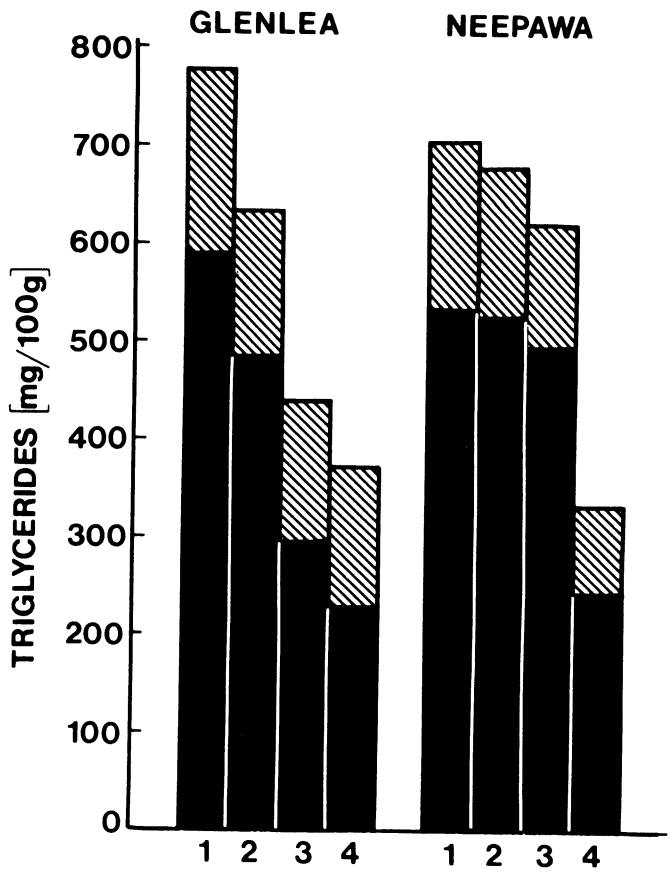

Fig. 1. Triglyceride content of Glenlea and Neepawa flours: **1**, sound; **2**, soaked; **3**, 18 h; **4**, 54 h; ■ = bound triglycerides; ▨ = free triglycerides.

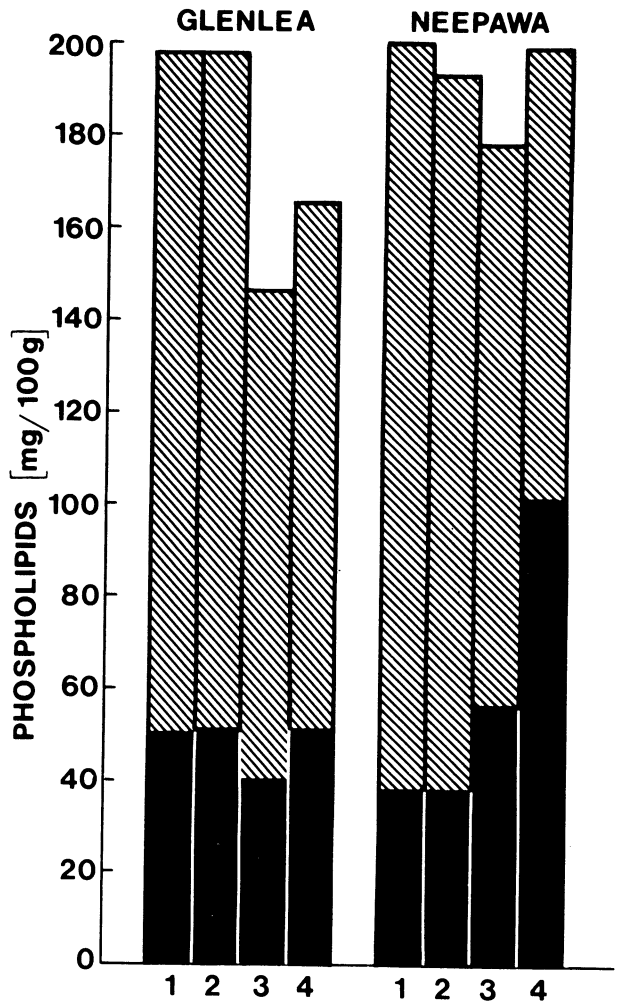

Fig. 2. Phospholipid content of Glenlea and Neepawa flours: **1**, sound; **2**, soaked; **3**, 18 h; **4**, 54 h; ■ = lysophospholipids; ▨ = remaining phospholipids.

TABLE I
Lipid Content of Flours[a]

	Sound			Soaked			18 h			54 h			Least Significant Difference		
	FL[b]	BL	TL	FL	BL	TL	FL	BL	TL	FL	BL	TL	FL	BL	TL
Glenlea															
NL[c]	713	350	1,063	594	280	874	408	279	687	349	279	628	72	40	104
GL	89	240	329	78	180	258	51	196	247	45	182	227	3	26	36
PhL	38	160	198	38	160	198	31	115	146	36	129	165	6	19	23
PL	127	400	527	116	340	456	82	311	393	81	311	392	13	45	56
Total	840	750	1,590	710	620	1,330	490	590	1,080	430	590	1,020	79	85	171
Neepawa															
NL	667	326	993	657	290	947	644	268	912	389	231	620	75	38	115
GL	85	242	327	80	210	290	71	199	270	58	183	241	9	30	40
PhL	48	152	200	43	150	193	45	133	178	63	136	199	8	19	22
PL	133	394	527	123	360	483	116	332	448	121	319	440	15	47	60
Total	800	720	1,520	780	650	1,430	760	600	1,360	510	550	1,060	92	83	183

[a] mg/100 g, Dry basis.
[b] FL = free lipids; BL = bound lipids; total lipids (TL) = FL + BL.
[c] NL = nonpolar lipids; GL = glycolipids; PhL = phospholipids; polar lipids (PL) = GL + PhL.

(1976) reported that the levels of PhL increased in wheat endosperm during the first two days of germination. However, the rates of incorporation of radioactive precursors into PhL indicated that, as well as synthesis, degradation and turnover of PhL occurs during early germination. The increase of lysophospholipids can originate from two sources: from the hydrolysis of PhL by a series of enzymes (Nolte et al 1974), and from the hydrolysis of starch by the amylases resulting in the liberation of starch lipids (Baisted and Stroud 1982).

Lipid Distribution of Modified Osborne Fractions

During dough mixing, substantial amounts of free flour lipids become bound to gluten proteins (Chung and Tsen 1975). Even the initial wetting of flour has an influence on the formation of these complexes (Zawistowska et al 1985). Lipid distribution between gliadin and glutenin and their components is largely dependent on whether the protein separations are made based on their molecular weight or on differences in solubility. As these proteins have specific lipid binding capacity, it is not surprising that results on the distribution of lipids in flour and gluten fractions obtained by different procedures are inconsistent (Békés et al 1983a, Chung et al 1978, Frazier et al 1981, Hoseney et al 1970, Ponte and De Stefanis 1969). Results can be legitimately compared only when identical separation methods have been used (Zawistowska et al 1984, 1985).

The total variation in lipid distribution (Fig. 3) includes changes in lipid content of individual Osborne fractions (Table II) and changes in dry material distribution of these fractions (Table III). In agreement with the protein distribution previously reported (Lukow and Bushuk 1984b), the most significant effect of germination on the dry material distribution is the change of the glutenin/residue; this value declined in the soaked sample and then increased during germination, indicating that a portion of insoluble residue became soluble in acetic acid. The gliadin fraction increased during germination in Neepawa, whereas in Glenlea soaking resulted in a significant decrease followed by an increase with germination.

TL of the modified Osborne fractions decreased significantly with germination for both cultivars (Table II). The rates of decrease were, in descending order: albumin-globulin, gliadin, glutenin, residue. Generally, FL and BL decreased during germination. However, BL of the albumin-globulin fraction of Glenlea increased during germination. There was no obvious trend with germination in the percentage of lipid in any fraction when the changes in dry material distribution were also taken into account (Table II, in parentheses).

Figure 3 shows the change during germination in TL content of the flours and the modified Osborne fractions. There were significant differences in lipid distribution of Glenlea and Neepawa in the sound samples (also discussed by Zawistowska et al 1984). However, these differences decreased systematically during germination such that after 54 h of germination, corresponding fractions from the two cultivars contained similar amounts of lipid.

Breadmaking Quality

A complex relationship exists between breadmaking quality and lipid content and composition of flour. Although changes in loaf volume resulting from variation of flour lipid have been well documented, no clear cause and effect relationship has been established (Chung and Pomeranz 1977, MacRitchie 1977). This

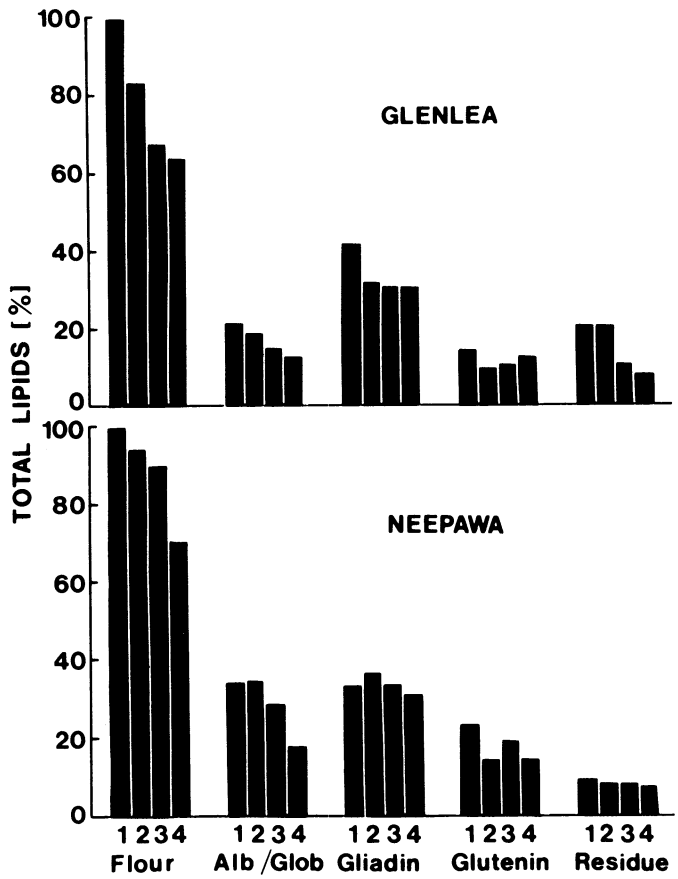

Fig. 3. Total lipids (%) of Glenlea and Neepawa flours and modified Osborne fractions: **1,** sound; **2,** soaked; **3,** 18 h; **4,** 54 h.

TABLE II
Lipid Content of Modified Osborne Fractions[a]

	Albumin/Globulin			Gliadin			Glutenin			Residue		
	FL[b]	BL	TL	FL	BL	TL	FL	BL	TL	FL	BL	TL
Glenlea												
Sound	5,630	1,600	7,230 (22.2)[c]	1,440	6,570	8,010 (42.2)	3,290	3,960	7,250 (14.8)	180	150	330 (20.8)
Soaked	5,280	2,180	7,460 (23.7)	1,410	6,000	7,410 (38.6)	3,270	3,610	6,880 (11.9)	170	130	300 (25.8)
18 h	5,050	2,480	7,530 (23.1)	1,040	5,060	6,100 (45.4)	2,840	3,980	6,820 (15.2)	100	100	200 (16.3)
54 h	2,960	2,770	5,730 (20.2)	906	4,140	5,046 (47.9)	2,150	3,510	5,660 (19.7)	50	90	140 (12.2)
LSD[d]	931	454	1,341	226	1,021	1,364	601	N.S.[e]	1,338	25	23	38
Neepawa												
Sound	8,470	2,640	11,110 (34.2)	1,730	7,190	8,920 (32.9)	5,690	2,800	8,490 (23.7)	50	110	160 (9.2)
Soaked	8,130	2,840	10,970 (36.9)	1,700	6,420	8,120 (38.5)	5,740	2,820	8,560 (15.5)	40	110	150 (9.1)
18 h	7,460	2,050	9,510 (31.9)	1,230	5,150	6,380 (37.2)	6,820	1,140	7,960 (21.9)	20	110	130 (9.0)
54 h	3,030	1,970	5,000 (24.9)	980	4,060	5,040 (43.6)	2,690	1,150	3,840 (20.8)	10	110	120 (10.7)
LSD	1,362	440	1,756	289	1,123	1,480	1,006	371	1,482	7	N.S.	29

[a] mg/100g, dry basis.
[b] FL = free lipids; BL = bound lipids; total lipids (TL) = FL + BL.
[c] Percent.
[d] LSD = least significant difference.
[e] N.S. = not significant.

TABLE III
Dry Material Distribution of Modified Osborne Fractions (%)

Sample	Albumin/Globulin	Gliadin	Glutenin	Residue
Glenlea				
Sound	4.16	7.15	2.79	85.90
Soaked	3.30	5.61	1.80	89.29
18 h	3.24	7.88	2.34	86.54
54 h	3.42	9.21	3.37	84.00
LSD[a]	0.45	1.18	0.56	1.18
Neepawa				
Sound	4.63	5.55	3.83	85.99
Soaked	3.99	5.62	1.85	88.52
18 h	4.17	7.26	3.43	85.14
54 h	4.62	8.03	5.00	82.34
LSD	0.62	1.27	0.62	0.92

[a]LSD = least significant difference.

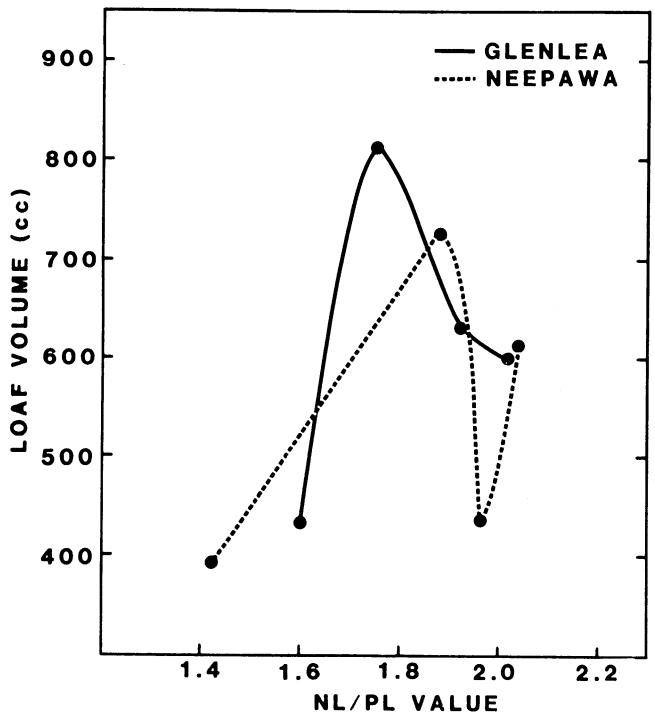

Fig. 4. Effect of nonpolar lipid/polar lipid (NL/PL) value on loaf volume of Glenlea and Neepawa flours.

study showed that the lipid content of flour decreased with progressive germination of both Glenlea and Neepawa. The effect of the flour NL/PL value on loaf volume (Lukow and Bushuk 1984a) was examined (Fig. 4). But, contrary to the results of Békés et al (1983c), the ratio of NL to PL was not negatively correlated with loaf volume. Considering that other wheat components that interact with lipids, particularly protein, are modified during germination and have a significant impact on breadmaking quality (Lukow and Bushuk 1984b), it is not surprising that the germinated wheat samples displayed a different trend than the sound wheats examined by Békés et al (1983c). However, the observation that maximum loaf volumes for both cultivars occurred at similar NL/PL values warrants further investigation. The resemblance of the Glenlea 18-h sample to the Neepawa sound sample in terms of lipid and protein characteristics may explain its improved breadmaking properties (Lukow and Bushuk 1984a). Furthermore, there were significant changes in the amount and composition of lipids associated with the various modified Osborne fractions of the flour proteins. These changes may contribute to the modification of breadmaking quality during germination in addition to the well-known alterations in enzymes, starch, and gluten proteins.

LITERATURE CITED

BAHL, J., BOUGAIN, G., and MONEGER, R. 1983. Évolution de la composition en lipides des grains de trois variétiés de Blé pendant la germination. Physiol. Veg. 21:475.

BAISTED, D. J., and STROUD, F. 1982. Soluble and particulate lysophospholipase in the aleurone and endosperm in germinating barley. Phytochem. 32:29.

BÉKÉS, F., ZAWISTOWSKA, U., and BUSHUK, W. 1983a. Protein-lipid complexes in the gliadin fraction. Cereal Chem. 60:371.

BÉKÉS, F., ZAWISTOWSKA, U., ZILLMAN, R., and BUSHUK, W. 1983b. Prediction of breadmaking quality of wheat cultivars from lipid content and composition. Cereal Foods World 28:565.

BOLLING, H., and EL BAYA, A. W. 1982. Changes in the lipid composition during germination and ripening of grain. Page 202 in: Proc. 7th World Cereal Bread Congres, Elsevier: Netherlands.

CHEN, C. H., and BUSHUK, W. 1970. Nature of proteins in triticale and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. Can. J. Plant Sci. 50:9.

CHUNG, O. K., and POMERANZ, Y. 1977. Wheat flour lipids, shortening and surfactants. Bakers Dig. 51:32.

CHUNG, O. K., and TSEN, C. C. 1975. Distribution of lipids in acid-soluble protein components as affected by dough-mixing and surfactants. Cereal Chem. 52:823.

CHUNG, O. K., POMERANZ, Y., and FINNEY, K. F. 1978. Wheat flour lipids in breadmaking. Cereal Chem. 55:598.

FRAZIER, R. J., DANIELS, N. W. R., and RUSSELL EGGITT, P. W. 1981. Lipid-protein interactions during dough development. J. Sci. Food Agric. 32:877.

HOSENEY, R. C., FINNEY, K. F., and POMERANZ, Y. 1970. Functional (breadmaking) and biochemical properties of wheat flour components. VI. Gliadin-lipid-glutenin interactions in wheat gluten. Cereal Chem. 47:135.

LUKOW, O. M., and BUSHUK, W. 1984a. Influence of germination on wheat quality. I. Functional (breadmaking) and biochemical properties. Cereal Chem. 61:336.

LUKOW, O. M., and BUSHUK, W. 1984b. Influence of germination on wheat quality. II. Modification of endosperm protein. Cereal Chem. 61:340.

MacRITCHIE, F. 1977. Flour lipids and their effects in baking. J. Sci. Food Agric. 28:53.

MacRITCHIE, F. 1981. Flour lipids: theoretical aspects and functional properties. Cereal Chem. 58:156.

MORRISON, W. R. 1978. Cereal lipids. Page 221 in: Advances in Cereal Science and Technology. Vol. II. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

NOLTE, D., REBMAN, H., and ACKER, L. 1974. Phosphatidspaltende Enzymes des Getreides. Getreide Mehl Brot 7:189.

PONTE, J. G., and DE STEFANIS, V. A. 1969. Note on separation and baking properties of polar and nonpolar wheat flour lipids. Cereal Chem. 46:325.

TAVENER, R. J. A., and LAIDMAN, D. L. 1972. The induction of triglyceride metabolism in the germinating wheat grain. Phytochem. 11:981.

VARTY, K., and LAIDMAN, D. L. 1976. The pattern and control of phospholipid metabolism in wheat aleurone tissue. J. Exp. Bot. 27:748.

ZAWISTOWSKA, U., BÉKÉS, F., and BUSHUK, W. 1984. Intercultivar variations in lipid content, composition, and distribution and their relation to baking quality. Cereal Chem. 61:527.

ZAWISTOWSKA, U., BÉKÉS, F., and BUSHUK, W. 1985. Gluten proteins with high affinity for flour lipids. Cereal Chem. In press.

[Received November 14, 1984. Revision received March 6, 1985. Accepted March 12, 1985.]