Intercultivar Variations in Lipid Content, Composition, and Distribution and Their Relation to Baking Quality[1]

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ABSTRACT

Lipid content and composition of flours and the distribution of dry matter, protein, and lipids in Osborne protein fractions of five hard red spring wheat cultivars with diverse baking quality were investigated. The hexane-soluble lipid (free-lipid) content but not the total lipid content of the flours showed marked differences among the cultivars. Applying prediction equations developed earlier resulted in excellent agreement between measured and predicted loaf volumes using the ratio of nonpolar to polar lipids in the free-lipid fraction. For the five cultivars, the albumin/globulin Osborne fraction contained 21.8–30.3% of the flour lipids. Analogous data for the gliadin, acetic acid soluble glutenin, and residue fractions were 27.5–39.8, 13.3–21.7, and 20.5–26.3%, respectively. The gliadin fraction contained relatively more polar lipid than did the glutenin fraction. For the five cultivars, the lipid content of both fractions was significantly correlated with loaf volume, which indicated that the lipid binding capacity of the gluten proteins, gliadin and glutenin, is an important factor in the breadmaking quality of flour. AUC (0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide)- and acetic acid-soluble fractions of glutenin had the same lipid binding capacity. Determination of the lipid content and composition of three fractions (I, >200,000; IIa, 27,500–75,000; and IIIa, 15,500–21,000) of Osborne gliadin fractionated by gel filtration on Sephadex G-200 showed that fraction I had an extremely high binding capacity for polar lipid. Only trace amounts of lipids were associated with the other fractions. Protein composition of fraction I (by SDS-PAGE) showed that it contained mostly the high-molecular weight gliadin components (>68,000) and small amounts of low-molecular weight components. For the five cultivars, the amount of aggregated fraction I was negatively correlated with the remix loaf volume. PAGE electrophoregrams of the gliadin fractions extracted directly from flour by 70% aqueous ethanol showed that the same gliadin components were involved in binding the lipids for the five cultivars. The intensity of some of the bands in the electrophoregram depended on whether lipids were in the starting material.

Recent studies in our and other laboratories (Bekes et al 1983a,b; Zawistowska et al 1984; Lukow et al 1984 and references therein) show that specific association of gluten proteins and lipids is an important factor in the breadmaking quality of wheat flour. Our study shows that intercultivar differences in protein quality (for breadmaking) may be related to the lipid binding capacity of specific gliadin and glutenin components.

MATERIALS AND METHODS

Flour Samples

Five hard red spring wheat varieties, Sinton, Neepawa, Era, Glenlea, and Tesopaco, each grown in western Canada at the same location, were selected because they had a relatively wide range of breadmaking qualities. Harvested in 1980, the wheat was stored for 1.5 years at 4°C. Flour was milled on a Buhler experimental mill. Flour yields, protein and ash contents, and remix loaf volumes (RLV) of the samples are given in Table I. The GRL-MPB (malt-phosphate-bromate) baking test was used to measure RLV (Kilborn and Tipples 1981).

Sample Preparation

Flour proteins were fractionated by the modified Osborne procedure of Chen and Bushuk (1970) into albumin/globulin, gliadin, acetic acid-soluble glutenin, and residue. The residue fraction was further extracted with AUC (0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide) solution as follows: 5 g of freeze-dried residue fraction was extracted with 40 ml of AUC solution by being gently shaken at 4°C for 2 hr, centrifuged for 30 min (1,860 × *g*), and the supernatant decanted. This was followed by a second, similar extraction for 1 hr. The combined supernatants and the residue were dialyzed against distilled water and freeze-dried, yielding AUC-soluble glutenin and final residue.

The Osborne gliadin fractions were fractionated by gel filtration chromatography on Sephadex G-200 into three subfractions: I (>200,000), IIa (27,500–75,000), and IIIa (15,500–21,000). Chromatography conditions were as follows: column size, 1 × 25 cm; material added to column, 15 mg in AU (0.1*M* acetic acid and 3*M* urea) solvent; flow rate, 3 ml per hr; and collection volumes, 1 ml. The protein content of the eluate was measured as absorbancy at 280 nm on a Hitachi model 100-40 spectrophotometer. Fraction IIa equals the sum of fractions II and III, and fraction IIIa equals the sum of fractions IV and V of our previous study (Bekes et al 1983a).

Extraction and Fractionation of Lipids

Hexane-soluble lipids (free lipids, FL) and water-saturated *n*-butanol-soluble lipids (bound lipids, BL) were extracted from 5 g of flour, 0.5 g of the Osborne fractions, and 0.2 g of fraction I of Osborne gliadin, as described by Bekes et al (1983a). The sum of FL and BL was defined as total lipid (TL). FL and BL were fractionated into NL (neutral lipid, eluted with chloroform), GL (glycolipid, eluted with acetone), and PhL (phospholipid, eluted with methanol). The sum of GL and PhL was PL (polar lipid). Lipids extracted from flour samples were examined further by thin-layer chromatography (TLC). Column chromatography and TLC procedures were as described by Bekes et al (1983a).

Lipid analyses of flour samples were replicated three times; those of the protein fractions, two times. Mean values of replicates are reported. The total average coefficient of variation (CV) for lipid content was 7% for flours and 8.7% for protein fractions. Total

Table I
Flour Yield[a], Grain Protein[a], Flour Protein[a], Ash Content[a], and Remix Loaf Volume (RLV)

Cultivar	Flour Yield (%)	Grain Protein (%)	Flour Protein (%)	Ash (%)	RLV (cc)
Sinton	73.5	13.6	12.7	0.37	920
Neepawa	72.6	12.9	12.2	0.37	800
Era	75.4	11.0	10.4	0.40	760
Glenlea	74.0	12.2	12.0	0.39	720
Tesopaco	68.8	12.0	11.1	0.42	680

[a] 14% moisture basis.

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average recovery of lipids from silicic acid column was 94.7% (90.2–98.7%). All lipid data are reported in mg/100 g sample on dry basis.

Analytical Procedures

Moisture, protein ($N \times 5.7$), and ash contents of flour samples were determined by standard methods (AACC 1983). Protein content of protein fractions was determined by the micromethod of Nkonge and Ballance (1982).

Osborne gliadin fractions and the 70% ethanol extracts of flours before and after extraction with *n*-hexane and with water-saturated butanol were examined by polyacrylamide gel electrophoresis (PAGE), according to the procedure of Bushuk and Zillman (1978), as modified by Gobran (1981). This modification used sodium lactate buffer solution instead of aluminum lactate.

Gliadin subfractions separated on a Sephadex G-200 column (I, IIa, and IIIa) were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using the method of Khan and Bushuk (1977).

RESULTS AND DISCUSSION

Lipid Content and Composition of Flours

The amounts of TL in the flours (Table II) were quite similar for the five cultivars (1,410–1,590). The average (1,492) is higher than the values published by Lin et al (1974) for the HRS wheat flours Chris, Selkirk, and Red River 68, and lower than our previously reported TL mean data for 26 HRS wheat flours (Bekes et al 1983c). These differences indicate that the lipid content of wheat cultivars of the same class can vary quite widely; both genotypic and environmental effects are evident.

Only small differences were seen among varieties in FL/BL values—eg, 1.43, 1.11, 1.11, 1.12, and 1.04 for cultivars Sinton, Neepawa, Era, Glenlea, and Tesopaco, respectively; average, 1.16 ± 0.15; CV, 1.32%. Hereinafter, data for the five cultivars are given in the order of decreasing loaf volumes. NL/PL values for total lipids, $(NL/PL)_{TL}$, were also similar (1.92, 1.88, 1.85, 2.02, and 2.07; average 1.95 ± 0.09; CV, 4.61%). On the other hand, the same parameter for free lipid, $(NL/PL)_{FL}$, showed a much higher variation (3.80, 5.02, 5.50, 5.61, and 7.19; average, 5.42 ± 1.22; CV, 23.28%).

NL/GL values were similar to NL/PL values; ratios for TL were similar, but those for FL varied widely. $(NL/GL)_{TL}$ values were 3.00, 3.03, 2.74, 3.23, and 3.29; $(NL/GL)_{FL}$ values were 5.87, 7.84, 7.33, 8.01, and 11.30. CV for $(NL/GL)_{TL}$ was 4.93 and 24.70% for $(NL/GL)_{FL}$.

Lipid compositions for the five cultivars were similar to those published by Lin et al (1974) for cultivars Chris, Selkirk, and Red River 68. Their flours contained a lower proportion of NL (50.8% average vs 65.9%) and a slightly greater proportion of GL and PhL (26.4 and 17.8% averages vs 21.8 and 12.3%, respectively).

TLC results showed that triglycerides was the major (40.3–48.6%) component of TL for all five cultivars. Our values for this component are somewhat higher than those of Lin et al (1974). Digalactosyl diglycerides (DGDG) was the second largest component (10.5–11.6%). The DGDG data are essentially identical to the values for cultivars Chris and Selkirk, reported by Lin et al (1974). DGDG contents of FL were 5.8, 5.6, 5.2, 4.6, and 4.0%; the variation among the five varieties was much higher than in TL (CV for FL was 14.7% and 3.5% for TL).

Polar lipids, especially the glycolipids, are an important factor in breadmaking quality (Bekes et al 1983a,b; Chung et al 1982; Pomeranz 1971). Our study showed that of the lipid parameters examined, the $(NL/GL)_{FL}$ and $(NL/PL)_{FL}$ values were the most variable among the cultivars. The validity of correlations among $(NL/GL)_{FL}$, $(NL/PL)_{FL}$, and loaf volume indicates that the amount of nonpolar lipids can modify the effect of polar or glycolipids. This is in general agreement with the findings of MacRitchie and Gras (1973), who showed that the effects of polar and nonpolar lipids on baking quality were different. As reported previously (Bekes et al 1983c), of the lipid data analyzed, $(NL/PL)_{FL}$ was most strongly correlated with loaf volume. Using the regression equation for loaf volume vs $(NL/PL)_{FL}$ values for flour, the predicted loaf volumes for the five cultivars used in our study agreed with actual values to within 5%. This result adds further support to our earlier claim that certain lipid data can be used to predict loaf volume of varieties in wheat breeding populations.

Distribution of Dry Material, Protein, and Lipid in Osborne Fractions

Protein recovery and distribution results for the Osborne

Table II
Lipid Content[a] and Composition of Flour Samples[b]

	Cultivar														
	Sinton			Neepawa			Era			Glenlea			Tesopaco		
	FL	BL	TL	FL	BL	TL	FL	BL	TL	FL	BL	TL	FL	BL	TL
NL	681	278	959	667	326	993	660	301	961	713	350	1,063	632	320	952
GL	116	203	319	85	242	327	90	260	350	89	240	329	56	233	289
PhL	63	118	181	48	152	200	30	139	169	38	160	198	32	137	169
PL	179	321	500	133	394	527	120	399	519	127	400	527	88	370	458
TL	860	600	1,460	800	720	1,520	780	700	1,480	840	750	1,590	720	690	1,410

[a] mg/100 g, dry basis.
[b] FL = free lipids; BL = bound lipids; TL (total lipids) = FL + BL; NL = chloroform eluate from silicic acid column (neutral lipids); GL = acetone eluate (glycolipids); PhL = methanol eluate (phospholipids); PL (polar lipids) = GL + PhL.

Table III
Dry Material (D) and Protein (P) Distribution of Protein Fractions[a]

	Albumin/Globulin[b]		Gliadin[b]		Glutenin[b]		Residue[b]		AUC Extract[c]		AUC Residue[d]		Recovery[e]	
Cultivar	D	P	D	P	D	P	D	P	D	P	D	P	D	P
Sinton	4.9	15.4	5.4	35.1	2.4	14.7	87.3	34.8	4.6	30.9	82.7	3.9	93.7	94.3
Neepawa	5.7	19.1	6.4	31.8	3.1	13.1	84.8	36.0	4.0	35.0	80.8	5.0	89.4	88.5
Era	5.8	13.4	6.8	32.5	2.6	17.4	84.8	36.7	4.6	32.4	80.2	4.3	94.7	89.7
Glenlea	5.9	14.8	6.9	30.0	2.9	20.1	84.3	35.1	5.5	31.4	78.8	3.7	91.6	92.6
Tesopaco	4.4	16.1	4.7	36.0	4.0	19.9	86.9	28.0	3.7	27.7	83.2	4.7	91.3	89.9

[a] Percentage on dry basis.
[b] Data for the four Osborne fractions normalized to 100% dry material and protein recovery.
[c] AUC extract of Osborne residue.
[d] Residue after AUC extraction of Osborne residue.
[e] Of total fractionation, including AUC extraction of Osborne residue.

fractions (Table III) showed good agreement with published results for other wheat varieties (Orth and Bushuk 1972). The albumin/globulin fraction represented approximately 5% of the dry material and contained 13.4–19.1% of the total protein.

Dry material proportions in the Osborne gliadin fraction varied from 4.7 to 6.9%. This fraction of cultivar Tesopaco contained the highest proportion of the flour protein; that of cultivar Glenlea, the lowest proportion. The proportions of dry matter showed the opposite trend. A similar effect of the nonprotein material was observed for the acetic acid-soluble glutenin fraction. Thus, the gliadin/glutenin values gave different rankings of the five cultivars on the basis of dry material and protein. The gliadin/glutenin values were 2.25, 2.06, 2.61, 2.37, and 1.17, based on dry material, and 2.38, 2.42, 1.86, 1.49, and 1.80, based on protein. For the five cultivars, both sets of gliadin/glutenin values were significantly linearly correlated with loaf volume (r_{dm} = 0.655* and r_{prot} = 0.677*).

When the AUC-soluble protein of the Osborne residue fraction was added to the soluble Osborne fractions, more than 95% of the flour protein was solubilized. This finding agrees with our earlier findings for other cultivars (Orth and Bushuk 1973). AUC solubilized approximately 5% of the dry material of the Osborne residue fraction. The ratio of the AUC-soluble and the acetic acid-soluble protein (2.10, 2.85, 1.89, 1.56, and 1.39) was significantly correlated with remix loaf volume (r = 0.626*). An analogous parameter for dry material (1.91, 1.29, 1.76, 1.89, and 0.90) gave a slightly higher correlation coefficient, but the level of significance remained unchanged (r = 0.690*). Thus, the inclusion of nonprotein constituents in the calculations did not affect the relationship between the ratio of the two fractions and remix loaf volume. Actually, the correlation coefficients were slightly higher, which suggests that in addition to the protein (content and quality) constituent of flour, the nonprotein constituents, especially those associated with gluten proteins, contribute to differences in loaf volume among wheat cultivars. Accordingly, a complete definition of gluten "quality" for breadmaking must include the contribution of relevant nonprotein constituents.

Lipid data for gliadin and glutenin fractions have been obtained by several different experimental procedures. In some cases, gluten (Olcott and Mecham 1947, Ponte and DeStefanis 1969, Hoseney et al 1970) or dough (Chung and Tsen 1975) was prepared and the protein fractions separated and analyzed for lipid. Gliadin and glutenin were separated on the basis of their different solubility (Olcott and Mecham 1947, Ponte and DeStefanis 1969) or on the basis of their molecular or aggregate size by gel filtration chromatography (Chung and Tsen 1975) or by ultracentrifugation (Hoseney et al 1970). Our results (Zawistowska et al 1984) showed that the two types of separation can lead to quite different lipid distributions in the two protein fractions. For example, in the gel filtration fractionation, subfraction I of the gliadin fraction (which contains most of the polar lipid of the gliadin) is isolated together with the glutenin fraction.

Furthermore, wetting the flour and mixing the dough alters the association of flour lipids with the proteins. Accordingly, the distribution of the lipids calculated from flour analyses (Table IV) cannot be compared with published data based on analyses of protein fractions prepared from gluten or dough. Chung and Tsen (1975) reported lipid distribution data for flour, but because our fractionation is quite different, comparisons cannot be made.

Table IV shows the TL content and composition (NL, PL, GL, and PhL) data for the five Osborne fractions. The proportion of the flour lipid in each fraction is in parentheses.

The albumin/globulin fractions contained 5,590–9,740 mg/100 g lipid, representing 21.8–30.3% of the flour lipid. The lipid of this fraction is rich in nonpolar lipids; the NL/PL values were 3.11, 4.72, 2.64, 4.09, and 2.41 for the five cultivars. GL formed 74.1, 72.3, 71.9, 77.5, and 70.0% of PL. The most striking difference among the cultivars was the high TL value for cultivar Tesopaco.

The Osborne gliadin fraction contained 8.01–9.14% lipid (27.5–39.8% of the flour lipid). The acetic acid-soluble glutenin fraction had slightly lower lipid content (7.25–8.49%) and contained 13.3–21.7% of the flour lipid. The two fractions had different NL/PL and GL/PL values. Although the gliadin fraction contained approximately equal amounts of NL and PL (NL/PL values were 0.916, 0.956, 1.01, 1.09, and 1.18), the glutenin fraction lipid had considerably more NL than PL (NL/PL values were 2.83, 2.55, 3.03, 2.37, and 2.94).

Polar lipid in the Osborne gliadin fraction was mainly glycolipid; GL/PL values were 0.811, 0.816, 0.772, 0.762, and 0.759. On the other hand, the polar lipid in the glutenin fraction was mostly phospholipid; PhL/PL values were 0.521, 0.744, 0.750, 0.615, and 0.516. This is consistent with the finding of Bourdet and Feillet (1967) that essentially all of the flour lipid phosphorus is associated with the glutenin fraction.

Lipid content of the Osborne residue fractions was quite low, the highest value, 0.47%, being for cultivar Glenlea. Because of their substantial dry material content, the lipid of the residue fractions represented a significant proportion (20.5–26.3%) of the flour lipid. The residue that remained after AUC extraction of the Osborne residue did not contain any lipid. For each cultivar, the lipid content and composition of the AUC-soluble fraction (of the Osborne residue) were practically identical to those of the Osborne glutenin fraction.

Table IV
Lipid Content[a] of Osborne Fractions and Lipid Expressed as Percent of Flour Lipid[b]

		Fraction			
Cultivar	Lipid	Albumin/ Globulin	Gliadin	Glutenin	Residue
Sinton	TL	7,930 (26.6)	9,140 (33.7)	8,230 (13.4)	440 (26.3)
	NL	6,000 (30.8)	4,370 (24.7)	6,080 (15.3)	320 (29.2)
	PL	1,930 (18.7)	4,770 (50.5)	2,150 (9.9)	120 (20.9)
	GL	1,430 (21.1)	3,870 (62.9)	1,030 (7.5)	30 (8.5)
	PhL	500 (14.0)	900 (27.5)	1,120 (15.2)	90 (43.3)
Neepawa	TL	6,010 (22.6)	8,920 (37.7)	8,490 (17.3)	400 (22.4)
	NL	4,960 (28.4)	4,360 (28.1)	6,100 (19.0)	285 (24.5)
	PL	1,050 (11.4)	4,560 (55.6)	2,390 (14.2)	115 (18.8)
	GL	760 (13.8)	3,720 (73.0)	610 (5.8)	30 (7.4)
	PhL	290 (8.5)	840 (27.0)	1,780 (27.5)	85 (37.0)
Era	TL	5,590 (21.8)	8,670 (39.8)	7,880 (13.9)	420 (24.5)
	NL	4,050 (24.4)	4,370 (30.9)	5,960 (16.1)	323 (28.5)
	PL	1,540 (17.1)	4,300 (56.2)	1,960 (9.8)	100 (16.9)
	GL	1,110 (19.8)	3,320 (69.7)	490 (4.0)	30 (6.5)
	PhL	430 (12.7)	980 (34.0)	1,470 (19.3)	70 (34.0)
Glenlea	TL	7,230 (26.7)	8,010 (34.8)	7,250 (13.3)	470 (25.2)
	NL	5,810 (32.3)	4,180 (27.2)	5,100 (14.0)	330 (26.5)
	PL	1,420 (15.7)	3,830 (50.0)	2,150 (11.8)	140 (22.5)
	GL	1,100 (19.5)	2,920 (60.7)	830 (7.3)	50 (12.5)
	PhL	320 (9.6)	910 (32.1)	1,320 (19.4)	90 (38.9)
Tesopaco	TL	9,740 (30.3)	8,250 (27.5)	7,640 (21.7)	330 (20.5)
	NL	6,890 (31.8)	4,470 (22.0)	5,700 (23.9)	250 (22.3)
	PL	2,850 (27.9)	3,780 (39.5)	1,940 (16.9)	80 (15.7)
	GL	2,000 (31.7)	2,870 (48.5)	940 (13.5)	20 (6.3)
	PhL	850 (21.7)	910 (24.8)	1,000 (23.2)	60 (30.3)

[a] mg/100 g.
[b] In parentheses.

Table V
Linear Correlation Coefficients for Gliadin and Glutenin Lipid Data and Remix Loaf Volume

Lipid	Gliadin	Glutenin
TL	0.879**[a]	0.688**
NL	0.309	0.613**
PL	0.643**	0.425*[b]
GL	0.924**	−0.119
NL/PL	−0.911**	0.498*
GL/PL	0.978**	−0.851**

[a] ** = Significant at 0.01 level.
[b] * = Significant at 0.05 level.

Lipid data for the Osborne gliadin and glutenin fractions were correlated with remix loaf volumes. The number of observations, five, is too low for a generalized conclusion, but it is significant that some lipid data showed a strong relationship with remix loaf volume (Table V). For high loaf volume potential, high glycolipid binding capacity of gliadin and high nonpolar lipid binding capacity of glutenin appear to be desirable. Because of the wide heterogeneity of gliadin (Woychik et al 1961) and glutenin (Huebner et al 1974, Khan and Bushuk 1979) components in amino acid composition, this conclusion underlines the importance of components or subunits that bind specific lipids. Kinetic studies of this binding could contribute to our understanding of breadmaking quality.

Lipids Associated with Gliadin Subfractions

Previous results for cultivar Neepawa showed that subfraction I (from Sephadex G-200) of pH-precipitation gliadin contained most (97%) of the PL of original gliadin (Bekes et al 1983a). On the other hand, subfraction III contained much less lipid, which was mainly NL. That work was extended to the Osborne gliadin fraction of the five cultivars used in our study.

As before (Bekes et al 1983a), chromatography of gliadin on Sephadex G-200 gave five peaks. However, to decrease the number of analyses in our study, we combined eluates of peaks II and III into subfraction IIa and those of peaks IV and V into subfraction IIIa (Table VI). For all five cultivars, subfraction I contained most of the lipid of the Osborne gliadin and the lipid was mainly GL. Subfraction lipid IIa contained much less lipid and was mainly NL. Only a trace of it was found in subfraction IIIa.

The data on breadmaking quality (Table VI) showed that subfractions I of cultivars of higher baking quality contained more lipid. Furthermore, the correlations among RLV and TL and RLV and GL were highly significant ($r = 0.876^{**}$ and 0.884^{**}, respectively). This leads to the conclusion that the functionality of flour glycolipids in breadmaking is through their association with specific gliadin components (ie, those that comprise subfraction I).

SDS-PAGE analysis of subfraction I showed that it contained lower molecular weight besides the high molecular weight components. Analogous results were obtained previously when the composition of subfraction I of the gliadin of cultivar Neepawa prepared by the pH-precipitation procedure (Bekes et al 1983a) was studied. Our result is consistent with the possibility that aggregates of some gliadin proteins and glycolipids were formed during the wetting of flour in the first extraction step of the Osborne procedure. However, the possibility that the gliadin-glycolipid complex was formed during desiccation of wheat endosperm after grain development cannot be excluded on the basis of results obtained so far.

Our results on the lipid content of subfraction I for cultivar Neepawa can be compared with those for the same subfraction of the gliadin prepared from gluten and reported previously (Bekes et al 1983a). In our study, subfraction I contained 86% of the original gliadin lipid, whereas the previous subfraction I contained 93%. Also, the proportion of subfraction I in Osborne gliadin was lower (27.5%) than in pH-precipitation gliadin (47%), which indicates that dough mixing (and gluten preparation) increased the proportion of low molecular weight protein components and lipid in subfraction I. Comparison of proportions of aggregated material in Osborne gliadin fraction among five cultivars showed that flours with better baking quality contained less subfraction I. For the five cultivars, the remix loaf volume was negatively correlated ($r = -0.768$) with the amount of this subfraction. This indicates that different amounts of lipid are required for aggregation high molecular weight gliadin subunits in subfraction I of cultivars with different baking characteristics. We showed in an earlier study (Bekes et al 1983a, b) that lipid is required for the aggregation of specific gliadin components.

Effect of Lipids in Gliadin Electrophoregrams

The PAGE electrophoregram of the 70% ethanol extract of grain meal or flour of wheat cultivars has important scientific and

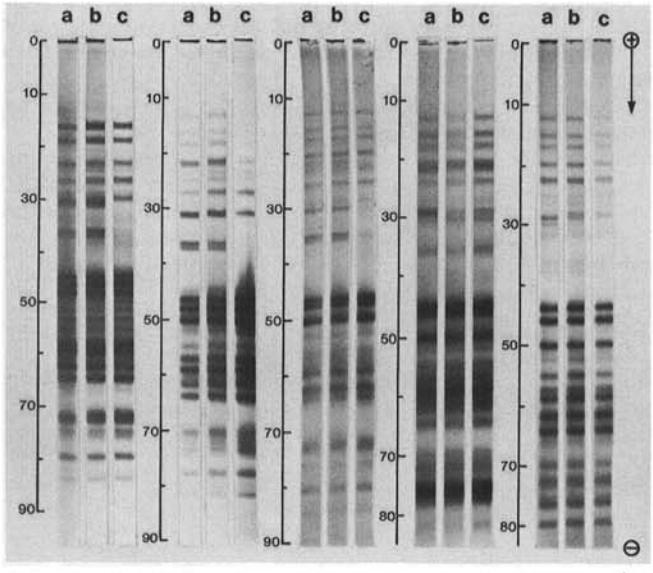

Fig. 1. PAGE patterns of gliadins. From left to right: cultivars Sinton, Neepawa, Era, Glenlea, and Tesopaco: a) gliadin extracted directly with 70% ethanol from flour; b) gliadin extracted with 70% ethanol from flour defatted with n-hexane; and c) gliadin extracted with 70% ethanol from flour totally defatted with water-saturated *n*-butanol.

Table VI
Dry Material Distribution and Lipid Content × 10^2[b] of Gliadin Subfractions

Cultivar	Subfraction I	Subfraction IIa	Subfraction IIIa
Sinton			
Dry matter	26.3	64.6	9.1
Lipid			
TL	291.5	22.5	1.5
NL	127.5	15.5	nd[c]
PL	164.0	7.0	nd[c]
GL	129.0	5.5	nd[c]
PhL	35.0	1.5	nd[c]
Neepawa			
Dry matter	27.5	61.5	11.0
Lipid			
TL	280.5	19.0	1.0
NL	126.5	14.0	nd[c]
PL	154.0	nd[c]	nd[c]
GL	127.0	trace	nd[c]
PhL	27.0	trace	nd[c]
Era			
Dry matter	34.8	54.5	10.7
Lipid			
TL	220.5	18.0	15.0
NL	105.5	13.0	nd[c]
PL	115.0	nd[c]	nd[c]
GL	88.5	4.5	nd[c]
PhL	26.5	trace	nd[c]
Glenlea			
Dry matter	29.7	61.4	8.9
Lipid			
TL	160.0	52.5	1.5
NL	63.5	37.0	nd[c]
PL	96.5	15.5	nd[c]
Gl	76.5	10.0	nd[c]
PhL	20.0	5.5	nd[c]
Tesopaco			
Dry Matter	40.6	49.4	10.0
Lipid			
TL	180.5	18.0	nd[c]
NL	88.5	17.5	nd[c]
PL	92.0	nd[c]	nd[c]
GL	70.0	trace	nd[c]
PhL	22.0	trace	nd[c]

[a] Percentage.
[b] mg/100 g.
[c] nd = not determined because of low TL.

practical significance. The electrophoregram can be used for cultivar identification and may contain information on agronomic traits and functional properties (Wrigley et al 1982). Accordingly, our previous observation (Bekes et al 1983a) that the electrophoregram is modified by the lipid status (amount and composition) may be important in this regard.

In our study of five cultivars with widely different electrophoregrams (Fig. 1), we extended the effects of lipids on the gliadin electrophoregram previously reported for cultivar Neepawa only. Again, the largest differences were in the 29–31.5 region (nomenclature of Bushuk and Zillman 1978) of the electrophoregrams. For cultivars Sinton, Neepawa, and Era, the intensity of band 31 decreased when the lipid was removed. For Sinton and Era, this band was absent for samples that did not contain any lipid. For cultivar Glenlea, intensity of band 29 decreased; for cultivar Tesopaco, the decrease was for bands 30 and 31.5.

Other cultivar specific effects were observed, some of which are not clearly visible in Fig. 1 but are based on examination of stained gels. For example, in region 10–20 for cultivar Sinton, the intensity of bands 16 and 19 increased when lipid was removed. For cultivars Neepawa and Era, bands 13.5, 15.5, and 17.5, and 21.5, respectively, were fainter for defatted samples. In the 35–38 region, intensity of bands 36 and 37.5 for cultivar Sinton and 37 and 38 for cultivar Tesopaco decreased after lipid was removed.

Increases in band intensity after defatting were seen in the high mobility region for some cultivars. The effect was particularly apparent for bands 72 and 81.5 for cultivar Sinton; bands 70, 72.5, 74.5, and 78 for cultivar Neepawa; and band 82 for cultivar Glenlea.

Bands in the 30–38 region showed another effect of experimental conditions: The blue stain of these bands gradually changed to a reddish-blue during soaking in water for two to three weeks. Caldwell and Kasarda (1978) saw a similar effect in electrophoregrams of wheat albumins and globulins, and Minetti et al (1978) saw a similarity in electrophoregrams of albumins, globulins, and gliadins. The reason for the color change is unknown.

The gliadin fraction of original flours of the five cultivars used in our study contained 11.5–13.5% lipid. The same fractions of flour from which free lipid had been removed by n-hexane contained 3.6–4.2% lipid, and those of flour that had been totally defatted with WSB contained no lipid. Accordingly, the differences in the gliadin electrophoregrams indicated above are presumed to be due to the presence of protein-lipid complexes. If this is so, it is surprising that the mobility of the particular gliadin components was not affected.

CONCLUSION

Analyses of the lipid constituent of flours of five HRS wheat cultivars with a wide range of baking quality showed marked variations in amount and composition. Also observed were differences in the distribution of lipid among the Osborne protein fractions. Evidence was presented on specific binding of lipid by gluten proteins. The quantity and quality of the bound lipid as well as the relative amounts of lipid-binding proteins in gluten are considered to be essential elements of gluten "quality" for breadmaking.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved methods of the AACC. Methods 08-01, 46-12, and 44-15, approved April 1961. The Association, St. Paul, MN.

BEKES, F., ZAWISTOWSKA, U., and BUSHUK, W. 1983a. Lipid-protein interactions in the gliadin fraction. Cereal Chem. 60:371.

BEKES, F., ZAWISTOWSKA, U., and BUSHUK, W. 1983b. Lipid-mediated aggregation of gliadin. Cereal Chem. 60:379.

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

BOURDET, A., and FEILLET, P. 1967. Distribution of phosphorus compounds in the protein fractions of various types of wheat flours. Cereal Chem. 44:457.

BUSHUK, W., and ZILLMAN, R. 1978. Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method, and nomenclature. Can. J. Plant Sci. 58:505.

CALDWELL, K. A., and KASARDA, D. D. 1978. Assessment of genomic and species relationships of *Triticum* and *Aegilops* by PAGE and by different staining of seed albumins and glutenins. Theor. Appl. Genet. 52:273.

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., POMERANZ, Y., and FINNEY, K. F. 1982. Relation of polar lipid content to mixing requirement and loaf volume potential of hard red winter wheat flour. Cereal Chem. 59:14.

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.

GOBRAN, F. 1981. Relationship between some physiocochemical properties of wheat proteins and breadmaking quality. M.Sc. thesis. Univ. of Manitoba, Winnipeg.

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.

HUEBNER, F. R., DONALDSON, G. L., and WALL, J. S. 1974. Wheat glutenin subunits. II. Compositional differences. Cereal Chem. 51:240.

KHAN, K., and BUSHUK, W. 1977. Studies of glutenin. IX. Subunit composition of sodium dodecyl sulfate-polyacrylamide gel electrophoresis at pH 7.3 and 8.9. Cereal Chem. 53:588.

KHAN, K., and BUSHUK, W. 1979. Studies of glutenin. XIII. Gel filtration, isoelectric focusing and amino acid composition studies. Cereal Chem. 56:505.

KILBORN, R. H., and TIPPLES, K. H. 1981. Canadian test baking procedures. I. GRL method and variations. Cereal Foods World 26:624.

LIN, M. J. Y., YOUNGS, V. L., and D'APPOLONIA, B. L. 1974. Hard red spring and durum wheat polar lipids. I. Isolation and quantitative determination. Cereal Chem. 51:17.

LUKOW, O. M., BEKES, F., and BUSHUK, W. 1983. Influence of germination on wheat quality. III. Modification of flour lipids. Submitted to Cereal Chem.

MacRITCHIE, F., and GRAS, P. W. 1973. The role of flour lipids in baking. Cereal Chem. 50:292.

MINETTI, M., PETRUCCI, T., CATTANEO, S., POCCHIARI, F., and SILANO, V. 1973. Studies of the differential staining of wheat albumins, globulins, and gliadins in polyacrylamide gel by aniline blue-black. Cereal Chem. 50:198.

NKONGE, C., and BALLANCE, G. M. 1982. A sensitive colorimetric procedure for nitrogen determination in micro Kjeldahl digest. J. Agric. Food Chem. 30:416.

OLCOTT, H. S., and MECHAM, D. K. 1947. Characterization of wheat gluten. I. Protein-lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction. Cereal Chem. 24:407.

ORTH, R. A., and BUSHUK, W. 1972. A comparative study of the proteins of wheat of diverse baking quality. Cereal Chem. 49:268.

ORTH, R. A., and BUSHUK, W. 1973. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50:106.

POMERANZ, Y. 1971. Glycolipid-protein interaction in breadmaking. Bakers Dig. 45:26.

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

WOYCHIK, J. H., BOUNDY, J. A., and DIMLER, R. J. 1961. Amino acid composition in wheat gluten. J. Agric. Food Chem. 9:307.

WRIGLEY, C. W., AUTRAN, J. C., and BUSHUK, W. 1982. Identification of cereal varieties by gel electrophoresis of the grain proteins. P. 211 in: Adv. Cer. Sci. Technol. Vol. V. Y. Pomeranz, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

ZAWISTOWSKA, U., BEKES, F., and BUSHUK, W. 1984. Gluten proteins with high affinity for flour lipids. Cereal Chem. In press.

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