# Gliadin in Crumb of Bread from High-Protein Wheat Flours of Varied Breadmaking Potential

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**ABSTRACT**

Gliadins extracted from flours and bread crumbs of six high-protein hard red winter wheats that varied widely in breadmaking potential were examined by polyacrylamide gel electrophoresis (PAGE) and high-pressure liquid chromatography (HPLC). Four of the HRW wheats consisted of two pairs of sister lines. The gliadins in the six flours (including the sister pairs) differed in their PAGE patterns. Generally, slow-moving (relative mobility [RM] values below 40) PAGE bands (ω-gliadins) extracted from bread crumb were more pronounced than those from wheat flour. The reverse was true for fast moving (RM values above 40) PAGE bands (α-, β-, and γ-gliadins). Generally, the change from flour to bread crumb was more pronounced in good breadmaking flours than in poor breadmaking flours. The flours also differed in their gliadin HPLC elution patterns. Differences in HPLC-separated gliadin bands between pairs of sister lines were small. There were relatively small changes (from flour to bread crumb) in HPLC elution bands below 20 min; elution bands above 20 min were consistently reduced in intensity from flour to bread crumb. The extent of reduction in peak intensity was much higher in good- than in poor-breadmaking quality flours. It is postulated that heat-labile α-, β-, and γ-gliadins (the highly hydrophobic gliadins) are modified during baking and that the modification may be related, in part at least, to differences in breadmaking potential of wheat flours.

The functional (breadmaking) properties of wheat flours and wheat proteins (mainly gluten) were described and reviewed by Pomeranz (1968, 1980), Finney et al (1982), Jones et al (1983), and Finney (1985). Those reports documented intervarietal differences in gliadins in flours that varied in breadmaking potential. Chromatographic and electrophoretic patterns of gliadins and their relation to breadmaking quality were demonstrated to be governed by genetics rather than environment.

Polyacrylamide gel electrophoretic (PAGE) patterns of gliadins show four groupings of bands designated α, β, γ, and ω (Jones et al 1959; Woychik et al 1961, 1964). Another classification designates sulfur-poor ω-gliadins and sulfur-rich α-, β- and γ-gliadins (Wrigley et al 1980, Tatham and Shewry 1985).

McCausland and Wrigley (1976) reported that gel electrophoretic patterns were modified as a result of baking. Such patterns still could provide the basis for distinction between wheat and rye. According to Schofield et al (1983) the baking performance of gluten declined on heating and was destroyed by 75° C. Extractability of gliadin proteins was unaffected by heating up to 75° C and decreased markedly after 100° C. Gliadin patterns were essentially unaltered up to 75° C, but at 100° C ω-gliadins dominated the patterns.

We know of no reported study comparing the effects of breadmaking on extractability of gliadin proteins and the electrophoretic and chromatographic patterns of the gliadins in crumb of bread baked from flours that varied in breadmaking potential. Such an investigation is the subject of this report.

We recently reported on changes in PAGE and high-pressure liquid chromatography (HPLC) patterns of gliadin proteins during baking of a composite hard red winter wheat flour (Menkovska et al 1987). HPLC and PAGE patterns have demonstrated an interaction of gliadin proteins during bread

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baking but not during dough mixing or fermentation. The PAGE $\omega$-gliadin bands with low relative mobility (RM) (<40) were more intense (stronger) and those with RM >40 ($\alpha$-, $\beta$-, and $\gamma$-gliadins) were less intense (weaker) when extracted from bread crumb than those extracted from the corresponding wheat flour. Highly hydrophobic gliadins, with longest HPLC elution times (>23 min), were more heat labile (and probably interacted more with other flour components) than the less hydrophobic gliadins (elution times <23 min). Heat lability of gliadin proteins during bread baking was confirmed by in vitro heat treatment of HPLC-isolated fractions (20–23 min and 23–26 min elution times) and subsequent analysis by PAGE and HPLC.

We report here on changes in PAGE and HPLC patterns of gliadins in six high-protein hard red winter wheat flours that varied widely in breadmaking potential. In the previous investigation (Menkovska et al 1987) we studied extracts from flour, mixed dough, fermented dough, bread crumb, and bread crust. The effects of adding 3% whey, soy flour, or milk solids on PAGE and HPLC patterns were insignificant. No consistent changes were observed as a result of dough mixing or fermentation, and the amount of gliadins from bread crust resolved by PAGE and HPLC was very small. Consequently, gliadin extracts from flour and bread crumb, only, were separated in this study.

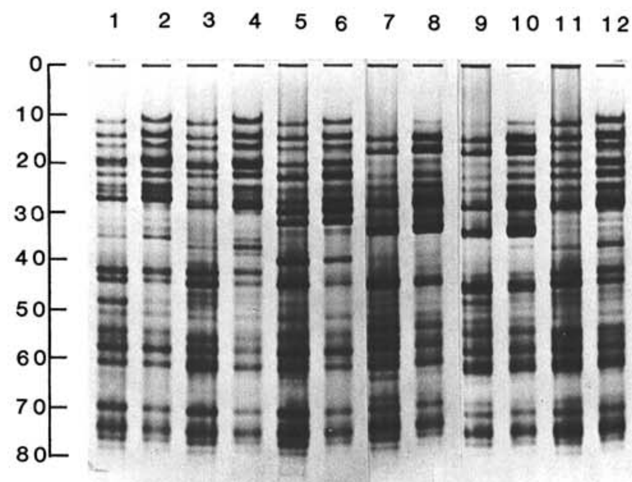


**Fig. 1.** Polyacrylamide gel electrophoretic patterns of gliadins from flours (**1, 3, 5, 7, 9,** and **11**) and corresponding bread crumbs (**2, 4, 6, 8, 10,** and **12**) of C.I. 12995, Shawnee, KS 644, KS 501097, KS 501099, and Ottawa Selection, respectively; 3 and 11 (and 4 and 12) and 7 and 9 (and 8 and 10) are sister line pairs.

## MATERIALS AND METHODS

The six hard red winter wheat flours used in this study are described in Table I; the wheats were grown in Manhattan, KS, in 1981. Shawnee and Ottawa Selection and KS 501099 and KS 501097 are pairs of sister lines. Breadmaking quality of the varieties or selections C.I. 12995 and Shawnee is good, Concho/2* Triumph intermediate, and KS 501099, KS 501097, and Ottawa Selection decreasing from poor to very poor. Good breadmaking quality is indicated by long mixing time (and, generally associated with it, good mixing tolerance, satisfactory water absorption, and, foremost, high loaf volume).

Moisture, ash, and protein were determined by AACC methods 44-15A, 08-101, and 46-11, respectively (AACC 1983). The wheat samples were milled on an experimental mill (Allis-Chalmers Mfg. Co., Milwaukee, WI) to produce a flour of about 72% extraction (Finney and Bolte 1985). Mixing time, baking water absorption, loaf volume, and crumb grain and texture were determined using a straight-dough baking procedure as described by Finney (1984). About 1 hr after baking, the bread crumb was separated from the crust, cut into small pieces, air-dried for about 48 hr, and ground to a fine powder in a mortar and pestle.

PAGE and HPLC analyses were done on 10-$\mu$l aliquots of the same 70% ethanol extracts from flour and bread crumb as described by Menkovska et al (1987).

## RESULTS AND DISCUSSION

The flours with long and medium mixing times (C.I. 12995, C.I. 14157 and KS 644) produced large loaves with good crumb grain. The flours with short mixing times produced small loaves with poor crumb grain. The oxidation requirements of flours with long mixing times were low, and those with short mixing times were high (data not shown). Whereas the two sister lines from Chiefkan-Tenmarq crosses were comparable in breadmaking quality, the two sister lines, Shawnee and Ottawa Selection, varied widely in their functional properties and bread quality (Table I).

PAGE and HPLC patterns of the gliadins extracted from the six flours and the corresponding bread crumbs are compared in Figure 1 and Figure 2, respectively. These patterns should be compared from three viewpoints: 1) genetically controlled differences in wheat flour gliadin proteins in all six samples; 2) genetically controlled differences in wheat flour gliadin proteins between two pairs of sister lines: Shawnee and Ottawa Selection, and KS 501097 and 501099; and 3) differences in PAGE and HPLC patterns between gliadins extracted from flours and corresponding bread crumbs. As stated before, in the comparison between sister lines, Shawnee is of good and Ottawa Selection of poor breadmaking quality, and KS 501097 and 501099 are both of poor breadmaking potential.

There was considerable variability in the numbers and intensities

**TABLE I**
**Description of Wheat Flours Used in This Study**[a]

| Wheat Variety[b] | Ash (%) | Protein (N × 5.7, %) | Water Absorption (%) | Mixing Time (min) | Loaf Volume (cm³) | Overall Breadmaking Quality |
|---|---|---|---|---|---|---|
| Qv-Tm × Mq. Oro (C.I. 12995) | 0.52 | 20.6 | 69.4 | 5 | 1,245 | Good |
| Shawnee o (C.I. 14157) | 0.52 | 18.7 | 69.6 | 4 3/8 | 1,328 | Good |
| Triumph (KS 644) | 0.43 | 17.8 | 68.2 | 3 | 1,286 | Intermediate |
| Chiefkan-Tenmarq Δ (KS 501097) | 0.44 | 18.6 | 67.9 | 7/8 | 875 | Poor |
| Chiefkan-Tenmarq Δ (KS 501099) | 0.43 | 19.1 | 67.8 | 3/4 | 818 | Poor |
| Ottawa Selection o (KS 699042) | 0.60 | 20.7 | 64.3 | 7/8 | 693 | Very poor |

[a]All results expressed on a 14% moisture basis.
[b]Selections or varieties followed by like symbols are sister lines.

of PAGE bands among the gliadins of the six flours (Fig. 1, patterns 1, 3, 5, 7, 9, and 11). The differences were large and consistent (for replicates) to provide a reliable basis for varietal identification and discrimination. Thus, for instance, gliadins from C.I. 12995 (pattern 1) and KS 644 (pattern 5) differed significantly between themselves and from any of the other gliadin patterns. The gliadin patterns of the sisters, Shawnee (pattern 3) and Ottawa Selection (pattern 11), were similar. However, some differences in band intensity were noted. Thus, for instance, the band with RM 20 was stronger (darker stained) than the bands with RM below 20 in the good quality Shawnee line, but not in the poor quality Ottawa Selection line. (The terms good quality and poor quality refer to breadmaking potential.) Similarly, whereas the band patterns of sister lines KS 501097 (pattern 7) and KS 501099 (pattern 9) were similar, they differed mainly in the presence of several strong bands in the RM region of about 65 (present in pattern 7 and absent in pattern 9).

In the context of this paper, the differences between gliadin patterns of wheat flours and bread crumbs are of greatest interest. The densities of the bands in the odd-numbered patterns (flours) were equal to or stronger than those in the even-numbered patterns (bread crumbs) for the same line. The change in intensity of the bands from extracts of flour to those from bread crumb was not equal, however, for various groups of gliadins and for various

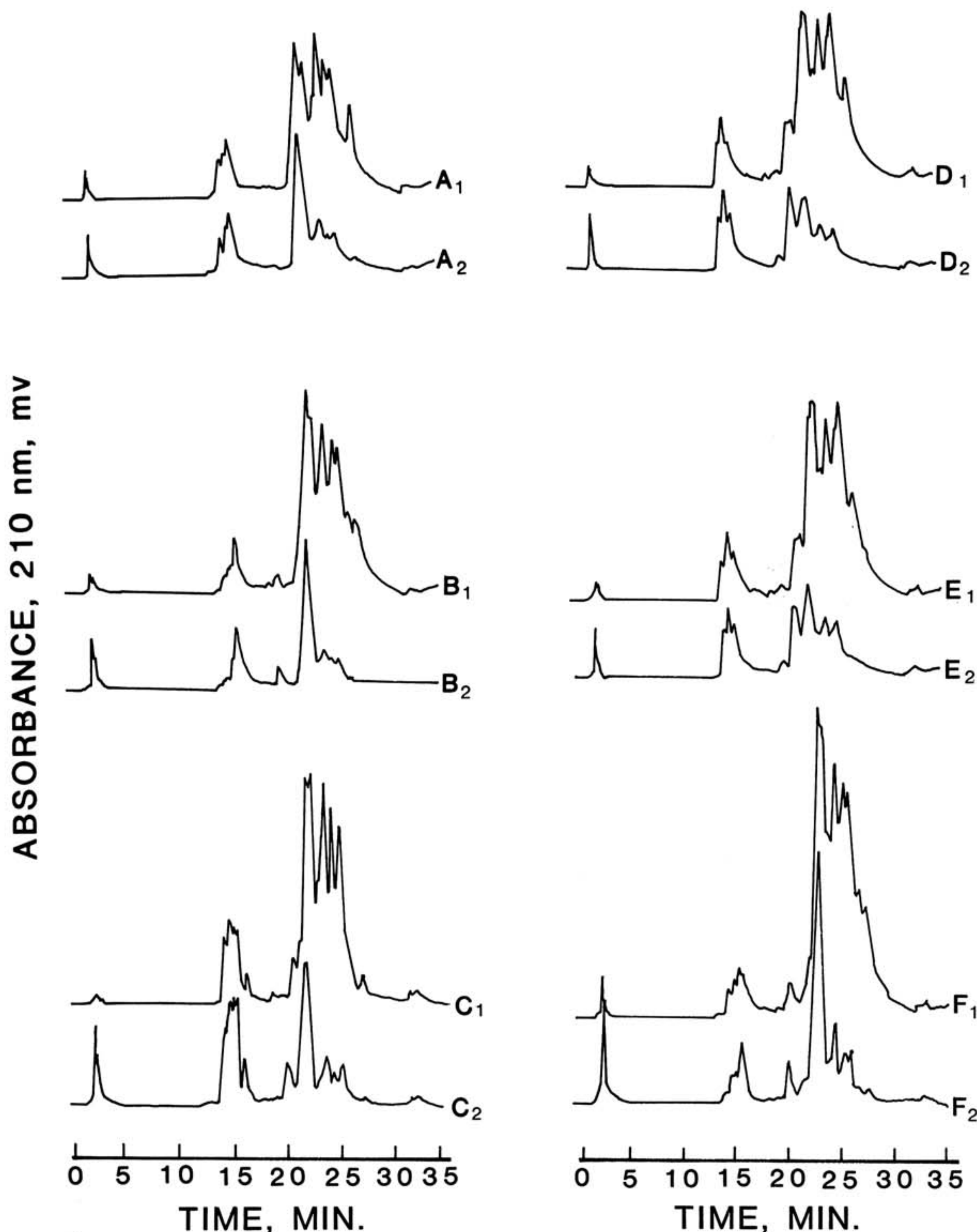


**Fig. 2.** High-performance liquid chromatographic elution patterns of gliadin proteins from flours (**1**) and corresponding bread crumbs (**2**) of C.I. 12995 (**A**), Shawnee (**B**), KS 644 (**C**), KS 501097 (**D**), KS 501099 (**E**), and Ottawa Selection (**F**); B and F and D and E are sister line pairs.

flours. Generally, the ω-gliadin bands (RM <40) were stronger in bread crumb than in wheat flour patterns. The α-, β-, and γ-gliadin bands generally were more intense in patterns from wheat flour extracts than from extracts of the corresponding bread crumb. This is consistent with the heat lability of the high-RM gliadin and heat stability of the low-RM ω-gliadins. The relative decrease from flour to bread crumb in high-RM gliadins was much greater in the good to intermediate breadmaking flours (C.I. 12995, Shawnee, and KS 644) than in the poor-breadmaking flours (KS 501097, KS 501099, and Ottawa Selection). The difference is particularly conspicuous for the sister pairs. Thus, whereas in Shawnee only weak bands with high RM values were present in the bread crumb, in the Ottawa Selection those bands were quite prominent. In the sister lines KS 501097 and 501099, there were prominent and strong high-RM bands in gliadins extracted from the flours and from the bread crumbs. The results point to higher reactivity (binding interaction, denaturation, or polymerization) of α-, β-, and γ-gliadins in good than in poor breadmaking flours.

As with PAGE bands (Fig. 1), HPLC elution patterns (Fig. 2) must be considered with regard to differences among all varieties (selections), differences between pairs of sister lines, differences among peaks of highly hydrophobic proteins (elution times above 23 min) and less hydrophobic proteins (elution times below 23 min), and changes from flour to bread crumb.

There were large differences in HPLC elution patterns among the gliadins of the six flours (Fig. 2, patterns $A_1$, $B_1$, $C_1$, $D_1$, $E_1$, and $F_1$). Those differences could provide a reliable basis for varietal differentiation and identification. The sister lines of different breadmaking potential (Shawnee, pattern $B_1$, and Ottawa Selection, pattern $F_1$) differed much more in the elution patterns than the sister lines of comparable breadmaking potential (KS 501097, pattern $D_1$, and KS 501099, pattern $E_1$). During the transition from flour to bread, there was a decrease in the size of the elution peaks. That decrease was larger for the highly hydrophobic proteins (elution times of 23 min and larger) than for the less hydrophobic proteins (elution times below 23 min; especially around 15 min). Comparison of changes from flour to bread crumb for the sister lines shows a decrease in peak heights from flour to bread crumb in highly hydrophobic proteins in the poor breadmaking lines, KS 501097 (D) and KS 501099 (E); still, some bands with elution times above 23 min are present in extracts of bread crumbs $D_2$ and $E_2$. For Shawnee and Ottawa Selection, we found a larger decrease in the 23-min peak and in the peaks with elution times above 23 min in $B_1$–$B_2$ than in $F_1$–$F_2$.

In summary, relatively fewer PAGE bands of high RM were present, and fewer highly hydrophobic proteins were eluted and fractionated by HPLC from bread crumb of good than of poor flours, indicating differences in interaction or heat lability. Consequently, it is postulated that heat-labile α-, β-, and γ-gliadins and highly hydrophobic gliadins are modified during baking and that the modification may be related, in part at least, to differences in breadmaking potential of wheat flours.


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