Characterization of Acetic Acid Soluble and Insoluble Fractions of Glutenin of Bread Wheat[1]

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ABSTRACT

Cereal Chem. 73(1):131–135

The breadmaking potential of wheat flour depends strongly on its protein content and quality. Research has shown that for bread wheat varieties of diverse breadmaking potential, loaf volume is inversely related to the amount of acetic acid soluble (AAS) glutenin fraction and directly related to the amount of acetic acid insoluble (AAI) glutenin fraction. The objective of the present research was to identify the molecular properties of glutenin controlling its solubility in acetic acid, which in turn might explain the significant correlations with loaf volume. Two wheat cultivars of diverse breadmaking quality (Glenlea and Katepwa) contained different proportions of AAS and AAI fractions. The AAS fractions contained similar amounts of glutenin but that of Katepwa contained significantly more gliadin. Both fractions of Glenlea contained significantly more high molecular weight glutenin subunit (HMW-GS) 7. Quantitative differences in individual HMW-GS and the ratio of HMW-GS to (low molecular weight) LMW-GS could not explain differences in solubility of glutenin. The higher content of the AAI fraction in the stronger cultivar Glenlea appears to be related to its higher content of HMW-GS 7 and weaker interaction with gliadin. Interaction of glutenin with gliadin appears to be related to the solubility of glutenin in acetic acid solution; the greater the interaction the higher the solubility and vice versa. The same phenomenon may be involved in dough development during mixing.

It has long been known that protein content and quality are important factors in the breadmaking potential of bread wheat cultivars (Finney and Barmore 1948). Protein quality is a poorly defined term and is presumed to depend on a complex interrelationship of physicochemical properties of many different protein molecules. The two major proteins of wheat flour are the gliadins and the glutenins. Gliadins are single polypeptide chains that are soluble under nonreducing conditions. Glutenin molecules comprise many polypeptides (subunits) crosslinked by disulfide bonds. The importance of glutenin as a protein quality factor in breadmaking potential was demonstrated by Orth and Bushuk (1972). Based on the solubility distribution of the proteins of 26 bread wheats varying in breadmaking quality, they showed that loaf volume was positively correlated (+0.85**) with residue protein or acetic acid insoluble (AAI) glutenin and negatively correlated (–0.86**) with acetic acid soluble (AAS) glutenin. The variation in solubility of glutenin was attributed to variation in molecular weight. The results obtained by Orth and Bushuk (1972) were in agreement with those of Pomeranz (1965), who reported earlier that flours of poor quality had a greater proportion of protein dispersible in 3*M* urea. Since then, much research has focused on the possible relationship between the structure of glutenin and its functionality in breadmaking. Later studies provided further evidence for a solubility-quality relationship (Field et al 1983, Moonen et al 1982, MacRitchie 1987, Marchylo et al 1990, Gao and Bushuk 1992) (for current review, see Schofield 1994). The proportions of glutenin that were unextractable in AUC (Huebner and Wall 1976), 2*M* urea (MacRitchie 1973), 0.1*M* acetic acid (MacRitchie 1978) and 0.5% sodium dodecyl sulfate (SDS) (Gupta et al 1993) were reported to correlate with breadmaking quality. Based on three fractionation procedures and reconstitution studies, Chakraborty and Khan (1988b) found that, regardless of the fractionation procedure, fractions containing larger amounts of glutenin gave the highest positive responses to loaf volume.

The presence or absence of specific high molecular weight glutenin subunits (HMW-GS) has been correlated with breadmaking quality (Orth and Bushuk 1973, Payne et al 1979, Payne et al 1981, Ng and Bushuk 1988). In addition, additive effects (Moonen et al 1983) and interactions (Kolster and Vereijken 1993) have been reported for the genes controlling the HMW-GS. Subsequent research has shown that the proportion of the intervarietal variation in breadmaking potential that can be attributed to differences in the composition of the HMW-GS fluctuates widely between 15 and 60% (for review, see Kolster 1992). In addition to HMW-GS composition, the ratio of HMW-GS to LMW-GS appears to be an important factor in breadmaking quality as reflected by dough strength. Wheat cultivars with greater dough strength were reported to have a higher HMW-GS to LMW-GS ratio (Gupta and MacRitchie 1991, Gupta et al 1992, MacRitchie and Gupta 1993). Graveland et al (1982, 1985) provided further support for the importance of subunit composition and the ratio of HMW-GS to LMW-GS in solubility of glutenin and breadmaking quality.

In addition to HMW-GS quality and composition, the quantity of glutenin or HMW-GS (MacRitchie 1987, Ng et al 1989, Hamer et al 1992, Kolster and Vereijken 1993) have also been reported as an important factor in breadmaking potential and dough properties. Recent studies lend further support for the importance of quantity as well as quality of HMW-GS (Gupta and MacRitchie 1994, Gupta et al 1994, Khatkar et al 1995).

The correlations between glutenin fractions and loaf volume reported by Orth and Bushuk (1972) has been supported by many studies over the last two decades. Although the AAS and AAI glutenins correlate with loaf volume, the structural differences between the two fractions have not been determined. Thus, the objective of this study was to determine which factors related to breadmaking quality could explain the intervarietal variation in solubility of glutenins in 0.05*M* acetic acid solution.

MATERIALS AND METHODS

Materials

Katepwa, a Canada Western Red Spring (CWRS) wheat of good breadmaking quality, and Glenlea, a Canada Western Extra Strong (CWES) wheat, were selected for this study because of their widely different dough strengths. Mixograph dough devel-

[1]Publication 256 of Department of Food Science, University of Manitoba, Winnipeg, MB. Presented in part at the 9th World Congress of Food Science and Technology, Budapest, Hungary, August 1995.

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Publication no. C-1996-0115-01R.

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opment time was more than two times longer for the CWES cultivar Glenlea (5.1 min) than for the CWRS cultivar Katepwa (2.4 min). Wheat samples were milled into straight-grade flour on a Bühler pneumatic laboratory mill (Bühler Bros., Inc., Uzwil, Switzerland). Protein contents (N × 5.7, 14% mb) were 13.6 and 13.3% for Glenlea and Katepwa, respectively. Chemical and technological tests were performed according to approved methods (AACC 1983). All chemicals were of analytical reagent grade or better.

Preparation of AAS and AAI Fractions of Glutenin

Flour proteins were fractionated into salt soluble (0.5*M* sodium chloride), alcohol soluble (70% ethanol), acetic acid (0.05*M*) soluble and insoluble fractions according to the modified Osborne fractionation method of Chen and Bushuk (1970) with some modifications. The salt soluble fraction was not dialyzed to separate the albumins from the globulins. Fractionation was performed at room temperature (21–23°C) instead of 4°C. AAS and AAI fractions were freeze-dried and stored at 4°C.

Purification of the AAS Fractions of Glutenin

Purification of the AAS fraction was required to remove most of the contaminating gliadin. A modification of the method developed by Fu and Sapirstein (1996), which isolates monomeric proteins from polymeric proteins by 50% 1-propanol extraction of flour followed by 70% 1-propanol precipitation, was used for this purpose. The AAS fraction (80 mg of protein) was dissolved in 50% 1-propanol (25 ml). The solution was neutralized with 0.1*N* NaOH and brought to a final concentration of 70% with a sufficient volume of 1-propanol. The solution was centrifuged at room temperature for 10 min at 20,000 *g*. The resulting residue consisted of glutenin and some co-precipitated ω-gliadins, and the supernatant contained most of the remaining gliadins (α-, β-, γ-, ω-). The purified AAS glutenin (70% 1-propanol-insoluble) was freeze-dried and used for subsequent analyses by electrophoresis and reversed-phase high-performance liquid chromatography (RP-HPLC).

The ω-gliadins migrate to a position intermediate between the HMW- and LMW-GS during electrophoresis and elute well before the HMW- and LMW-GS during RP-HPLC separation. Hence, the purified AAS fractions studied by densitometry and RP-HPLC are considered gliadin-free because the quantitation of glutenin is not confounded by their presence. Thus, the isolation method developed by Fu and Sapirstein (1996) can be successfully applied to the AAS protein fraction as well as flour.

Electrophoresis

Polyacrylamide gel electrophoresis under acidic pH (A-PAGE) was performed at constant current (50 mA) for 4 hr (Sapirstein and Bushuk 1985). Total acrylamide concentration was 6%. Gel temperature was maintained at 20°C by circulating water through the apparatus. PAGE in the presence of sodium dodecyl sulfate (SDS-PAGE) with and without 2-mercaptoethanol (reducing and nonreducing conditions) was performed in a cooled vertical slab gel unit (Hoefer Scientific Instruments, San Fernando, CA). A 3.0% stacking gel and 17.3% separating gel were used at 10 mA per gel for 20 hr (Ng et al 1988). The wheat cultivar Neepawa, official grading standard of the CWRS class (Ng et al 1988), was run with A-PAGE and SDS-PAGE gels as a reference standard.

Densitometry

Three replicate SDS-PAGE gels of the purified gliadin-free AAS fractions and the AAI fractions of Glenlea and Katepwa were scanned at 600 nm with a BioRad Video Densitometer (model 620; HP 3396A integrator). Integrated areas obtained for individual HMW-GS peaks and the LMW-GS region were used for calculations. Integrated areas for ω-gliadin peaks were excluded from glutenin quantitation, but they were used to determine the relative amount of ω-gliadins co-precipitated in the purified AAS fractions.

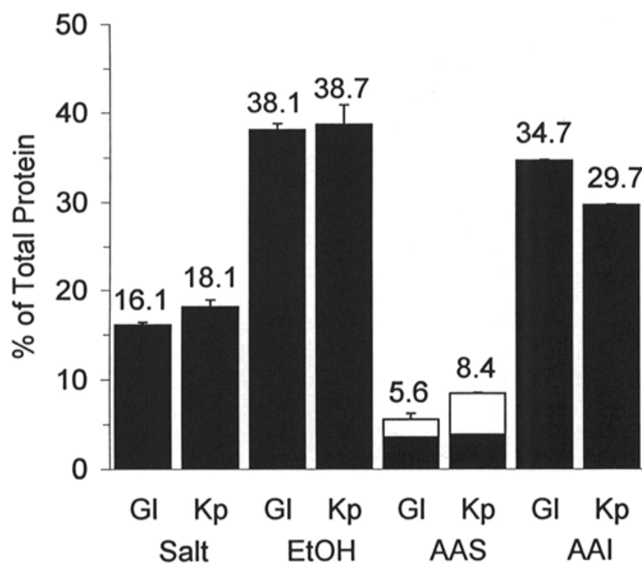

Fig. 1. Comparison of % total protein for modified Osborne fractions of Glenlea (Gl) and Katepwa (Kp) wheat cultivars. The acetic acid soluble (AAS) fractions show the amount of gliadin (❑) and glutenin (■). Standard deviations (±) are indicated by bars; values for each faction, except the EtOH fractions, are significantly different (α = 0.05).

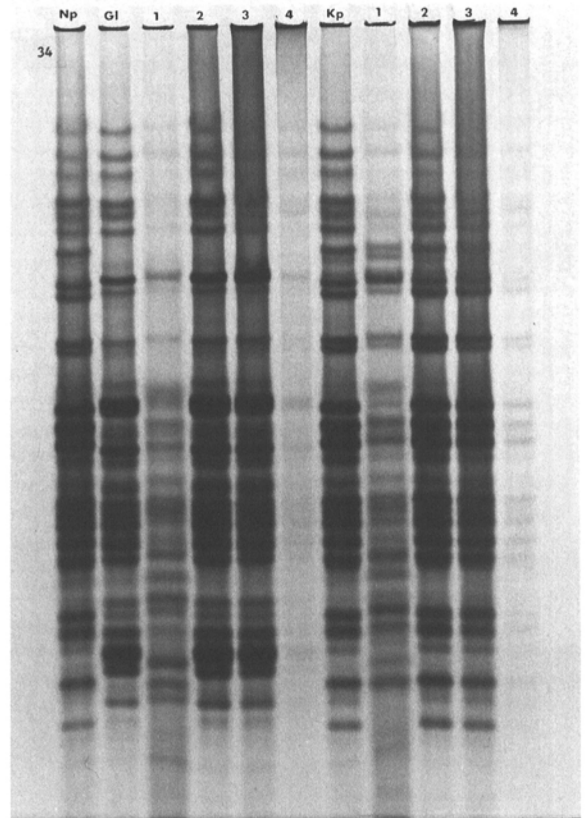

Fig. 2. Electrophoregram from polyacrylamide gel electrophoresis under acidic pH (A-PAGE) of room temperature modified Osborne fractions of Glenlea (Gl) and Katepwa (Kp). Lanes 1–4: Salt-soluble, ethanol-soluble, crude acetic acid soluble, acetic acid insoluble, respectively. Wheat cultivar Neepawa (Np) was run as a reference standard.

RP-HPLC

Samples were prepared for RP-HPLC as described by Fu and Sapirstein (1996). Briefly, flour (50 mg) and AAI fractions (5 mg of protein) were extracted twice with 50% 1-propanol (1 ml) and the pellets were washed with 50% 1-propanol. The pellets thus obtained and the purified AAS fractions (5 mg of protein) were reduced with a Tris-HCl buffer solution (0.08*M*; 100–200 μl; pH 7.5) containing 50% 1-propanol and 1% dithiothreitol for 1 hr at 60°C. The reduced protein was then alkylated with an equivalent volume of the Tris-HCl buffer solution containing 50% 1-propanol and 14% 4-vinylpyridine for 15 min at 60°C. The supernatant obtained by centrifugation was syringe-filtered (0.45 μm) into a HPLC microvial and sealed.

Samples (5 μl) were immediately analyzed by RP-HPLC as described by Fu and Sapirstein (1996). Chromatograms were recorded from the signal detected at 214 nm. Integrated areas obtained for individual HMW-GS peaks and the LMW-GS region were used for calculations. Integrated areas for ω-gliadin peaks were excluded from glutenin quantitation, but they were used to determine the relative amount of ω-gliadins co-precipitated in the purified AAS fractions.

Statistical Analyses

All statistical analyses were executed using SAS 6.0 software (SAS Institute 1990; on a HP 9000/380 microcomputer). Analysis of variance (ANOVA) and Duncan's multiple range test were performed to determine significant differences between cultivars and fractions. All experiments were performed at least in duplicate. Replicated results are reported as means.

RESULTS AND DISCUSSION

All fractions obtained by the modified Osborne fractionation, except the ethanol-soluble, were significantly different between cultivars (Fig. 1). Consistent with the earlier results of Orth and Bushuk (1972) and more recent results of Chakraborty and Khan (1988a), the present study showed that the weaker flour (Katepwa) contained more AAS protein than did the stronger flour (Glenlea): 8.4 and 5.6%, respectively. Also, as reported earlier (Orth and Bushuk 1972), Katepwa contained less AAI protein than did Glenlea: 29.7 and 34.7%, respectively. Earlier studies interpreted these results as reflecting a substantive difference in the solubility of glutenin as it was assumed that the preceding extraction of the flour with 70% ethanol solution had removed all of the gliadin, and that the residue, which was further fractionated by extraction with acetic acid solution, contained mostly glutenin.

Analysis of the crude AAS protein by A-PAGE showed that this fraction contained substantial quantities of gliadin along with glutenin (Fig. 2, lane 3). Purification of this crude fraction resulted in AAS glutenin essentially free of α-, β-, and γ-gliadins. This can be seen in Figure 3 by comparing the unreduced, crude AAS fractions (electrophoregram A, lanes G3 and K3) to the unreduced, purified AAS fractions (electrophoregram B, lanes G3 and K3). The AAI fractions (Fig. 2, lane 4) contained relatively little gliadin contamination.

The amount of gliadin in the AAS fraction appears to be genotype dependent. The actual values obtained were 2.04 ± 0.24% and 4.62 ± 0.20% (total flour protein basis) for Glenlea and Katepwa, respectively (Fig. 1). The difference in the glutenin content of the two AAS fractions was relatively small, but the fraction of the weaker cultivar Katepwa contained slightly more (3.82 ± 0.25%) glutenin than did the fraction of the stronger cultivar Glenlea (3.52 ± 0.33%). When the amount of glutenin was adjusted for the content of ω-gliadins that co-precipitated with glutenin (Table I), this small difference was practically eliminated. On the basis of the small number of samples (two), the difference in AAS glutenin content between the cultivars was not statistically significant. Examination of a larger number of wheat cultivars of more widely diverse quality than that covered by the two samples used in the present study would be helpful in clarifying what appears to be a discrepancy between the published results and the results obtained in this study.

In the context of the objective of this study, the results showed some interesting, albeit not large, differences between AAS and AAI glutenin. SDS-PAGE patterns (Fig. 3) showed that the two glutenin fractions were the same in terms of presence of bands in the HMW- and LMW-GS regions. Densitometric analysis of the SDS-PAGE patterns showed only minor quantitative differences in the relative amounts of the GS (Table I). The AAS glutenin contained slightly less subunits 5 and 10 than did the AAI glutenin. It should be recalled that these two subunits are controlled by genes on the 1D chromosome and, according to several authors (Orth and Bushuk 1973, Payne et al 1981, Ng and

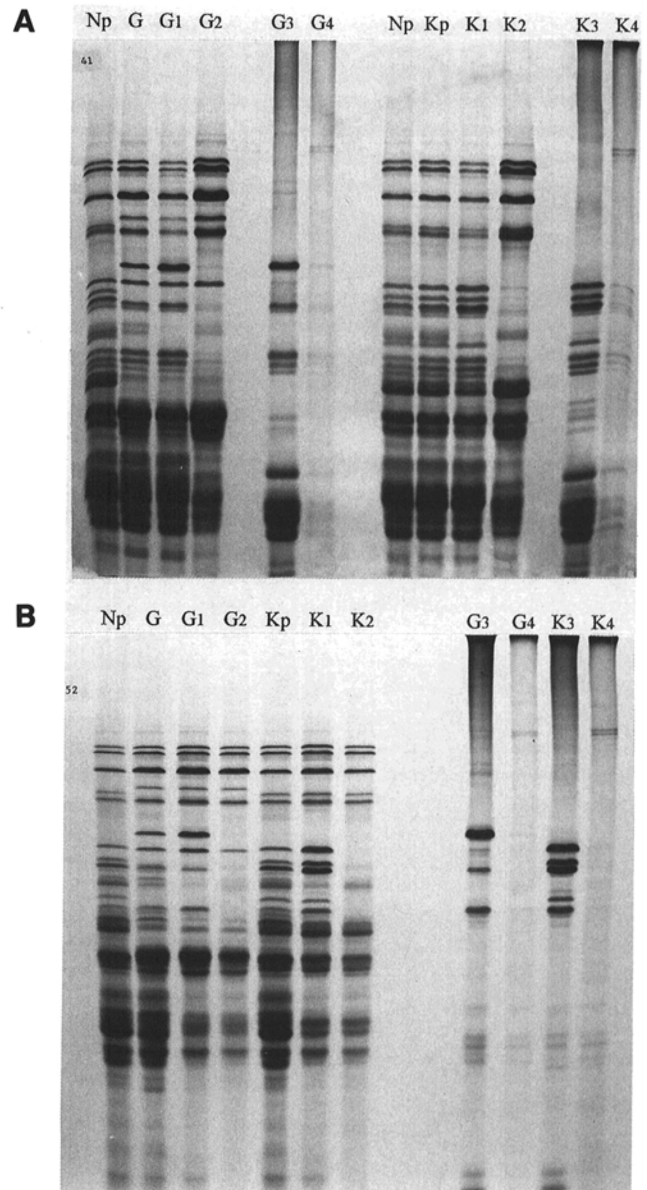

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electrophoregrams of reduced and unreduced acetic acid soluble (AAS) and acetic acid insoluble (AAI) fractions of Glenlea (G) and Katepwa (Kp). Wheat cultivar Neepawa (Np) was run as a reference standard. Reduced and unreduced AAS fractions (G1, K1 and G3, K3, respectively); reduced and unreduced AAI fractions (G2, K2 and G4, K4, respectively). **A,** unpurified AAS fraction (G1, K1) contaminated with gliadins (G3, K3). **B,** purified AAS fractions (G1, K1)containing negligible gliadins, except for ω-gliadins (G3, K3).

Bushuk 1988), they are especially important to breadmaking quality of common wheat. Also, the ratio of HMW-GS to LMW-GS calculated from the densitometric data, though not significant, was higher for the AAI fraction than for the AAS fraction for both cultivars. This data is generally consistent with solubility as the glutenin molecules of higher molecular mass would be less soluble.

HPLC comparison of AAS and AAI glutenin showed similar trends to those obtained by SDS-PAGE and densitometry but there were some substantive differences. HPLC results showed that the AAS glutenin of both cultivars contained significantly more of subunit 2* than did the AAI glutenin (Table I); the same trend was apparent in the densitometric analysis but was not statistically significant. Differences in the amounts of other HMW-GS were observed for each cultivar but these were not consistent when the results for the two cultivars were compared. Also, the HPLC ratio of HMW-GS to LMW-GS was very similar for the AAS and AAI fractions of Glenlea and the AAS fraction of Katepwa; the ratio was lower for the AAI fraction for Katepwa. In general, the two methods provide similar results. Differences may be attributable to the different detection methods used, peptide bonds being detected in RP-HPLC and dye-binding in densitometry. The reason for the discrepancy between the HPLC and SDS-PAGE and densitometry results warrants further investigation.

CONCLUSION

In conclusion, this study has shown that the AAS and AAI glutenins are quite similar in subunit composition and content. Small differences were observed, which appear to be consistent with the solubility of the two fractions of glutenin but may not be sufficient to account for the observed difference in solubility in acetic acid solution. It appears, therefore, that the previously-reported (Orth and Bushuk 1972, Gupta and MacRitchie 1991) explanation of the variability in glutenin solubility based on molecular weight is plausible. However, proof of this hypothesis must await the development of a method for accurate determination of the molecular weight of polymeric glutenin molecules. Other properties of glutenin, such as concentration of intermolecular disulfide crosslinks, secondary and tertiary structure, etc., may contribute to its solubility, but as yet there is no clear evidence to support this speculation.

A possible molecular basis of the large difference in dough mixing properties of the two cultivars used in this study deserves further comment. The two cultivars have the same 1D HMW-GS, 5 + 10, which are considered more important to breadmaking quality than the other HMW-GS. Also, both have the 1A HMW-GS, 2*. They differ in the 1B HMW-GS; Glenlea has 7 + 8, whereas Katepwa has 7* + 9. Subunits 7 and 7* are the same by HPLC, but differ slightly by SDS-PAGE (Marchylo et al 1992). Glenlea contains relatively more of the subunit 7 than Katepwa. Subunit 8 is similar to subunit 9 but is slightly higher in molecular mass. We can conclude that the large difference in dough properties cannot be explained by the small difference in HMW-GS composition. The influence of differences in LMW-GS composition remain to be discovered by future research.

The observation that a substantial quantity of gliadin is not extracted by the 70% ethanol solution used in the modified Osborne solubility fractionation (Chen and Bushuk 1970) has not been reported previously. Essentially all of the alcohol-insoluble gliadin was extracted by the 0.05*N* acetic acid solution used in the next step of fractionation. The alcohol insolubility of some of the gliadin is attributed to its strong aggregation with glutenin. Furthermore, it appears that the proportion of gliadin that is not extracted depends on the genotype. In the present study, the AAS fraction of Katepwa, the weaker cultivar, contained substantially more gliadin than did the same fraction of Glenlea, the extra strong cultivar.

The genotype-specific aggregation of gliadin and glutenin may have implications in the functional properties of wheat flour doughs during mixing. It is generally believed that during dough mixing, glutenin polymers are hydrated, unfolded, and stretched; together with the gliadins, they form a continuous viscoelastic gluten network. The time required for this process (i.e., the dough development time in the mixograph or the farinograph) would depend on the degree of intrinsic interaction between the glutenins and gliadins in wheat flour. Cultivars that have more aggregated gliadin-glutenin in the flour (i.e., a higher gliadin content in the AAS fraction) would require less mixing for optimum development, and vice versa. This explanation is plausible for the results obtained in the present study for two diverse wheat cultivars. The conclusions presented here are consistent with two other recent studies, the results of which suggest that gliadin-glutenin interaction is a factor in the solubility behavior of the protein fractions during mixing (Hay et al, *in press*) and in the dynamic rheological properties of glutens (Khatkar et al 1995). This hypothesis remains to be confirmed by experiments on a larger number of wheat cultivars covering a wider range of dough mixing properties.

TABLE I
Relative Amounts (%) of High Molecular Weight Glutenin Subunits (HMW-GS) and Low Molecular Weight Glutenin Subunits (LMW-GS) and Ratios of HMW-GS to LMW-GS for Acetic Acid Soluble (AAS) and Acetic Acid Insoluble (AAI) Fractions of Glenlea and Katepwa Obtained by Densitometry and High-Performance Liquid Chromatography (HPLC)[a,b]

Subunit or Region	Densitometry				Reversed-Phase HPLC			
	Glenlea		Katepwa		Glenlea		Katepwa	
	AAS	AAI	AAS	AAI	AAS	AAI	AAS	AAI
HMW-GS 2*	15.8 ± 0.6 a	14.6 ± 1.5 a	16.7 ± 1.0 a	14.9 ± 4.4 a	12.4 ± 0.1 a	11.7 ± 0.3 b	14.8 ± 0.0 c	13.1 ± 0.3 d
HMW-GS 5	19.9 ± 1.2 a	22.1 ± 1.2 a	19.2 ± 1.0 a	22.4 ± 6.8 a	18.6 ± 0.1 a	21.8 ± 0.1 b	26.2 ± 1.2 c	24.6 ± 0.1 c
HMW-GS 7/7*	37.2 ± 1.5 a	35.0 ± 1.2 ab	29.6 ± 0.7 bc	24.5 ± 6.5 c	43.0 ± 1.1 a	40.4 ± 0.1 b	29.6 ± 0.7 c	29.8 ± 0.4 c
HMW-GS 8/9	9.4 ± 1.4 a	7.9 ± 0.6 a	9.2 ± 1.2 a	8.2 ± 2.4 a	12.6 ± 1.9	11.6 ± 0.2	14.1 ± 0.5	14.4 ± 0.1
HMW-GS 10	17.7 ± 2.3 a	20.4 ± 4.0 a	25.4 ± 2.1 a	29.9 ± 20.2 a	13.4 ± 0.6 a	14.6 ± 0.4 ab	15.6 ± 0.8 b	18.1 ± 0.9 c
Total HMW-GS	35.2 ± 6.0 a	40.6 ± 7.0 a	28.4 ± 5.5 a	39.0 ± 15.3 a	34.8 ± 0.2 a	34.5 ± 0.1 a	35.4 ± 0.8 a	30.2 ± 0.3 b
LMW-GS region	61.2 ± 6.4 a	56.6 ± 7.6 a	68.1 ± 6.4 a	56.9 ± 14.6 a	65.2 ± 0.2 a	65.5 ± 0.1 a	64.6 ± 0.8 a	69.8 ± 0.3 b
Ratio of HMW to LMW-GS	0.59 ± 0.15 a	0.74 ± 0.21 a	0.42 ± 0.11 a	0.78 ± 0.53 a	0.54 ± 0.0 a	0.52 ± 0.0 a	0.54 ± 0.0 a	0.44 ± 0.0 b
ω-Gliadin (%) in AAS[c]	17.9 ± 2.1	ND[d]	23.7 ± 0.9	ND	13.9 ± 1.0	ND	20.8 ± 1.5	ND

[a] Means of duplicates (for HPLC) and triplicates (for densitometry) ± standard deviation.
[b] Values with the same letter are not significantly different ($\alpha = 0.05$).
[c] Excluded from calculations of relative amounts of AAS glutenin.
[d] Not determined.

ACKNOWLEDGMENTS

B. Dupuis gratefully acknowledges the financial assistance from the Natural Sciences and Engineering Research Council of Canada in the form of a 1967 Science and Engineering Scholarship and from the American Association of Cereal Chemists in the form of a Graduate Fellowship sponsored by A. E. Staley Mfg. Company. Other financial assistance was provided by NSERC and the United Grain Growers.

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[Received April 4, 1995. Accepted October 23, 1995.]