

Intercultivar Variation in the Quantity of Monomeric Proteins, Soluble and Insoluble Glutenin, and Residue Protein in Wheat Flour and Relationships to Breadmaking Quality

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ABSTRACT

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A new fractionation procedure based on differential solubility was applied to wheat flour proteins to evaluate the relationship between protein fractions and functionality for breadmaking. Flour was initially extracted with 50% 1-propanol. Monomeric proteins (mainly gliadins) and soluble glutenin contained in the 50% propanol soluble extract were fractionated by selective precipitation of the glutenin by increasing the concentration of 1-propanol to 70%; monomeric proteins remain in the supernatant. Insoluble glutenin in the 50% propanol insoluble residue was extracted using 50% 1-propanol containing 1% dithiothreitol (DTT) at 60°C. Protein in the final residue was extracted using SDS with or without DTT. It comprised mainly *Glu-1D* high molecular weight glutenin subunits and nongluten polypeptides. For seven Canadian cultivars of diverse breadmaking quality, there was relatively little variation in the percentage of flour protein corresponding to monomeric proteins (48–52%) and residue protein (14–18%).

In contrast, intercultivar variation in soluble and insoluble glutenin was substantial, with contents of 10–20% and 12–28% of flour protein, respectively. Soluble and insoluble glutenin were also highly correlated with physical dough properties, accounting for 83–95% of the variation of individual dough rheological parameters (except dough extensibility), and ≈74% of the variation in loaf volume. In contrast, monomeric and residue protein fractions were poorly associated with breadmaking quality. However, among the four protein fractions, only residue protein was significantly correlated ($r = -0.79$) with dough extensibility. The flour sample with the highest and lowest concentrations of insoluble and soluble glutenin, respectively, as well as marginally the lowest concentrations of monomeric and residue proteins was Glenlea, a cultivar of the Canada Western Extra Strong Red Spring wheat class which characteristically possesses distinctly strong dough mixing properties.

The uniqueness of wheat flour doughs for breadmaking derives from a balance of two protein-related rheological properties, viscosity and elasticity (Bushuk 1985). Viscosity is generally associated with the monomeric proteins of wheat endosperm, mainly gliadins, which are single-chain polypeptides. On the other hand, the elasticity of dough derives from the glutenin component, which comprises polydisperse polymers of disulfide-bonded polypeptides. This fundamental distinction of structure and functionality of two very different types of proteins is not always reflected in chemical fractionation work where the overlapping solubilities of gliadin and glutenin proteins is known to be a longstanding problem in cereal chemistry research since the original proposals of Osborne (1907, 1924).

Numerous protein solubility schemes have been reported for gliadin and glutenin fractionation (Mifflin et al 1983), and many of these commence with Osborne's traditional approach of either water or salt solutions as the initial solvents for albumin and globulin proteins. Recent work (Dupuis et al 1996, Fu and Sapirstein 1996) indicates that the initial hydration of flour with water-salt solutions is the principal factor contributing to the subsequent difficulties in the fractionation of gliadin and glutenin proteins. Under these preparative conditions, the glutenin fraction is substantially contaminated with gliadins, as has been previously observed (Orth and Bushuk 1973, Bietz and Wall 1975) but not quantified.

In the absence of initial extraction of flour with water-salt solutions, glutenin free of monomeric proteins can be prepared from the residue after direct extraction of flour with dimethyl sulfoxide (Burnouf and Bietz 1989, Gupta and MacRitchie 1991) or with 50% 1-propanol (Singh et al 1991, Fu and Sapirstein 1996). However, under these conditions, a significant amount of glutenin is also extracted along with the monomeric proteins (Fu and Sapirstein 1996). Soluble glutenin is presumed to com-

prise polymers of smaller size than those of insoluble glutenin (MacRitchie 1992).

Apart from a limited number of studies on Osborne-type ethanol-soluble glutenin (Bietz and Wall 1973, 1980; Huebner and Bietz 1993), there is little information on the nature, amount, intergenotype variability, and quality relations of soluble glutenin directly extractable from flour with nonreducing solvents such as aqueous alcohol or acetic acid. On the other hand, good evidence exists that the amount of unextractable polymeric protein following direct extraction of flour with 50% 1-propanol (Sapirstein and Johnson 1996, Bean et al 1998) or 0.05M acetic acid (Orth and O'Brien 1976) is closely related to dough strength.

An alternative protein fractionation strategy to study intercultivar variability of soluble or insoluble glutenin, plus other endosperm proteins, employs the use of size-exclusion high-performance liquid chromatography (SE-HPLC) of SDS-extractable protein from endosperm either without or with sonication. Quantitation of excluded protein in SE-HPLC separations of direct SDS extracts of flour and sonicated residues provides a measure of relatively small (soluble, extractable) and large (insoluble, extractable by sonication) polymeric protein, respectively. This approach has been successfully used to obtain strong relationships between extractable or unextractable glutenin and breadmaking quality (Dachkevitch and Autran 1989, Singh et al 1990a, Gupta et al 1993, Gupta and MacRitchie 1994). However, it is worth noting that sonication of total or SDS-unextractable flour protein followed by SE-HPLC would confound glutenin and other quantitatively minor polymeric proteins in endosperm, such as triticins and high molecular weight (HMW) albumins, as all these different proteins would elute together in the excluded protein fraction (Singh et al 1990b).

Recently, we developed a new flour protein fractionation procedure (Fu and Sapirstein 1996) largely based on the differential solubility of wheat endosperm proteins in different aqueous solutions of 1-propanol. The procedure yields four protein fractions, one monomeric in nature and three polymeric fractions corresponding to soluble glutenin, insoluble glutenin, and residue protein. The objective of this study was to apply this fractionation procedure to determine the extent of the intercultivar variation in these protein fractions and their relationships to breadmaking quality.

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MATERIALS AND METHODS

Wheat Cultivars and Quality Measurements

Samples of seven Canadian wheat cultivars were used representing a diverse set of breadmaking quality types belonging to six commercial wheat classes (Table I). Glenlea, Roblin, Katepwa, Biggar, and AC Karma were milled to straight-grade flour on a Buhler pneumatic laboratory mill. SWS-52 and Harus were similarly milled on an Allis Chalmers laboratory mill. Dough mixing properties were determined using a 2-g computerized mixograph (National Manufacturing, Lincoln, NE). Mixograph properties for all samples, except SWS-52 and Harus, were determined at 62% absorption. For SWS-52 and Harus, 55% absorption was used. Extensigraph data were obtained according to Approved Method 54-10 (AACC 1995). The remix-to-peak bake test (Kilborn and Tipples 1981) was used to determine loaf volume under optimized conditions of water absorption and dough development.

Protein Fractionation

The overall fractionation scheme (Fig. 1) is similar to that previously described (Fu and Sapirstein 1996) and contains a number of modifications to account for the reduction in the scale of the procedure used in this study. Flour (100 mg) was extracted twice with 1 mL of 50% (v/v) 1-propanol for 30 min at 23°C in a micro-centrifuge tube with intermittent vortexing, and centrifuged for 3 min at 2,200 × g. The pellet was washed with the same solution for 1 min and centrifuged for 3 min at 15,000 × g. The supernatants were combined. This pooled fraction is referred to as 50PS protein, which comprises monomeric proteins and soluble glutenin. The resulting 50% 1-propanol insoluble residue containing insoluble glutenin and some nonprolamins is referred to as the 50PI fraction. This fraction was further extracted at 60°C, three times with 1.0 mL of 50% 1-propanol containing 1% (w/v) DTT for 1.0, 0.5, and 0.5 hr, respectively. After each extraction, centrifugation was conducted for 5 min at 15,000 × g at room temperature. The pooled supernatants comprised the insoluble glutenin fraction. Protein in the residue was referred to as residue protein.

The 50PS protein, containing monomeric proteins and soluble glutenin, was fractionated using a macroscale procedure as previously described (Fu and Sapirstein 1996). Briefly, soluble glutenin was isolated by selective precipitation of the 50PS fraction with 70% (v/v) 1-propanol (70PS). The resulting 70% 1-propanol-insoluble (70PI) precipitate, which contains some ω-gliadins and polymeric glutenin, was reduced and alkylated for reversed-phase (RP) HPLC analysis, which facilitated separation and quantification. The sum of 70PS protein plus ω-gliadins in the 70PI fraction constituted monomeric proteins. 70PI protein less the constituted soluble glutenin.

The nature of the insolubility of residue protein was also investigated. Freshly prepared residue fraction was mixed with 0.2 mL of 5% (w/v) N-ethylmaleimide (NEMI) in 50% 1-propanol at 60°C for 1 hr. The mixture was subsequently dried. Protein in the

dried residue was extracted with 2% SDS solution without reductant and the extract was analyzed by SDS-PAGE. To determine whether or not NEMI could efficiently inactivate DTT, 0.5 mL of 50% 1-propanol containing 1% DTT was mixed with 0.5 mL of 50% 1-propanol and 5% NEMI. The mixture was incubated for 1 hr at 60°C. Subsequently, 50 mg of flour was extracted with 0.2 mL of this solution, and the extract was examined by SDS-PAGE to determine the presence or absence of HMW glutenin subunits (GS).

The protein (N × 5.7) contents of the various fractions were determined by the Kjeldahl procedure (Method 46-11A, AACC 1995). Three independent replicates of the protein fractionation procedure were performed and mean results are reported.

Analytical

SDS-PAGE was conducted according to Ng and Bushuk (1987) except that the stacking and separating gel concentrations were altered to 3.5 and 14.0%, respectively, and separation was for 4 hr at 25 mA. Freeze-dried protein fractions were dissolved in buffer and prepared for SDS-PAGE as previously described (Fu and Sapirstein 1996). RP-HPLC analysis for determining the proportion of ω-gliadins in 70PI glutenin was conducted as previously described (Fu and Sapirstein 1996).

Statistical Analysis

The protein fractionation data were statistically evaluated by the one-way analysis of variance (ANOVA) procedure using SAS/STAT System software ver. 6.12 (SAS Institute, Cary, NC). The Duncan's multiple range test was applied to compare the mean values. Correlation analysis was performed using the same statistical software program.

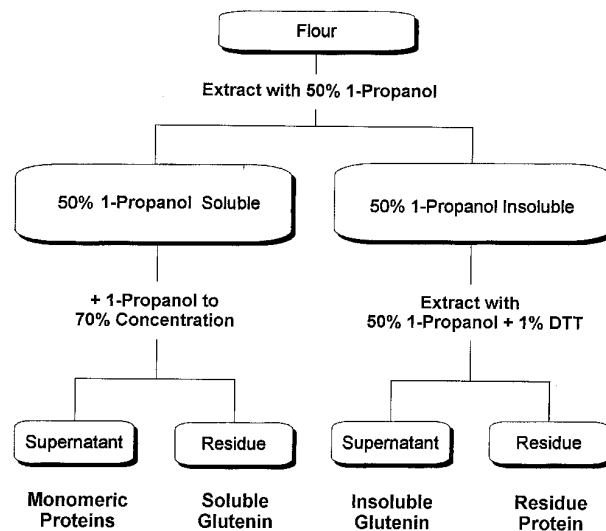


Fig. 1. Protein fractionation scheme.

TABLE I
Quality Characteristics^a of Flour Samples

Cultivar ^b	Wheat Class ^c	Flour Protein (%)	Mixograph		Extensigraph			Loaf Volume (mL)
			DDT (min)	WIP (%)	R _{max} (BU)	Ext (cm)	Area (cm ²)	
Glenlea	CWESRS	13.7 ± 0.1	5.3 ± 0.1	211 ± 13	1,000 ± 50	19.0 ± 0.2	296 ± 12	828 ± 25
Biggar	CPSR	12.5 ± 0.1	3.7 ± 0.0	141 ± 3	720 ± 7	22.5 ± 0.4	234 ± 6	970 ± 7
Roblin	CWRS	13.6 ± 0.0	3.4 ± 0.1	141 ± 4	560 ± 14	23.0 ± 0.7	196 ± 7	1010 ± 35
Katepwa	CWRS	13.3 ± 0.1	3.5 ± 0.1	123 ± 2	590 ± 14	16.7 ± 1.1	146 ± 3	913 ± 18
AC Karma	CPSW	10.9 ± 0.2	2.6 ± 0.1	69 ± 2	390 ± 11	18.0 ± 1.1	108 ± 6	603 ± 25
SWS-52	CWSWS	9.5 ± 0.0	1.9 ± 0.0	42 ± 1	245 ± 3	17.5 ± 0.3	61 ± 5	423 ± 4
Harus	CEWW	9.5 ± 0.1	2.0 ± 0.1	30 ± 1	170 ± 2	16.0 ± 0.6	41 ± 2	355 ± 7

^a Time to peak dough development (DDT), work input to peak (WIP), maximum resistance (R_{max}), extensibility (Ext).

^b Ranked according to decreasing dough strength as measured by mixograph dough work input to peak development and extensigraph area.

^c CWESRS = Canada Western Extra Strong Red Spring, CPSR = Canada Prairie Spring Red, CWRS = Canada Western Red Spring, CPSW = Canada Prairie Spring White, CWSWS = Canada Western Soft White Spring, CEWW = Canada Eastern White Winter.

RESULTS AND DISCUSSION

Flour Quality

Several important breadmaking quality properties of the flour samples were evaluated (Table I). These results were consistent with the expected quality characteristics of the parent wheat classes. For example, true to its "extra strong" class designation, Glenlea had exceptionally strong dough characteristics (i.e., mixograph dough development and extensigraph maximum resistance and area). In quantitative terms, it is noteworthy that compared to the Canada Western Red Spring cultivars Roblin and Katepwa, which can be described as strong mixing flours, the mixograph and extensigraph parameters for Glenlea were more than 53 and 73% higher in magnitude, respectively. It seems likely that the distinct dough rheological properties of Glenlea are closely related to its equally distinct endosperm protein quality as primarily reflected in the glutenin fraction.

Flour Protein Solubility

For the seven flours, between 57 and 70% of flour protein was directly extracted with 50% 1-propanol, and 12–28% more protein (insoluble glutenin) was solubilized by exhaustive extraction of the 50PS residue with 50% 1-propanol under reducing conditions (Table II). Despite the exhaustive extraction conditions, a significant proportion of flour protein (14–18%) remained in the final residue. The amounts of 50PS protein obtained in this study is higher when compared to that obtainable by Osborne fractionation, where combined values of water and salt soluble and alcohol soluble protein fractions generally do not exceed 50% of total flour protein (Orth and Bushuk 1972, Tanaka and Bushuk 1972, Dupuis et al 1996). Osborne fractionation is generally conducted in the cold (4°C), which likely limits protein extractability. Kruger et al (1988) applied Osborne fractionation at 60°C for all solvent extracts, and reported 58–64% of total flour protein solubilized in combined sequential extracts of different flours using 0.5M NaCl and 50% 1-propanol.

For insoluble glutenin and residue protein, our results are not comparable to those obtained by Osborne fractionation, which identifies soluble and insoluble glutenin as acetic acid soluble and residue protein, respectively. We obtained significantly higher and lower amounts of insoluble glutenin and residue protein, respectively (compare results in Table II with Fig. 1 of Dupuis et al 1996). Furthermore, a significant proportion (>50% in some cases) of the Osborne acetic acid soluble glutenin fraction, depending on cultivar, is comprised of gliadin proteins (Dupuis and Bushuk 1996, Dupuis et al 1996, Fu and Sapirstein 1996); cultivars with lower dough mixing requirements appear to have higher amounts of gliadin in this Osborne fraction (Dupuis and Bushuk 1996,

Dupuis et al 1996). These studies indicate that the protein content of the Osborne acetic acid soluble fraction does not accurately reflect the amount of soluble glutenin in flour.

Fractionation of 50PS protein into monomeric proteins and soluble glutenin (Table III) showed that the intercultivar variation in the amount of protein in the 50PS fraction was attributable to soluble glutenin (coefficient of variation [CV] = 25.9%), which comprised 10–19% of total flour protein across samples. Expressed as a percentage of total glutenin in flour, soluble glutenin content ranged from 25% (Glenlea) to 59% (Harus) for the strongest and weakest cultivar samples, respectively. By contrast, there was relatively little variation in the quantity of monomeric proteins among the diverse flours that were studied (CV = 2.9%); monomeric protein contents of the flours were 48–52%.

Intercultivar variation in 50PI protein (Table III) was relatively high, and ranged from 30–42% of total flour protein. For each cultivar sample, the quantity of 50PI protein was in close agreement with the sum of insoluble glutenin plus residue protein (Table II) whose amounts were determined directly using a microscale procedure. Thus, a reliable determination of insoluble glutenin content of flour can be made with a relatively simple sequential extraction procedure using 50% 1-propanol without and with reductant.

SDS-PAGE Composition of Flour Protein Fractions

For all cultivars that were studied, reduced 50PS protein (Fig. 2, lanes PS) was qualitatively identical in SDS-PAGE composition to the total protein extract (lane TP). As previously shown (Fu and Sapirstein, 1996), 50% 1-propanol efficiently extracts essentially all monomeric proteins (gliadins, albumins and globulins), plus some glutenin. The relative content of polymeric glutenin in the 50PS and 50PI fractions, as judged by SDS-PAGE band intensities of HMW-GS, appeared to closely correspond to the relative amounts of soluble and insoluble glutenin (Tables II and III) which in turn were closely related to dough strength. For example, for the very strong mixing cultivar Glenlea, the concentration of HMW-GS in the 50PS fraction was substantially less than that in 50PI protein (compare Glenlea lanes PS and PI in Fig. 2). In contrast, for the very weak mixing cultivar Harus, the concentration of HMW-GS bands in 50PS appeared to be marginally greater than in the 50PI protein (compare Harus lanes PS and PI in Fig. 2). Extraction of the 50PS residue with 50% 1-propanol plus DTT yielded a glutenin fraction of very high purity (Fig. 2, lanes PI), indicated by the presence of only HMW and LMW subunits. These results are consistent with those reported previously (Byers et al 1983, Singh et al 1991, Fu and Sapirstein 1996).

SDS-PAGE of residue protein (Fig. 2, lanes FR) revealed the presence of some HMW-GS and other proteins whose identity

TABLE II
Proportion of Flour Protein in Sequential Extracts of Wheat Flour by a Microscale Procedure Using Solutions of Propanol and Propanol Plus Reductant

Cultivar	50% 1-Propanol Soluble Protein	Insoluble Glutenin ^a	Residue Protein	Protein Recovery (%)
Glenlea	57.2 ± 0.8g ^b	28.3 ± 0.4a	14.5 ± 0.2c	97.9 ± 1.3
Biggar	59.8 ± 0.3f	25.5 ± 0.3b	14.7 ± 0.4c	97.4 ± 0.6
Roblin	61.7 ± 0.3e	23.8 ± 0.1c	14.4 ± 0.1c	98.6 ± 0.5
Katepwa	62.9 ± 0.5d	19.9 ± 0.2d	17.2 ± 0.2ab	98.3 ± 0.8
AC Karma	64.8 ± 0.6c	20.5 ± 0.2d	14.7 ± 0.3c	96.8 ± 0.9
SWS-52	68.9 ± 0.4b	14.5 ± 0.6e	16.6 ± 0.8b	96.6 ± 1.1
Harus	69.6 ± 0.3a	12.6 ± 0.4f	17.8 ± 0.5a	97.1 ± 1.0
Mean	63.6	20.7	15.7	
CV (%) ^c	7.2	27.5	9.1	

^a 50% 1-propanol and 1% dithiothreitol soluble protein after extraction of flour with 50% 1-propanol.

^b Means of three replicates ± standard deviation. Means in columns with the same letter are not significantly different ($P < 0.05$) among cultivars.

^c Coefficient of variation.

TABLE III
Proportion of Flour Protein Comprising Monomeric Proteins, Soluble Glutenin, and 50% 1-Propanol Insoluble (50PI) Protein Using a Macroscale Fractionation Procedure

Cultivar	Monomeric Protein	Soluble Glutenin ^a	50PI Protein	Soluble Glutenin (%) ^b
Glenlea	48.3 ± 0.7d ^c	9.6 ± 0.5f	42.1 ± 0.6a	25.3
Biggar	48.5 ± 0.7d	12.4 ± 0.5d	39.1 ± 0.6b	32.7
Roblin	51.5 ± 0.5ab	11.0 ± 0.4e	37.5 ± 0.4c	31.6
Katepwa	51.3 ± 1.1ab	12.6 ± 0.7d	36.1 ± 0.5d	38.8
AC Karma	50.2 ± 0.4bc	15.5 ± 0.8c	34.3 ± 0.3e	43.1
SWS-52	49.8 ± 0.9c	19.4 ± 0.7a	30.8 ± 0.4f	57.2
Harus	51.9 ± 0.6a	17.8 ± 1.0b	30.3 ± 0.3g	58.6
Mean	50.2	14	35.7	41
CV ^d	2.9%	25.9%	12.0%	31.2%

^a Determined by difference: 100 – (monomeric protein + 50 PI protein).

^b Soluble glutenin as a percentage of the sum of soluble glutenin and insoluble glutenin in Table II.

^c Means of three replicates ± standard deviation. Column means with the same letter are not significantly different ($P < 0.05$) among cultivars.

^d Coefficient of variation.

could not be established. It is noteworthy that although other glutenin subunits were more abundant in flour (e.g., *Glu-B1* x-type subunit 7), the HMW-GS composition of residue protein mainly corresponded to subunits controlled by the *Glu-D1* locus: HMW-GS 5+10 in Glenlea and Katepwa, and HMW-GS 2+12 in Biggar and Harus samples. The SDS-PAGE result also indicates that residue protein does not likely contain any significant amount of LMW-GS. Although some reduced residue protein bands had mobilities similar to LMW-GS (Fig. 2, lanes FR), there was no clear correspondence between LMW-GS in the insoluble glutenin fraction (Fig. 2 lanes PI) and co-migrating bands of residue protein. It is tempting to speculate whether residue protein, as obtained in this study, comprises glutenin composed of only HMW-GS, as this has not been reported previously in normal euploid wheat.

Nature of Residue Protein Insolubility

Very little residue protein was solubilized in the third extraction cycle with 50% 1-propanol containing 1% DTT, indicating that the residue protein was not soluble in this solvent. It seemed plausible that the insolubility of the residue protein in 50% 1-propanol with 1% DTT was due to incomplete reduction of all disulfide bonds in the absence of a strong disaggregating agent such as SDS. An experiment was conducted to test this hypothesis. Fresh residue protein was treated with a sufficient concentration of the sulfhydryl-blocking agent NEMI to fully inactivate any residual DTT. SDS-PAGE results showed that a mixture of DTT and NEMI in 50% 1-propanol had apparently no reducing activity on glutenin as indicated by the absence of identifiable HMW-GS (Fig. 3A). However, a relatively HMW protein band with a relative mobility similar to that of HMW-GS 7 or 7* was clearly visible by SDS-PAGE (arrowhead, Fig. 3A). Whether this band was a HMW-GS, or alternatively an oligomer of LMW-GS, or a nongluten polypeptide was not determined. On the other hand, extraction of residue protein after

NEMI treatment using SDS solution without reductant produced SDS-PAGE results (Fig. 3B) essentially identical to those obtained by fully reducing residue protein in SDS (Fig. 2, lanes FR).

These results indicate that 50% 1-propanol with 1% DTT insoluble residue protein exists as reduced subunits, and is either not extractable or soluble in the alcohol-based solvent system. Residue protein has been described as structural protein in previous studies (Byers et al 1983, Kruger et al 1988). The insolubility of the residue protein in 50% 1-propanol with 1% DTT is probably due to strong noncovalent interaction among constituent subunits. This was indicated by the presence of noticeable protein staining in the slots of the gel slab that is evident in the reduced SDS-PAGE results of the final residue (Fig. 2, lanes FR), which showed a preponderance of *Glu-D1* subunits. These subunits appear to be important in maintaining such interactions, since even after three extractions with 50% 1-propanol under reducing conditions, these subunits still remain in the residue as insoluble or unextractable protein, and could only be extracted in SDS. The nature of the insolubility or unextractability of glutenin in the residue in 50% 1-propanol with 1% DTT, and an explanation for the predominance of *Glu-D1* subunits in this fraction requires further investigation. The functionality of these subunits in breadmaking may, in part, involve their ability to form highly insoluble aggregates.

SDS-PAGE also revealed that the final residue appears to contain a relatively large proportion of nongluten proteins that were insoluble in 50% 1-propanol, even after reduction. The 50% 1-propanol insoluble residue before reduction was extracted with SDS solution without reductant. Electrophoregrams (Fig. 3C) showed that this residue contained essentially no monomeric proteins; presumably these were removed by the prior extraction with 50% 1-propanol. This indicates that the remaining nongluten proteins in the final residue were originally associated with the polymeric protein fraction. Similar polypeptides have been previously observed in

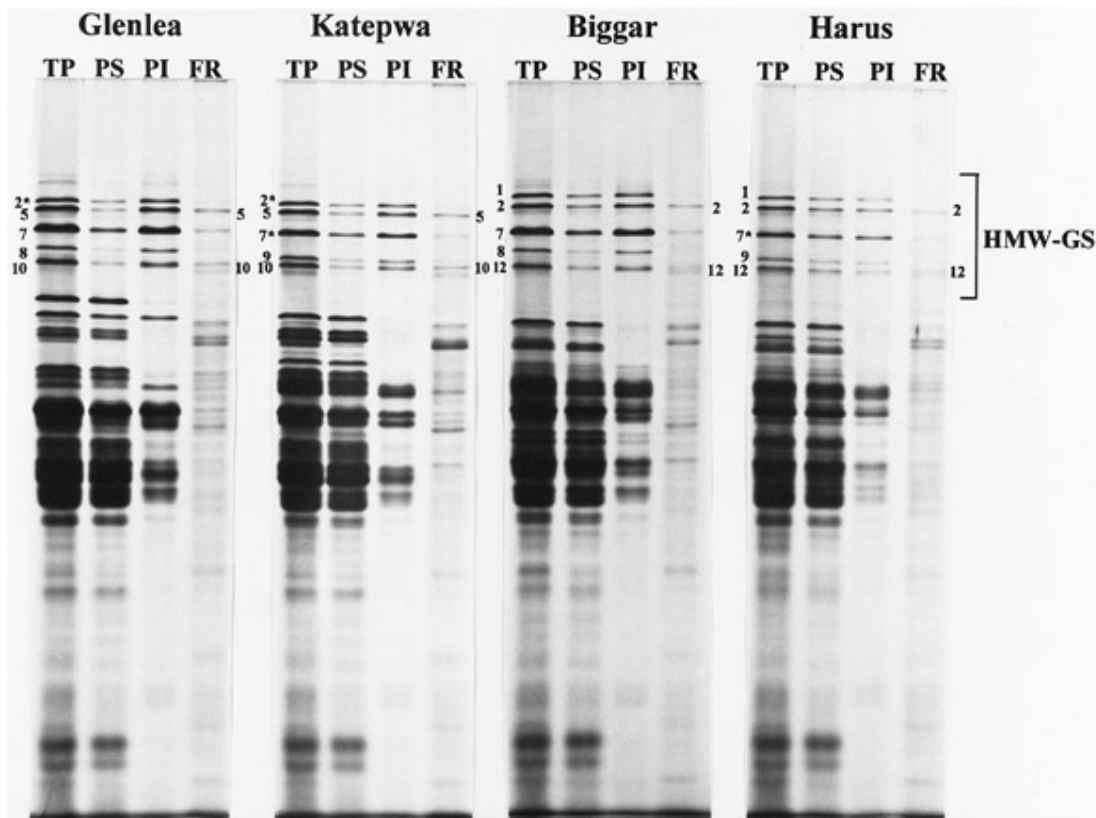


Fig. 2. SDS-PAGE patterns of proteins in sequential extracts of Glenlea, Katepwa, Biggar, and Harus wheat cultivars. TP = direct SDS and dithiothreitol (DTT) extract of flour (control); PS = 50% 1-propanol soluble flour protein, PI = 50% 1-propanol with 1% DTT extract of 50% 1-propanol insoluble residue; FR = SDS-DTT extract of final residue after 50% 1-propanol and 50% 1-propanol with 1% DTT extractions. HMW-GS = high molecular weight glutenin subunit compositions. *Glu-D1* subunits 5+10 or 2+12 predominate in the final residue.

Osborne glutenin fractions (Bietz and Well 1975), glutenin purified using dimethyl sulfoxide (Burnouf and Bietz 1989), and in the polymeric fraction of wheat flour protein (Gupta and Shepherd 1987; Gupta et al 1993). Genetic control and biochemical properties of these proteins were studied by Gupta et al (1991). However, it is still not clear whether or not these nongluten polypeptides are covalently linked with glutenin subunits in polymeric glutenin.

Relationships Between Protein Solubility Fractions and Breadmaking Properties

The CV results (Table II) showed that, compared to the intercultivar variability associated with 50PS protein (CV = 7.2%) or residue protein (CV = 9.1%), the intercultivar variation in insoluble glutenin content (CV = 27.5%) was from three to four times larger. The intercultivar variation in the proportion of flour protein com-

prising soluble glutenin was similarly at a high level (CV = 25.9%, Table III). The concentrations of both soluble and insoluble glutenin in flour protein are therefore mainly genotypic characteristics, which provide some explanation for the very high correlations that were found between these fractions and breadmaking quality (see below). Interestingly, when soluble and insoluble glutenin contents were expressed in terms of concentrations in flour (Table IV), the relative levels of intercultivar variation were quite different. Whereas, the intercultivar variability of insoluble glutenin content of flour was accentuated (range = 1.2–3.9%, CV = 39%), intercultivar variation in the concentration of soluble glutenin in flour was considerably reduced (range = 1.3–1.7%, CV = 10.5%). Among the four principal protein fractions that were studied, it is noteworthy that only insoluble glutenin provided a measure of protein quality that most closely paralleled the technological quality of the

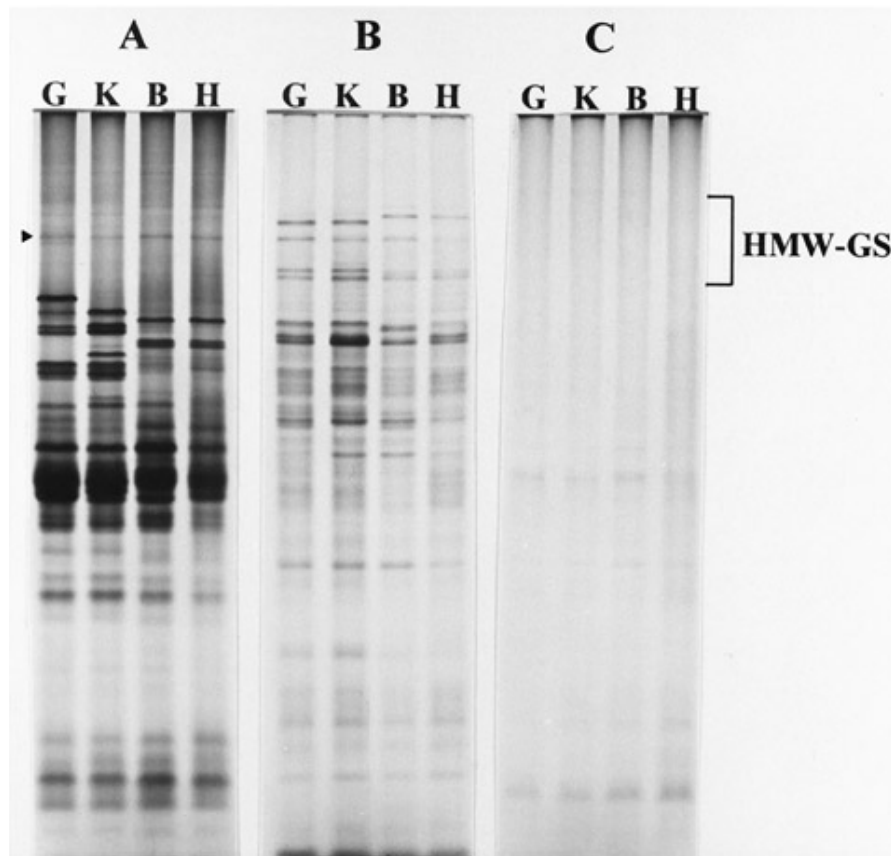


Fig. 3. SDS-PAGE under nonreducing conditions. **A**, Flour proteins extracted with 1% dithiothreitol (DTT) with 5% N-ethylmaleimide (NEMI) and 50% 1-propanol. **B**, SDS extracts of NEMI-treated residue of flours sequentially extracted with 50% 1-propanol and 50% 1-propanol with 1% DTT. **C**, SDS extracts of residue of flours initially extracted with 50% 1-propanol. G, K, B, and H = Glenlea, Katepwa, Biggar, and Harus wheat cultivars, respectively.

TABLE IV
Variation of Some Glutenin Compositional Factors as a Percentage of Flour and Flour Protein^a

Cultivar	% Insoluble Glutenin		% Soluble Glutenin		Ratio of Insoluble to Soluble Glutenin
	Flour Protein	Flour	Flour Protein	Flour	
Glenlea	28.3	3.88	9.6	1.32	2.95
Biggar	25.5	3.19	12.4	1.55	2.06
Roblin	23.8	3.24	11	1.5	2.16
Katepwa	19.9	2.65	12.6	1.68	1.58
AC Karma	20.5	2.23	15.5	1.69	1.32
SWS-52	14.5	1.38	19.4	1.84	0.75
Harus	12.6	1.2	17.8	1.69	0.71
Mean	20.7	2.54	14	1.61	1.65
CV ^b	27.5	39.3	25.9	10.5	49.1

^a Derived from flour protein contents in Table I and insoluble and soluble glutenin measures in Tables II and III.

^b Coefficient of variation.

different flours regardless of whether or not insoluble glutenin measures were normalized to account for intercultivar differences in flour protein contents (Fig. 4).

The correlation coefficients between the various protein fractions and breadmaking quality parameters are shown in Table V. The two crude fractions (50PS and 50PI) were very strongly negatively and positively correlated, respectively, with all quality parameters except dough extensibility. These fractions accounted for 90–95% of the variation in dough strength parameters (time to peak dough development, work input to peak, maximum resistance, extensibility, and area) and ≈70% of the variation in sample loaf volume. The basis for these strong relationships was found upon further fractionation of the 50PS and 50PI fractions into constituent subfractions. In the case of 50PS protein, which comprised monomeric proteins and soluble glutenin, only the latter fraction was significantly (negatively) correlated with breadmaking quality. Soluble glutenin accounted for 83–90% of the variation in dough strength properties and 79% of the variation in loaf volume. Similarly for 50PI protein, which contained insoluble glutenin and residue protein, only the insoluble glutenin fraction was significantly (positively) correlated with the quality parameters; 81–94% of the variation in dough strength parameters and 69% of the variation in loaf volume was explained.

It was interesting that the amount of final residue protein in total flour protein was negatively related to all quality parameters, in contrast to the positive correlations involving the insoluble glutenin component in the same 50PI protein fraction. This provides further evidence of the utility of the 1-propanol fractionation procedure in isolating functionally distinct protein fractions. The negative, albeit weak, relationship between the final residue protein and quality is consistent with results obtained by MacRitchie (1987) for the more insoluble fractions of gluten using dilute HCl as a solvent. As noted above, residue protein was mainly a mixture of some HMW glutenin subunits and nongluten proteins. While the contribution of residue protein to the formation of functional polymeric protein in flour for breadmaking is not clear, among all the protein fractions that were studied, only residue protein was significantly correlated ($r = -0.79$) with extensigraph extensibility. We found this result surprising, as dough extensibility is an attribute normally associated with the viscous property of doughs which should be more related to gliadins and the monomeric protein component of flour in general. However, unusual or inconsistent associations between dough extensibility and protein fractionation results have been reported previously. Gupta et al (1992) found that the total glutenin concentration in flour was strongly positively related to dough extensibility, but not total glutenin concentration expressed as a proportion of flour protein. In another study (Gupta et al 1993), percent unextractable glutenin in total protein or in total flour generally showed poor relationships with dough extensibility. Jia et al (1996) found Chopin alveograph dough extensibility (index G) values for one cultivar grown in many locations to be closely related to total SDS insoluble protein, and SE-HPLC peaks corresponding to both glutenin and gliadin fractions. As well, significant correlations with dough extensibility and LMW-GS by ELISA

responses have also been reported (Andrews and Skerritt 1996), although only a small proportion (<36%) of the variation in dough extensibility was explained. Most recently, Bangur et al (1997) found that most of the variation in dough extensibility could be

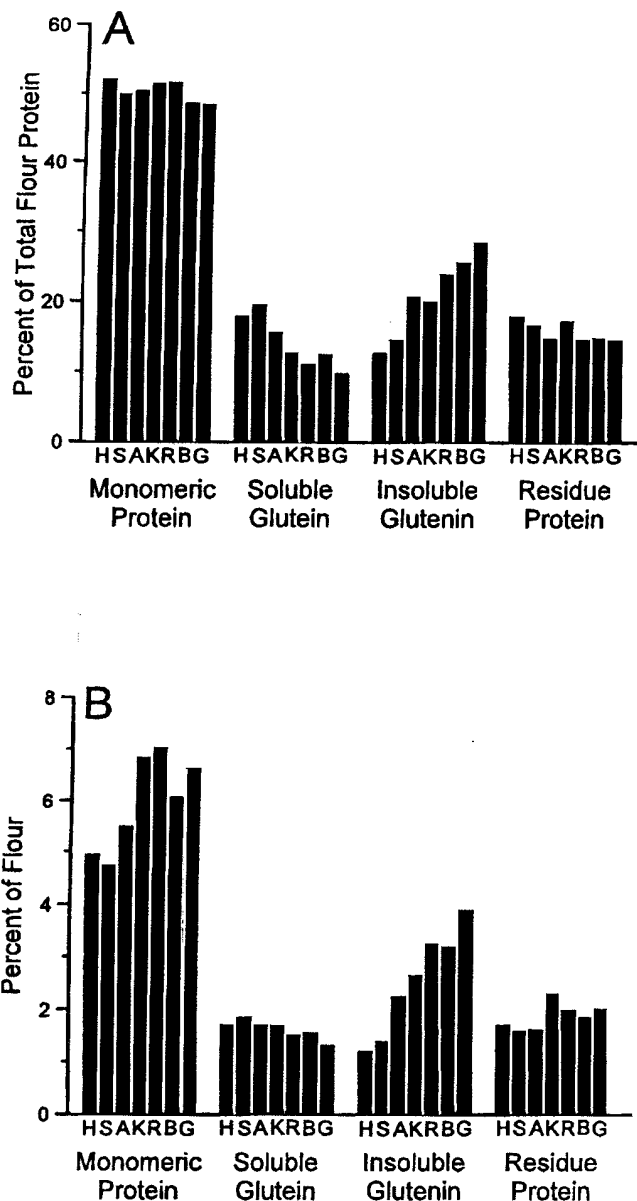


Fig. 4. Distribution of flour protein in four principal solubility fractions expressed as a percentage of total flour protein (A) and percentage of flour (B). Cultivar samples are ranked in order of increasing dough mixing strength. H = Harus, S = SWS-52, A = AC Karma, K = Katepwa, R = Roblin, B = Biggar, R = Roblin, G = Glenlea.

TABLE V
Correlation Coefficients Among Quality Parameters^a and Protein Fractions^b of Wheat Flour Samples

Quality Parameter	50PS	50PI	Monomeric Proteins	Soluble Glutenin	Insoluble Glutenin	Residue Protein
DDT	-0.95*** ^c	0.96***	-0.55	-0.92**	0.90**	-0.56
WIP	-0.98***	0.98***	-0.53	-0.95***	0.94**	-0.65
R_{max}	-0.98***	0.98***	-0.65	-0.91**	0.94**	-0.64
Ext	-0.61	0.6	-0.32	-0.59	0.69	-0.79*
Area	-0.99***	0.99***	-0.64	-0.92**	0.97***	-0.74
Loaf volume	-0.85*	0.83*	-0.23	-0.89**	0.83*	-0.60

^a Mixograph time to peak dough development (DDT), work input to peak (WIP), extensigraph maximum resistance (R_{max}), and extensibility (Ext).

^b 50% 1-Propanol soluble (50PS) and insoluble (50PI) protein.

^c *, **, and *** = Significance at $P < 0.05$, 0.01, and 0.001, respectively.

explained by differences in flour polymeric protein content assessed by SE-HPLC of SDS-sonication enhanced extracts of flour. It would be a fair observation that dough extensibility is a complex rheological characteristic whose biochemical basis is not well understood.

The overall distribution of the four principal solubility fractions in flour protein and their relationship to dough mixing requirements is illustrated in Fig. 4. Whereas the soluble and insoluble glutenin fractions clearly vary with dough strength in inverse and direct fashions respectively, the respective associations between monomeric and residue proteins and dough mixing requirements were marginal at best. This result is a reflection of the previously noted correlations between the protein fractions and dough mixing requirements, and can be largely explained by the intrinsic level of variation among samples which was maximal for the glutenin fractions ($CV \approx 27\%$) and minimal for monomeric and residue protein fractions ($CV \approx 8\%$). Thus, while the viscoelasticity of gluten can be greatly influenced or modified by changing the ratio of gliadin to glutenin (Khatkar et al 1995, Janssen et al 1996), this compositional factor is probably only infrequently related to intercultivar differences in dough mixing strength. This is because the size distribution of glutenin appears to have a larger effect (Gupta et al 1993), and variation in gliadin or monomeric protein content in flour protein is usually small, as found in this study and in other reports (Orth and Bushuk 1972, Kruger et al 1988).

The strong positive relationship found between insoluble glutenin and flour quality for breadmaking is consistent with many previous reports that strong mixing varieties contain more (unextractable) glutenin than weaker ones (Pomeranz 1965, Orth and Bushuk 1972, Orth and O'Brien 1976, Moonen et al 1983, Kruger et al 1988, Marchylo et al 1989, Gupta et al 1993, Gupta and MacRitchie 1994). The parallel influence of both soluble and insoluble glutenin in explaining so much of the variation in breadmaking quality attributes derives from the very high interdependence of these two protein fractions. The correlation between the proportion of soluble and insoluble glutenin in flour protein was very high ($r = -0.90$). This result can be explained by a difference in the average molecular size of the glutenin in these two fractions. It seems plausible that insoluble glutenin contains a distinctly higher proportion of large-sized polymers than is present in the soluble glutenin fraction, a view which has been expressed elsewhere (Gupta et al 1993). From the standpoint of protein functionality, the larger the average polymer size, the smaller will be the specific surface area available for interaction with solvents and other proteins as well. It follows that there should be a close inverse relationship between the proportions of insoluble and soluble glutenin in flour. Furthermore, it has been suggested that not all of the polymeric protein contributes to dough strength, only a fraction above a certain molecular size (the critical molecular size for effective entanglements) (MacRitchie 1992). The results obtained in this study support this hypothesis. By quantifying the amount of protein in flour either unextractable with 50% 1-propanol or subsequently extractable with 50% 1-propanol plus reductant, an excellent measure of large glutenin polymer can be made that closely relates to its functionality in breadmaking (Sapirstein and Johnson 1996).

Finally, it is interesting to note that the flour sample with the highest and lowest percentages of insoluble and soluble glutenin in flour protein, respectively, as well as marginally the lowest concentration of monomeric and residue proteins was Glenlea, a cultivar of the Canada Western Extra Strong Red Spring class, which characteristically has distinctly strong dough mixing properties. These compositional results provide new evidence to explain the biochemical nature of the extra strong dough properties of Glenlea wheat.

CONCLUSIONS

SDS-PAGE and statistical analysis showed that protein fractionation using different solutions of 1-propanol provided a sharp separation of flour proteins from a diverse set of wheats into

functionally distinct fractions. While the genotypic base of this study was small, application of the protein fractionation scheme in our laboratory with seven other samples, including pure cultivars and commercial wheat samples, has yielded similar results to those reported here (Suchy 1997). Two key features of the fractionation were the absence of salt, which can result in cross-contamination of glutenin with monomeric proteins, and the sequential separation of polymeric protein into three subfractions, (soluble and insoluble glutenin, and residue protein). The distinction between soluble glutenin and monomeric proteins was the most time-consuming aspect of the fractionation procedure due to the co-precipitation of some ω -gliadins with glutenin at the 70% 1-propanol precipitation step. While ω -gliadins were effectively separated from glutenin and quantified by RP-HPLC, we have explored a simpler approach using 0.75M NaI to selectively remove ω -gliadins from the 70PI (soluble) glutenin fraction which eliminates the need for HPLC (Fu 1996). This method will be described later.

Among the four protein fractions that were studied, results related to residue protein were especially intriguing in terms of chemistry, composition, and apparent functionality. This quantitatively minor fraction was very distinct in several aspects: 1) it was unextractable in the propanol-DTT solvent, although subunits in residue protein were apparently reduced; 2) it was devoid of identifiable LMW-GS; 3) the *Glu-D1* subunits predominated, in terms of HMW-GS composition; and 4) it was the only protein fraction that was significantly correlated (negatively) with dough extensibility. These findings point to the need for more work to gain a better understanding of residue protein composition, structure, and functionality.

Compared to the relatively complex determination of soluble glutenin as described in this article, or the equally complex procedure involving size-exclusion chromatography of SDS extractable protein, insoluble glutenin is very easily quantified. The purity of this fraction in terms of HMW-GS and LMW-GS composition, together with the very high correlations that were found for technological parameters of dough strength, indicate the value of propanol-DTT soluble protein of 50PI residues as an accurate measure of large glutenin polymer in endosperm. Also, determination of insoluble glutenin by the propanol fractionation method appears to have practical value as a relatively simple and effective test for discerning protein quality differences among different genotypes, particularly in relation to dough mixing requirements. A preliminary study in this regard has been performed and very promising results were obtained (Sapirstein and Johnson 1996). It seems clear that the concentration in wheat endosperm of insoluble glutenin and the level of expression of subunits contained therein, are critical factors in determining the distribution of molecular size of glutenin and related functional characteristics of flour, including protein solubility and dough rheology.

ACKNOWLEDGMENTS

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LITERATURE CITED

- American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9th ed. Method 46-11A. Method 54-10. The Association: St. Paul, MN.
- Andrews, J. L., and Skerritt, J. H. 1996. Wheat dough extensibility screening using a two-site enzyme-linked immunosorbent assay (ELISA) with antibodies to low molecular weight glutenin subunits. *Cereal Chem.* 73:650-657.
- Bangur, R., Batey, I. L., McKenzie, E., and MacRitchie, F. 1997. Dependence of extensograph parameters on wheat protein composition measured by SE-HPLC. *J. Cereal Sci.* 25:237-241.
- Bean, S. R., Lyne, R. K., Tilley, K. A., Chung, O. K., and Lookhart, G. L.

1998. A rapid method for quantitation of insoluble polymeric proteins in flour. *Cereal Chem.* 75:374-379.
- Bietz, J. A., and Wall, J. S. 1973. Isolation and characterization of gliadin-like subunits from glutenins. *Cereal Chem.* 50:537-547.
- Bietz, J. A., and Wall, J. S. 1975. The effect of various extractants on the subunit composition and association of wheat glutenin. *Cereal Chem.* 52:145-155.
- Bietz, J. A., and Wall, J. S. 1980. Identity of high molecular weight gliadin and ethanol-soluble glutenin subunits of wheat: Relation to gluten structure. *Cereal Chem.* 57:415-421.
- Bietz, J. A., Shepherd, K. W., and Wall, J. S. 1975. Single-kernel analysis of glutenin: Use in wheat genetics and breeding. *Cereal Chem.* 52:513-532.
- Burnouf, T., and Bietz, J. A. 1989. Rapid purification of wheat glutenin for reversed-phase high-performance liquid chromatography: Comparison of dimethyl sulfoxide with traditional solvents. *Cereal Chem.* 66:121-127.
- Bushuk, W. 1985. Flour proteins: structure and functionality in dough and bread. *Cereal Foods World* 30:447-451.
- Byers, M., Mifflin, B. J., and Smith, S. J. 1983. A quantitative comparison of the extraction of proteins fractions from wheat grain by different solvents, and of the polypeptide and amino acid composition of the alcohol-soluble proteins. *J. Sci. Food Agric.* 34:447-462.
- Dachkevitch, T., and Autran, J.-C. 1989. Prediction of baking quality of bread wheats in breeding programs by size-exclusion high performance liquid chromatography. *Cereal Chem.* 66:448-456.
- Dupuis, B., and Bushuk, W. 1996. Variation in high molecular weight glutenin subunit composition in various solubility fractions of flours of diverse dough strength. Pages 262-266 in: *Gluten 96, Proc. Int. Gluten Workshop*, 6th. C. W. Wrigley, ed. RACI: Melbourne.
- Dupuis, B., Bushuk, W., and Sapirstein, H. D. 1996. Characterization of acetic acid soluble and insoluble fractions of glutenin of bread wheat. *Cereal Chem.* 73:131-135.
- Fu, B. X. 1996. Biochemical properties of wheat gluten proteins in relation to breadmaking quality. PhD thesis. Department of Food Science, University of Manitoba: Winnipeg, Canada.
- Fu, B. X., and Sapirstein, H. D. 1996. Procedure for isolating monomeric and polymeric glutenin of wheat. *Cereal Chem.* 73:143-152.
- Gupta, R. B., and Shepherd, K. W. 1987. Genetic control of LMW glutenin subunits in bread wheat and association with physical dough properties. Pages 13-19 in: *Proc. Int. Workshop on Gluten Proteins*, 3rd. R. Lasztity and F. Bekes, eds. World Scientific Publishers: Singapore.
- Gupta, R. B., and MacRitchie, F. 1991. A rapid one-step one dimensional SDS-PAGE procedure for analysis of subunit composition of glutenin in wheat. *J. Cereal Sci.* 14:105-109.
- Gupta, R. B., Batey, I. L., and MacRitchie, F. 1992. Relationships between protein composition and functional properties of wheat flours. *Cereal Chem.* 69:125-131.
- Gupta, R. B., and MacRitchie, F. 1994. Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1* of bread wheats: Biochemical basis of the allelic effects on dough properties. *J. Cereal Sci.* 19:19-29.
- Gupta, R. B., Shepherd, K. W., and MacRitchie, F. 1991. Genetic control and biochemical properties of some high molecular weight albumins in bread wheat. *J. Cereal Sci.* 13:221-235.
- Gupta, R. B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *J. Cereal Sci.* 18:23-41.
- Huebner, F. R., and Bietz, J. A. 1993. Improved chromatographic separation and characterization of ethanol-soluble wheat proteins. *Cereal Chem.* 70:506-511.
- Janssen, A. M., Van Vliet, T., and Vereijken, J. M. 1996. Rheological behaviour of wheat glens at small and large deformations. Effects of gluten composition. *J. Cereal Sci.* 23:33-42.
- Jia, Y.-Q., Fabre, J.-L., and Aussenac, T. 1996. Effects of growing location on response of protein polymerization to increased nitrogen fertilization for the common wheat cultivar Soissons: Relationship with some aspects of the breadmaking quality. *Cereal Chem.* 73:526-532.
- Khatkar, B. S., Bell, A. E., and Schofield, J. D. 1995. The dynamic rheological properties of glens and gluten sub-fractions from wheats of good and poor bread making quality. *J. Cereal Sci.* 22:29-44.
- Kilborn, R. H., and Tipples, K. H. 1981. Canadian test baking procedures. I. GRL remix method and variations. *Cereal Foods World* 26:624-628.
- Kruger, J. E., Marchylo, B. A., and Hatcher, D. 1988. Preliminary assessment of a sequential extraction scheme for evaluating quality by reversed-phase high-performance liquid chromatography and electrophoretic analysis of gliadins and glutenins. *Cereal Chem.* 65:208-214.
- MacRitchie, F. 1987. Evaluation of contributions from wheat protein fractions to dough mixing and breadmaking. *J. Cereal Sci.* 6:259-268.
- MacRitchie, F. 1992. Physicochemical properties of wheat proteins in relation to functionality. *Adv. Food Nutr. Res.* 36:1-87.
- Marchylo, B. A., Kruger, J. E., and Hatcher, D. W. 1989. Quantitative reversed-phase high-performance liquid chromatographic analysis of wheat storage proteins as a potential quality prediction tool. *J. Cereal Sci.* 9:113-130.
- Mifflin, B. J., Field, J. M., and Shewry, P. R. 1983. Cereal storage proteins and their effects on technological properties. Pages 255-319 in: *Seed Proteins*. J. Daussant, J. Mosse, and J. Vaughan, eds. Academic Press: New York.
- Moonen, J. H. E., Scheepstra, A., and Graveland, A. 1983. The positive effects of the high molecular weight subunits 3+10 and 2* of glutenin on the breadmaking quality of wheat cultivars. *Euphytica* 32:735-742.
- Ng, P. K. W., and Bushuk, W. 1987. Glutenin of Marquis as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Cereal Chem.* 64:324-327.
- Orth, R. A., and Bushuk, W. 1973. Studies of glutenin. I. Comparison of preparative methods. *Cereal Chem.* 50:106-114.
- Orth, R. A., and Bushuk, W. 1972. A comparative study of the proteins of wheats of diverse baking qualities. *Cereal Chem.* 49:268-275.
- Orth, R. A., and O'Brien, L. 1976. A new biochemical test of dough strength of wheat flour. *J. Aust. Inst. Agric. Sci.* 42:122-124.
- Osborne, T. B. 1907. The proteins of the wheat kernel. Publ. No. 84, Carnegie Institution of Washington: Washington, DC.
- Osborne, T. B. 1924. *The Vegetable Proteins*. 2nd ed. Longmans, Green and Co.: London.
- Pomeranz, Y. 1965. Dispersibility of wheat proteins in aqueous urea solutions—New parameter to evaluate breadmaking potentialities of wheat flour. *J. Sci. Food Agric.* 16:586-593.
- Sapirstein, H. D., and Johnson, W. J. 1996. Spectrophotometric method for measuring functional glutenin and rapid screening of wheat quality. Pages 494-497 in: *Gluten 96, Proc. Int. Gluten Workshop*, 6th. C. W. Wrigley, ed. RACI: Melbourne.
- Singh, N. K., Donovan, R., and MacRitchie, F. 1990a. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. *Cereal Chem.* 67:161-170.
- Singh, N. K., Donovan, R., and MacRitchie, F. 1990b. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. *Cereal Chem.* 67:150-161.
- Singh, N. K., Shepherd, K. W., and Cornish, G. B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *J. Cereal Sci.* 14:203-208.
- Suchy, J. 1997. Physicochemical studies of sds gel protein and its value for prediction of wheat breadmaking quality. MSc thesis. Department of Food Science, University of Manitoba: Winnipeg, Canada.
- Tanaka, K., and Bushuk, W. 1972. Effect of protein content and wheat variety on solubility and electrophoretic properties of flour proteins. *Cereal Chem.* 49:247-257.

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Erratum

CEREAL CHEMISTRY, Vol. 75, No. 4, July-August 1998

Intercultivar Variation in the Quantity of Monomeric Proteins, Soluble and Insoluble Glutenin, and Residue Protein in Wheat Flour and Relationships to Breadmaking Quality

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On page 501, the last line of the third paragraph of the left column should read: "70PI protein less the ω -gliadins constituted soluble glutenin." On page 504, in the first line of the first paragraph of the left column, the reference should be: "(Bietz and Wall 1975)".

In addition, the caption for Figure 1 and definitions in Table I on page 501 are corrected as shown below.

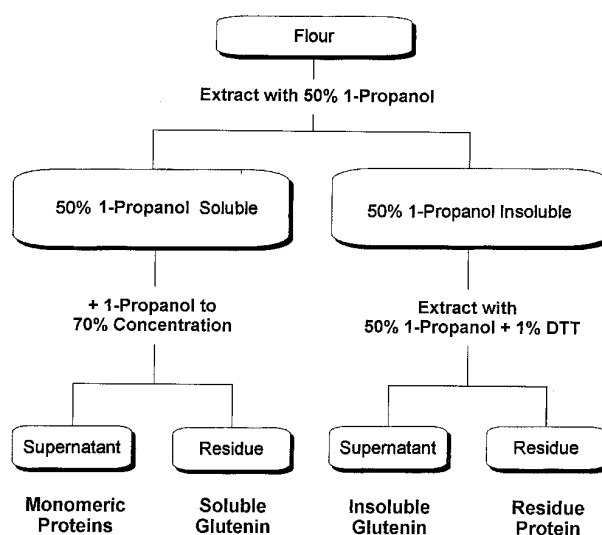


Fig. 1. Protein fractionation scheme. ω -Gliadins contained in the residue of 50% 1-propanol soluble protein precipitated by 70% 1-propanol were quantified by RP-HPLC to correct the proportions of monomeric proteins and soluble glutenin.

TABLE I
Quality Characteristics^a of Flour Samples

Cultivar ^b	Wheat Class ^c	Flour Protein (%)	Mixograph		Extensigraph			Loaf Volume (mL)
			DDT (min)	WIP (%T × min)	R_{max} (BU)	Ext (cm)	Area (cm ²)	
Glenlea	CWESRS	13.7 ± 0.1	5.3 ± 0.1	211 ± 13	1,000 ± 50	19.0 ± 0.2	296 ± 12	828 ± 25
Biggar	CPSR	12.5 ± 0.1	3.7 ± 0.0	141 ± 3	720 ± 7	22.5 ± 0.4	234 ± 6	970 ± 7
Roblin	CWRS	13.6 ± 0.0	3.4 ± 0.1	141 ± 4	560 ± 14	23.0 ± 0.7	196 ± 7	1010 ± 35
Katepwa	CWRS	13.3 ± 0.1	3.5 ± 0.1	123 ± 2	590 ± 14	16.7 ± 1.1	146 ± 3	913 ± 18
AC Karma	CPSW	10.9 ± 0.2	2.6 ± 0.1	69 ± 2	390 ± 11	18.0 ± 1.1	108 ± 6	603 ± 25
SWS-52	CWSWS	9.5 ± 0.0	1.9 ± 0.0	42 ± 1	245 ± 3	17.5 ± 0.3	61 ± 5	423 ± 4
Harus	CEWW	9.5 ± 0.1	2.0 ± 0.1	30 ± 1	170 ± 2	16.0 ± 0.6	41 ± 2	355 ± 7

^a Time to peak dough development (DDT), work input to peak (WIP) (% of torque × min), maximum resistance (R_{max}), extensibility (Ext).

^b Ranked according to decreasing dough strength as measured by mixograph dough work input to peak development and extensigraph area.

^c CWESRS = Canada Western Extra Strong Red Spring, CPSR = Canada Prairie Spring Red, CWRS = Canada Western Red Spring, CPSW = Canada Prairie Spring White, CWSWS = Canada Western Soft White Spring, CEWW = Canada Eastern White Winter.