Changes in Some Physicochemical Properties of Flour Proteins Due to Partial Reduction with Dithiothreitol[1]

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ABSTRACT

Changes in protein solubility, patterns of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and weight of SDS-insoluble gel protein and its rheological properties during reduction of wheat flour proteins by dithiothreitol (DTT) were investigated for two Canadian hard red spring wheats of diverse strength. SDS-PAGE results for the water-soluble protein fraction showed that changes in solubility and gel protein properties with gradually increasing reduction resulted from increasing differential release of water-soluble glutenin oligomers (fragments of polymeric glutenin comprising several subunits). Oligomers contained mainly y-type high M_r glutenin subunits (8 and 10 for Glenlea and 9 and 10 for Katepwa) or B-type low M_r glutenin subunits. The protein of Katepwa flour (strong) released water-soluble oligomers at a lower DTT concentration than did the protein of Glenlea (extra strong). With increasing DTT concentration, the amount of the y-type subunits (indicated by SDS-PAGE band intensity) and the solubility of total protein in SDS solution increased, whereas the gel protein weight and its elasticity decreased. The results showed that the proteins of the extra strong Glenlea flour were more resistant to reduction by DTT than were the proteins of the weaker Katepwa flour. The implication of these results in the molecular basis of wheat flour strength for breadmaking is discussed.

Glutenin is a major storage protein of wheat endosperm and is responsible for most of the intercultivar variation in breadmaking quality (Orth and Bushuk 1972, Payne 1987). Several molecular structural features have been identified as contributors to the unique functionality of glutenin in the breadmaking process (for current review see Schofield 1994). Glutenin is a polymer of some 20 polypeptides, called subunits, joined by interpolypeptide disulfide (S-S) bonds. In addition to stabilizing the tertiary structure of glutenin, the S-S bonds appear to be involved directly in the maturation of flour during storage or by addition of oxidizing improvers and in dough development during mixing or fermentation.

Many different experimental approaches have been used to study the contribution of S-S bonds to the breadmaking potential of wheat flour (review in Pomeranz 1988), or to obtain information on the arrangement of S-S bonds in glutenin polymers (Wall 1967, Graveland et al 1985, Werner et al 1992, Köhler et al 1994). Earlier, we reported that drastic changes in dough properties occurred upon addition of trace amount of S-S reducing dithiothreitol (DTT) (Ng et al 1991, Gao et al 1992). To obtain further information on the nature of polypeptides released by gradual reduction of S-S bonds of glutenin, we extended our earlier study to include reduction in a more dilute system, i.e., a dispersion instead of a dough. Two Canadian hard red spring wheat cultivars that differ widely in bread baking strength were used in an attempt to obtain information that may be useful for explaining differences in strength on the basis of fundamental properties of flour glutenin.

MATERIALS AND METHODS

Flours

Flours of two Canadian hard red spring wheat cultivars, Glenlea and Katepwa, were milled from grain grown in 1991. The two cultivars differ in baking strength; Glenlea is classified as extra strong whereas Katepwa is strong but substantially weaker than Glenlea. Wheat, flour and dough strength will be used synonymously. Strength is defined as the ability to carry a substantial amount of weaker flour and produce a loaf of high volume in the baking test (Irvine and McMullan 1960). Glenlea flour contained 14.4% protein (dry basis) and Katepwa 14.2%.

Acetic Acid-Insoluble Protein

The acetic acid-insoluble fraction was prepared by extracting gluten twice with 0.05*M* acetic acid solution. The gluten had been previously washed out with 2% NaCl solution (Kim and Bushuk 1995).

Determination of Protein

Protein content (N × 5.7) of flours and fractions was determined by the AACC approved method 46-12 (AACC 1983).

Partial Reduction

Partial reduction of flour was achieved in an aqueous dispersion. Flour (2 g, dwb) was weighed into a 40-ml ultracentrifuge tube and dispersed in 10 ml of 0, 0.01, 0.05, 0.1, 0.5, 1.0, or 100 m*M* DTT solution. The dispersions were shaken on a Thermolyne mixing-shaker at setting 10 (Maxi-Mix III, type 65800, Dubuque, IA) for 1 hr at room temperature.

Separation of Water-Soluble and -Insoluble Protein

The partially reduced flour dispersions were centrifuged at 12,000 × *g* for 15 min at 20°C. The supernatants, containing water-soluble proteins, were used for N analysis and electrophoresis. The residues, containing water-insoluble proteins, were washed by dispersing in 30 ml of water (to further remove residual water-soluble protein), centrifuged at 12,000 × *g* for 15 min at 20°C, and freeze-dried. The dry residue was used for electrophoresis.

Preparation of SDS Soluble and Insoluble Gel Protein

SDS solution (2.25%, 20 ml) was added to the aqueous flour dispersions in the centrifuge tube (as above) to give a final SDS concentration in the mixture of 1.5%. The mixture was shaken gently for 10 min at room temperature, and then centrifuged at 70,000 × *g* for 30 min at 25°C. The supernatant, containing SDS-soluble protein, was used for N analysis and electrophoresis. The

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SDS-insoluble protein, which forms a gel on top of the mainly starch layer, was carefully scraped off and its weight determined and expressed in grams per gram of flour. The gel was used for dynamic rheological measurements.

Rheological Measurements

Dynamic rheological measurements on the gel were performed using a VOR rheometer (Bohlin Rheology, Lund, Sweden) in the oscillation shear mode. The C-14 conical cylinder measuring system had a concentric cylinder and a 1.4-ml volume cup. Measurements were made at a strain of 0.0105 and a frequency of 1.0 Hz at 25°C. At this strain, all gel samples were in the linear viscoelastic region (data not shown). Because the rheological behavior of the gels changed with time, all measurements were made 10 min after preparation.

Electrophoresis

SDS-PAGE was performed according to Kim and Bushuk (1995), with a 14% gel system. Aliquots of protein extracts were mixed with buffer stock (1.0:0.4) solution containing 20 g of glycerol, 12.5 ml of 1.0*M* Tris-HCl solution, 24 ml of water, 8 g of SDS and 20 mg of pyronine Y. For complete reduction of the protein in the extract at 5% mercaptoethanol, the buffer stock solution containing 17.8% (v/v) mercaptoethanol was used. The same amount of protein was applied to each lane. Electrophoresis was done at 25 mA/gel.

For two-step electrophoresis, a lane of gel obtained from electrophoresis under nonreducing condition (first step) was cut out and equilibrated with buffer (stock buffer solution diluted with water 0.4:1.0) containing 5% mercaptoethanol for 1 hr at 37°C. The slice was carefully inserted into a long slot of the separating gel. Condition of the second electrophoresis were the same as the first.

Standard proteins (Bio Rad) were used to estimate relative molecular mass (M_r): myosin (200.0 kDa), β-galactosidase (111.6 kDa), phosphorylase B (97.0 kDa), serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa) and aprotinin (6.5 kDa).

TABLE I
Protein Extracted with Water (% of Flour Protein) from Glenlea and Katepwa Flours in the Presence of Increasing Concentrations of Dithiothreitol (DTT)[a]

	Glenlea		Katepwa	
DTT (m*M*)	% Protein	% Increase	% Protein	% Increase
0	6.8 ± 0.6	0	9.6 ± 1.2	0
0.01	7.0 ± 0.2	0.2	11.2 ± 0.6	1.6
0.05	7.2 ± 0.8	0.4	12.1 ± 0.6	2.5
0.1	7.9 ± 0.7	1.1	13.4 ± 0.2	3.8
0.5	11.2 ± 0.4	4.4	17.0 ± 0.6	7.4
1.0	11.3 ± 0.5	4.5	17.2 ± 0.4	7.6
100	9.7 ± 0.3	2.9	16.1 ± 0.2	6.5

[a] Mean and standard deviation of three replicate determinations.

TABLE II
Protein Extracted with 1.5% Sodium Dodecyl Sulfate Solution (% of Flour Protein) from Glenlea and Katepwa Flours in the Presence of Increasing Concentrations of Dithiothreitol (DTT)[a]

	Glenlea		Katepwa	
DTT (m*M*)	% Protein	% Increase	% Protein	% Increase
0	68.4 ± 1.4	0	77.5 ± 1.2	0
0.01	69.7 ± 0.8	1.3	80.2 ± 1.3	2.7
0.05	72.7 ± 0.7	4.3	84.8 ± 0.8	7.3
0.1	76.6 ± 1.3	8.2	87.9 ± 1.1	10.4
0.5	95.3 ± 1.5	26.9	98.5 ± 0.3	21.0
1.0	>99	>30.2	>99	>21.5
100	>99	>30.2	>99	>21.5

[a] Mean and standard deviation of three replicate determinations.

RESULTS

Protein Solubility in Water

Without addition of DTT (control), the protein of Katepwa flour was more soluble in water than the protein of Glenlea (Table I). Upon addition of DTT, the amount of water-soluble protein increased and was highest at 1.0 m*M* DTT, where the increase was 4.1% for Glenlea and 7.6% for Katepwa. Further increase in DTT concentration to 100 m*M* caused a slight decrease in the amount of protein soluble in water for both flours. The reason for this decrease is not clear and requires further study. At all identical DTT concentrations, the percentage of water-soluble protein in Glenlea flour was lower than that in Katepwa flour.

Protein Solubility in 1.5% SDS

Table II gives the change in flour protein solubility in 1.5% SDS solution after reduction by increasing concentrations of DTT. Without DTT, the protein of Katepwa was about 10% more soluble than that of Glenlea; similar results were reported by Gao and Bushuk (1993). Protein solubility in SDS solution increased markedly upon addition of DTT. At each identical DTT concentration, less protein was solubilized for Glenlea than for Katepwa.

SDS-PAGE Results

Water-Soluble and -Insoluble Protein. Under nonreducing conditions (Fig. 1), the SDS-PAGE patterns for Glenlea, reduced by up to 0.1 m*M* DTT, showed no differences. But some qualitative differences in the patterns for Katepwa, compared to those of the control, began to appear from 0.05 m*M* DTT. For DTT concentrations of 0.5 and 1.0 m*M*, some new bands, albeit somewhat diffuse, appeared for both wheats.

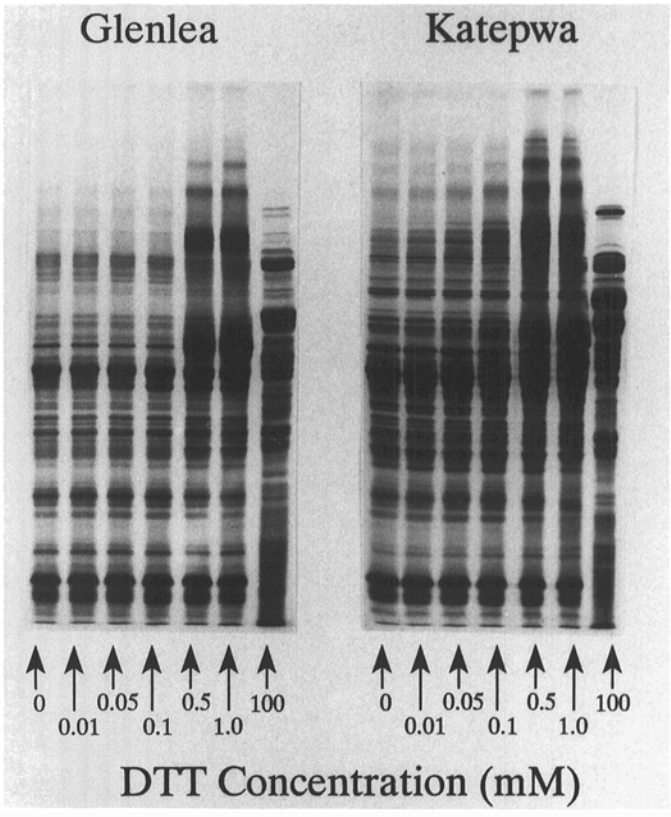

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns under nonreducing conditions of the water-soluble proteins of Glenlea and Katepwa flours after treatment with different concentrations of dithiothreitol (DTT).

At 100 m*M* DTT, y-type high molecular weight glutenin subunits (HMW-GS) (9 and 10 for Katepwa and 8 and 10 for Glenlea) appeared in the patterns. This result indicates that these subunits in reduced form are soluble in water. The amount of HMW-GS in the water-soluble fraction is small compared with that which remained in the water-insoluble residue.

When the water-soluble protein was analyzed by SDS-PAGE under reducing conditions (Fig. 2), the patterns showed that the relative staining intensity of certain protein bands increased markedly as DTT concentration increased. This indicates that partial reduction in a flour-water dispersion produced some protein fragments that are soluble in water. Protein bands that showed a substantial increase in intensity can be divided into three groups on the basis of their M_r.

The first group is y-type HMW-GS. The water-soluble protein of Katepwa, obtained without addition of DTT, also contained trace amounts of y-type HMW-GS. It is not clear whether the Katepwa flour contained these oligomers as indigenous components or whether they were produced by reducing substances present in the flour. Hussain and Lukow (1994) recently reported that after total reduction of glutenin the y-type HMW-GS were differentially more soluble in water than the x-type HMW-GS. The pattern for Katepwa only showed the presence of x-type HMW-GS (2*, 5, and 7) as very faint bands.

The second group of bands were in the M_r range 56–63 kDa. These bands may be the HMW albumins present in wheat flour as disulfide-linked polymers and as single-chain polypeptides (Gupta et al 1991).

The third group of bands were M_r 45 kDa for Glenlea and 45 and 50 kDa for Katepwa. To confirm the identity of these bands, a comparison of the SDS-PAGE patterns of the water-insoluble residue with those of the acetic acid-insoluble fraction (presumed to be glutenin) was made (Fig. 3). The water-insoluble residue contained fewer B-type low molecular weight glutenin subunits (LMW-GS) (indicated by asterisk) than did the acetic acid-insoluble residue. This is especially striking for the Katepwa treated with 0.5 m*M* DTT. The results in Figures 2 and 3 indicate that glutenin fragments comprising of some B-type LMW-GS are also soluble in water.

Results in Figure 3 also show that the water-insoluble residue of flour treated with 100 m*M* DTT still contained large amounts of y-type HMW-GS. The bands of these subunits were more intense than the equivalent bands for the water-soluble fraction (compare Figs. 2 and 3).

Two-Step SDS-PAGE of Water-Soluble Proteins. Two-step electrophoresis was performed to determine the subunit composition of the water-soluble glutenin oligomers. The results (Fig. 4 for Glenlea and Fig. 5 for Katepwa) showed that the oligomers with M_r of ≈70 kDa (under nonreducing conditions), comprised mainly the B-type LMW-GS. For Katepwa, additional faint bands were observed at 37 kDa. These are probably C-type LMW-GS (Graveland et al 1985).

Results of two-step electrophoresis showed that glutenin oligomers of only LMW-GS can have M_r values equal to those of dimers or trimers of HMW-GS reported previously (Gao et al 1992). In contrast, Werner et al (1992) did not detect any oligomers comprising only LMW-GS in their experiments on partial reduction of SDS-complexed glutenin.

Only trace amounts (faint bands visible in gels) of y-type HMW-GS were detected by the two-step electrophoresis, despite the fact that the amount of sample applied on the first step (I) of the two-step electrophoresis was 1.5× of the amount of the sample under reducing conditions (II).

In summary, SDS-PAGE patterns for the water-soluble protein of the flours treated with DTT showed evidence of glutenin oligomers containing y-type HMW-GS or B-type LMW-GS. Results

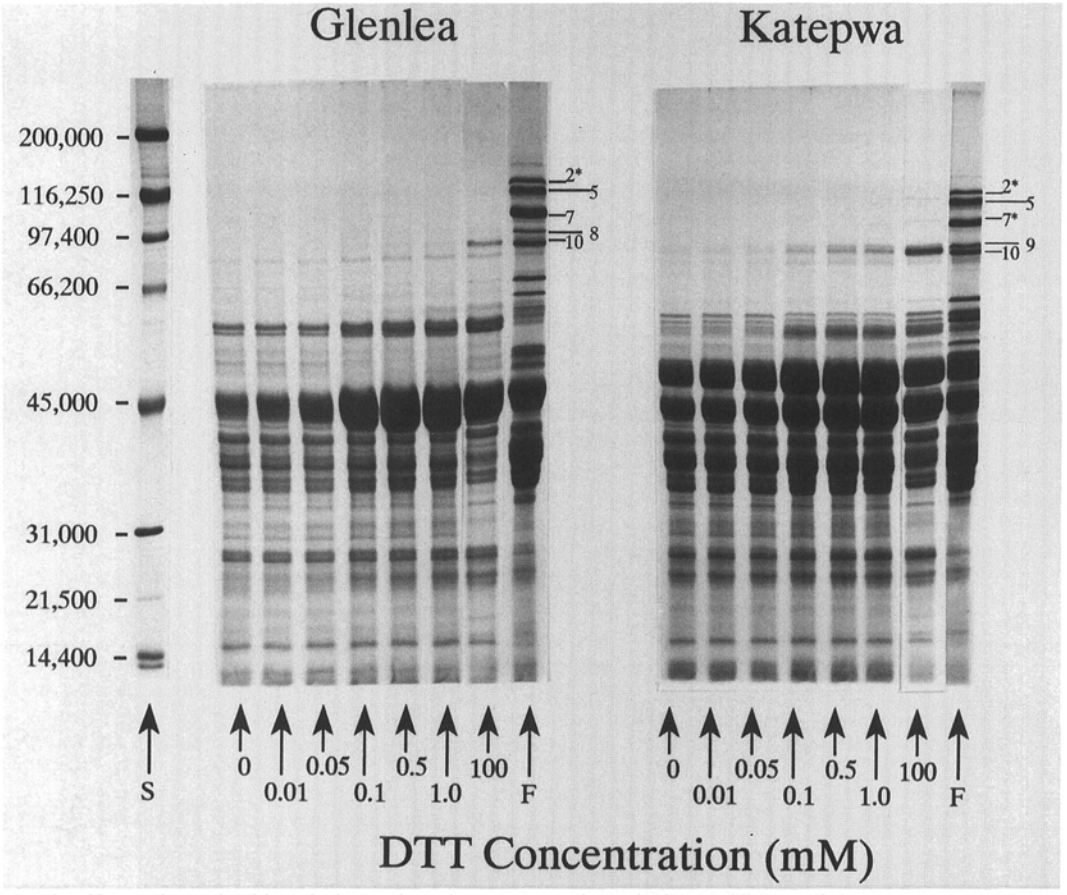

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns under reducing conditions of the water-soluble proteins of Glenlea and Katepwa flours after treatment with different concentrations of dithiothreitol (DTT). S = standard proteins. F = total protein of untreated flour.

further showed that, based on subjective comparison of stained bands, there was a clear cultivar difference in the patterns. For Katepwa, water-soluble oligomers were observed at a lower DTT concentration than for Glenlea. Also, at a specific DTT concentration, Katepwa released a greater amount of water-soluble oligomer than did Glenlea.

SDS-Soluble Proteins. Under nonreducing conditions (Fig. 6), the SDS-PAGE patterns of the SDS-soluble protein of untreated and treated (up to 0.1 m*M* DTT) flours were the same. At this level of reduction, there was no evidence of release of any HMW-GS. The patterns under reducing conditions for the same DTT treatments (Fig. 7) showed that the staining intensity of equivalent bands, for example the HMW-GS, increased continually up to 0.1 m*M* DTT. These results indicate that some oligomers produced by the DTT concentrations used are still too large to enter the gel. These observations are consistent with those reported earlier for the dough system (Gao et al 1992).

At 0.5 and 1.0 m*M* DTT, the SDS-PAGE patterns under nonreducing conditions showed that some HMW-GS were released along with oligomers. Glutenin oligomers comprising only HMW-GS (Graveland 1985, Gao et al 1992, Werner et al 1992), and those comprising a single HMW-GS and several LMW-GS (Gao et al 1992) have been reported previously.

The patterns for the SDS-soluble protein up to 1.0 m*M* DTT under reducing conditions (Fig. 7) showed that the staining intensity of specific bands (e.g., HMW-GS) increased more strongly with DTT concentration for Katepwa than for Glenlea.

The SDS-PAGE patterns of the SDS-soluble protein of each flour at 100 m*M* DTT were essentially the same under nonreducing and reducing conditions (Figs. 6 and 7), indicating that all

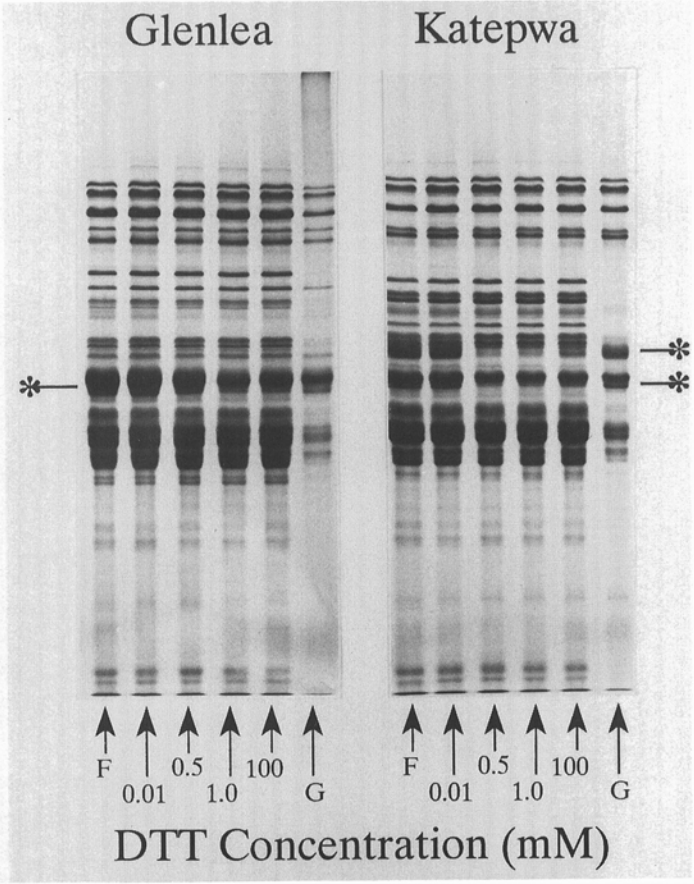

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns under reducing conditions for the 0.05*M* acetic acid-insoluble and the water-insoluble proteins of Glenlea and Katepwa flours after treatment with different concentrations of dithiothreitol (DTT). * = low molecular weight glutenin subunits. F = untreated flour. G = residue protein of untreated flour extracted with 0.05*N* acetic acid solution.

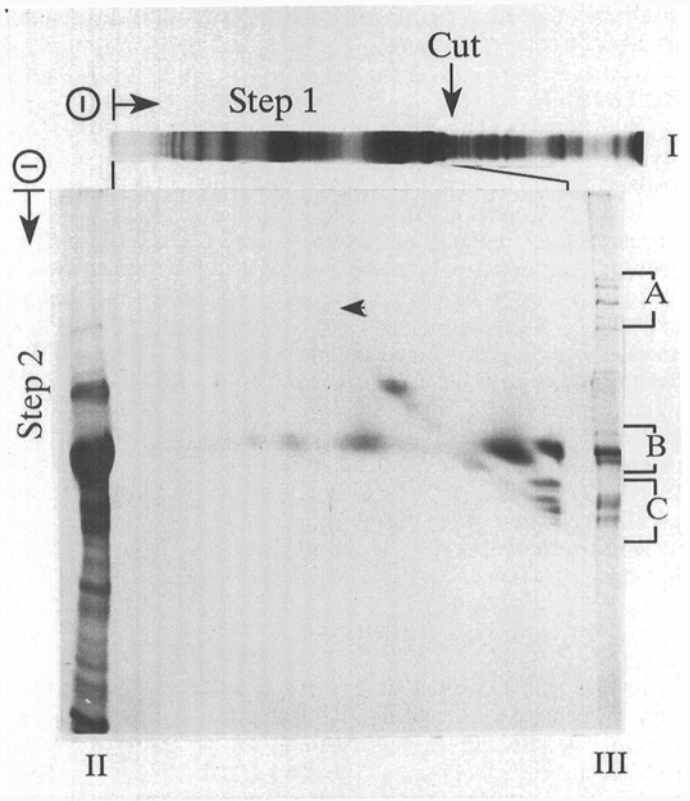

Fig. 4. Two step electrophoresis of the water-soluble proteins of Glenlea flour treated with 0.5 m*M* dithiothreitol. I = water-soluble proteins under nonreducing conditions, II = water-soluble proteins under reducing conditions, III = residue protein of flour extracted with 0.05*N* acetic acid solution under reducing conditions. Arrowhead indicates y-type high molecular weight glutenin subunits.

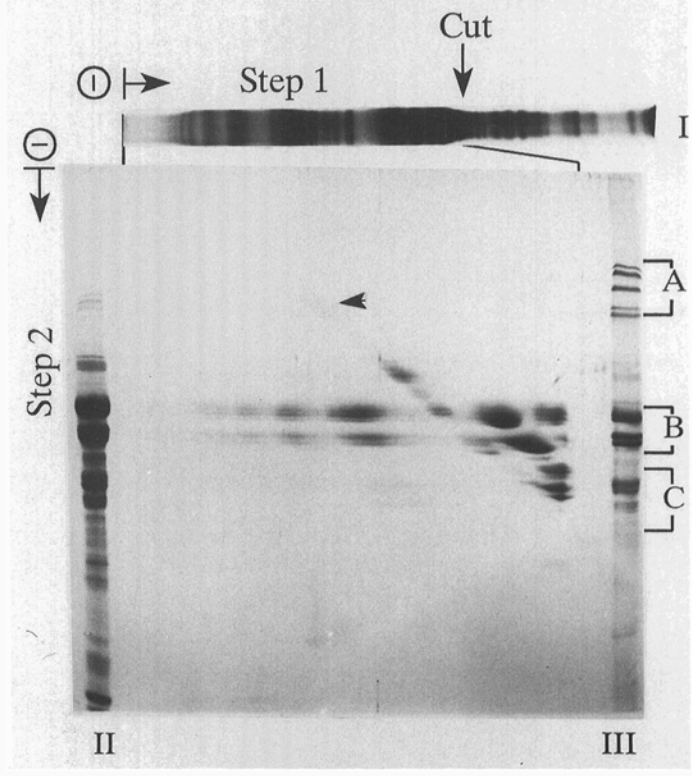

Fig. 5. Two step electrophoresis of the water-soluble proteins of Katepwa flour treated with 0.5 m*M* dithiothreitol. I = water-soluble proteins under nonreducing conditions, II = water-soluble proteins under reducing conditions, III = residue protein of flour extracted with 0.05*N* acetic acid solution under reducing conditions. Arrowhead indicates y-type high molecular weight glutenin subunits.

disulfides of glutenin were reduced (in the flour-water dispersion) at this DTT concentration.

Gel Protein

Table III gives the weight of gel obtained for the two flours used in this study after treatment with different amounts of DTT. Without DTT, Glenlea yielded more gel than did Katepwa. As DTT concentration increased, gel weight decreased for both wheats, but at different rates. For a specific DTT concentration, gel weight for Glenlea was always higher than that for Katepwa.

Figure 8 shows the change in rheological properties (storage modulus G' and phase angle δ) of the gel obtained from flours treated with increasing concentration of DTT. Without DTT treatment, the Glenlea gel had higher G' and lower δ values, indicating a more elastic gel. As the DTT concentration increased G' decreased and δ increased. The percentage decrease in G' for treatments of 0.01, 0.05, and 0.1 m*M* DTT were 7.2, 29.5, and 73.7% for Glenlea and 40.5, 74.1, and 99.5% for Katepwa. Glenlea gels were more elastic initially and lost less elasticity upon treatment with a specific concentration of DTT than did the Katepwa gels.

DISCUSSION

Gradual reduction of flour proteins in an aqueous dispersion with DTT caused an increase in the amount of water-soluble protein. The protein that contributed to this increase was mainly glutenin oligomer comprising mostly the y-type HMW-GS or B-type LMW-GS. The reason why the oligomers containing the y-type HMW-GS are differentially more soluble in water is probably that the y-type HMW-GS are more hydrophilic (in order of subunits 10 ,9, and 8) than the x-type subunits (in order of subunits 7, 5, and 2*). These relative hydrophilicities are based on published results of reversed-phase high-performance liquid chromatography (Marchylo et al 1989, Kawka et al 1992, Magnus and Khan 1992). The easier (lower concentration of DTT) release of oligomers containing y-type HMW-GS by Katepwa than by Glenlea can be partially attributed to the difference in one y-type HMW-GS (9 in Katepwa, which is more hydrophilic than 8 in Glenlea) and the lower amount of subunit 7, which is more hydrophobic than any of the other y-type subunits in the two cultivars (Marchylo et al 1989)

As the degree of reduction increased, more flour protein became soluble in 1.5% SDS solution. Concomitant with the increase in solubility, gel protein weight and its elasticity decreased. SDS-PAGE patterns (under nonreducing conditions) showed that the loss of gel properties occurred at a degree of reduction (up to 0.1 m*M*) at which the size of some resulting oligomers was still too large to enter the separating gel. These results were analogous to those obtained for doughs in which glutenin had been partially depolymerized by overmixing (Orsel et al 1992, Weegels et al 1993).

When the amount (band intensity) of the y-type HMW-GS in the water-soluble fraction was related to the change in protein solubility or in gel-protein properties, there appeared to be a negative relationship for DTT concentrations up to 0.1 m*M*. The greater the amount of y-type HMW-GS released, the higher the solubility and the lower the gel weight and its elasticity.

According to the models for the structure of polymeric glutenin (Graveland et al 1985, Kasarda 1989, Gao et al 1992), reduction of only a few rheologically effective disulfide bonds would have a large influence on dough properties. Such disulfides would be located near the middle of the glutenin polymer as was postulated by MacRitchie (1993). The results obtained in the present study indicated further that glutenin oligomers that are soluble in water appear to be joined to the glutenin polymer by rheologically ef-

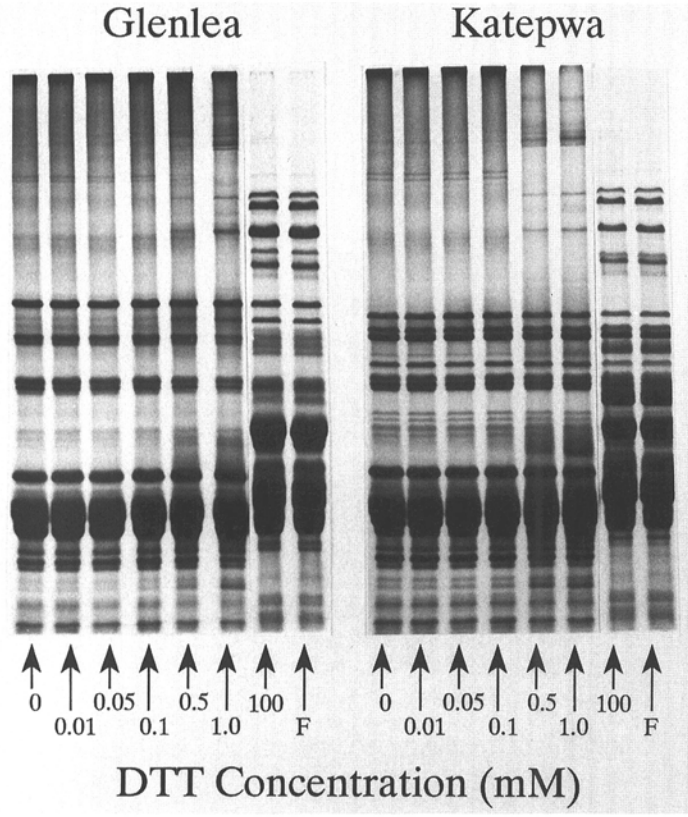

Fig. 6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns under nonreducing conditions of the sodium dodecyl sulfate soluble proteins of Glenlea and Katepwa flours treated with different concentrations of dithiothreitol (DTT). F = untreated flour under reducing conditions.

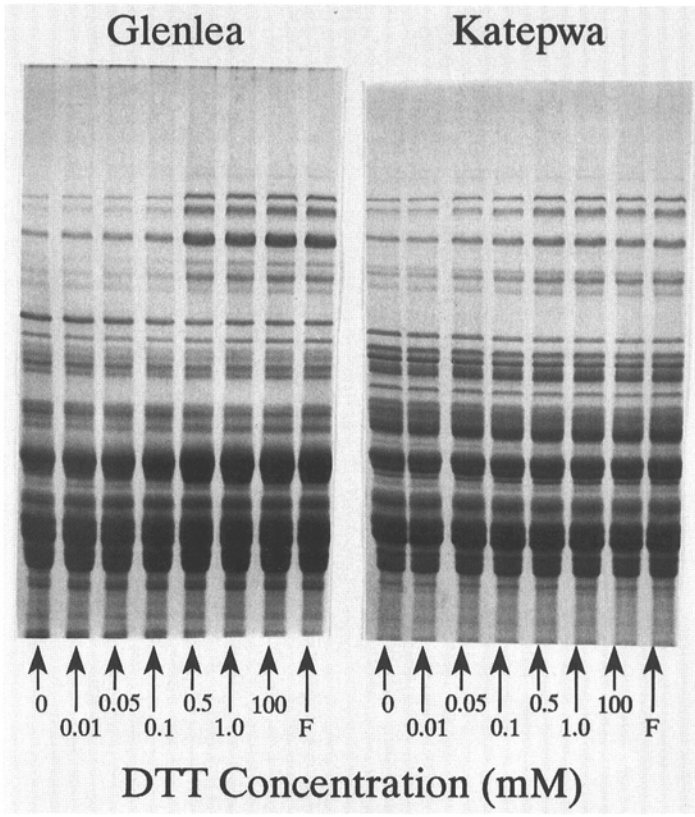

Fig. 7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns under reducing conditions of the sodium dodecyl sulfate soluble proteins of Glenlea and Katepwa flours treated with different concentrations of dithiothreitol (DTT). F = untreated flour under reducing conditions.

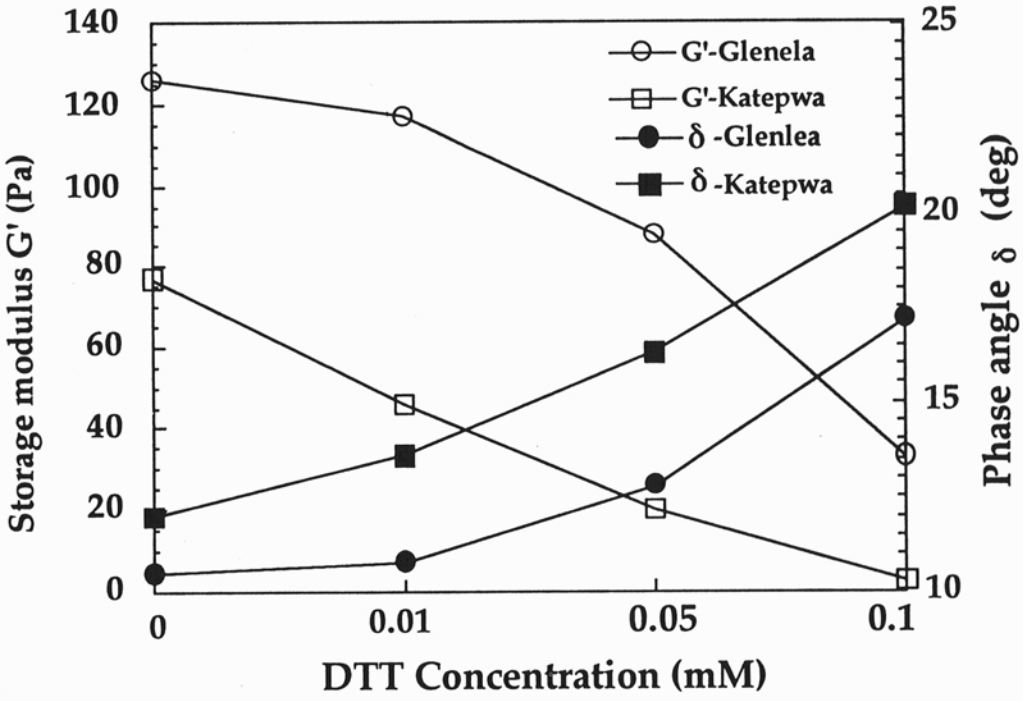

Fig. 8. Changes in G' and phase angle δ of the sodium dodecyl sulfate-insoluble protein gels obtained from Glenlea and Katepwa flours treated with increasing concentrations of dithiothreitol (DTT).

TABLE III
Weight (grams per gram of Flour) of Gel Protein Obtained for Glenlea and Katepwa Flours in the Presence of Increasing Concentrations of Dithiothreitol (DTT)[a]

DTT (m*M*)	Glenlea	Katepwa
0	2.98 ± 0.13	2.73 ± 0.05
0.01	2.94 ± 0.12	2.65 ± 0.04
0.05	2.84 ± 0.06	2.10 ± 0.07
0.1	2.49 ± 0.13	1.08 ± 0.11
0.5	0.15 ± 0.02	0

[a] Mean and standard deviation of three replicate determinations.

fective disulfide bonds. These oligomers contain relatively more y-type than x-type HMW-GS.

This study showed that the glutenin oligomers produced by mild reduction contain mainly y-type HMW-GS or B-type LMW-GS, which are soluble in water. Presumably, the hydrophilic nature of these oligomers facilitates reduction of neighboring disulfides in aqueous system. As an extension of this observation, it is postulated that analogous reduction by water-soluble SH compounds (e.g., glutathione) occurs as the first step in S-S interchanges during dough development by mixing. Disulfides that are buried in hydrophobic regions of polymeric glutenin would be initially inaccessible to reduction by water-soluble reducing substances. Application of this concept to the mechanism of dough development by mixing suggests that the main change that occurs during mixing toward peak development is the conversion of a relatively hydrophobic system to one that is more hydrophilic, thus facilitating interactions in an aqueous environment. Differences in glutenin hydrophobicity that were observed for different wheat varieties (Chung and Pomeranz 1979, Popineau 1985, Magnus and Khan 1992) would explain well-known intervarietal differences in breadmaking strength.

ACKNOWLEDGMENT

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LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. The Association: St. Paul, MN.

CHUNG, K. H., and POMERANZ, Y. 1979. Acid-soluble proteins of wheat flours. II. Binding to hydrophobic gels. Cereal Chem. 56:196.

GAO, L., and BUSHUK, W. 1993. Solubilization of glutenin in urea/SDS solutions at elevated temperature. J. Cereal Sci. 16:81.

GAO, L., NG, P. K.W., and BUSHUK, W. 1992. Structure of glutenin based on farinograph and electrophoretic results. Cereal Chem. 69:452.

GRAVELAND, A., BOSVELD, P., LICHTENDONK, W. J., MARSEILLE, J. P., MOONEN, J. H. E., and SCHEEPSTRA, A. 1985. A model for the molecular structure of the glutenins from wheat flour. J. Cereal Sci. 3:1.

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.

HUSSAIN, A., and LUKOW, O. M. 1994. Characterization of 1B/1R translocation in wheat using water extractable protein concentrate. Euphytica 78:109.

IRVINE, G. N., and McMULLAN, M. E. 1960. The Remix baking test. Cereal Chem. 37:603.

KASARDA, D. D. 1989. Glutenin structure in relation to wheat quality. Pages 277-302 in: Wheat is Unique. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

KAWKA, A., NG, P. K.W., and BUSHUK, W. 1992. Equivalence of high molecular weight glutenin subunits prepared by reversed-phase high-performance liquid chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 69:92.

KIM, H. R., and BUSHUK, W. 1995. Salt sensitivity of acetic acid-extractable proteins of wheat flour. J. Cereal Sci. 21:241.

KÖHLER, P., BELITZ, H.-D., and WIESER, H. 1994. Disulphide bonds in wheat gluten. Pages 79-88 in: Gluten Proteins 1993. Assoc. Cereal Res.: Detmold, Germany.

MacRITCHIE, F. 1993. Aspect of protein structure/composition that determine dough strength and extensibility, Pages 134-140 in: Gluten Proteins 1993. Assoc. Cereal Res.: Detmold, Germany.

MAGNUS, E. M., and KHAN, K. 1992. Hydrophobic properties of reduced and alkylated acetic acid-soluble glutenins—Fractionation by hydrophobic interaction chromatography. Cereal Chem. 69:607.

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.
NG, P. K. W., XU, C., and BUSHUK, W. 1991. Model of glutenin structure based on farinograph and electrophoretic results. Cereal Chem. 68:321.
PAYNE, P. I. 1987. The genetical basis of breadmaking quality in wheat. Aspects Appl. Biol. 15:79.
POPINEAU, Y. 1985. Fractionation of acid-soluble proteins from wheat gluten by hydrophobic interaction chromatography: Evidence for different behaviour of gliadin and glutenin proteins. J. Cereal Sci. 3:29.
POMERANZ, Y. 1988. Composition and functionality of wheat flour components. Pages 219-370 in: Wheat: Chemistry and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
ORSEL, R., LICHTENDONK, W. J., WEEGELS, P. L., and HAMER, R. J. 1992. Relationships between dynamic rheological properties and biochemical properties of dough; dependence on mixing time. Cereal Foods World 37:541.
ORTH, R. A., and BUSHUK, W. 1972. A comparative study of the proteins of wheats of diverse baking quality. Cereal Chem. 49:268.
SCHOFIELD, J. D. 1994. Wheat proteins: Structure and functionality in milling and breadmaking. Pages 73-106 in: Wheat Production, Properties and Quality. W. Bushuk and V. F. Rasper, eds. Blackie Academic and Professional: Glasgow.
WALL, J. S. 1967. Review of wheat proteins. Pages 73-106 in: National Conference on Wheat Utilization Research, 5th. USDA/ARS: Washington, DC.
WEEGELS, P. L., HAMER, R. J., and SCHOFIELD, J. D. 1994. Depolymerisation and polymerisation of individual glutenin subunits in *situ* in dough-implications for the structure of gluten. Pages 57-66 in: Gluten Proteins 1993. Assoc. Cereal Res.: Detmold, Germany.
WERNER, W. E., ADALSTEINES, A. E., and KASARDA, D. D. 1992. Composition of high molecular-weight glutenin subunit dimers formed by partial reduction of residue glutenin. Cereal Chem. 69:535.

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