Structure of Glutenin Based on Farinograph and Electrophoretic Results[1]

L. GAO, P. K. W. NG, and W. BUSHUK

ABSTRACT

The objective of this study was to obtain additional information on the molecular structure of glutenin in relation to its functionality in doughs during breadmaking. In this context, doughs from the flour of the Canadian hard red spring wheat cultivar Katepwa were mixed in a farinograph in the absence and presence of a disulfide reducing agent, dithiothreitol (DTT). The glutenin of the control dough and of the partially reduced dough was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) without and with reduction during electrophoretic analysis. At a low concentration of DTT (20 μmol/50 g of flour), the farinograph properties were markedly affected, but no high molecular weight (HMW) subunits were liberated as indicated by SDS-PAGE without reduction. At higher concentrations of DTT (80–3,000 μmol/50 g of flour), several types of glutenin subunit oligomers and all of the oligomer subunits (2*, 5, 7, 9, and 10) were liberated gradually with increasing DTT concentration. HMW glutenin oligomers as three close-moving bands in SDS-PAGE, with molecular weights higher than that of the largest HMW subunit (2*), were observed for doughs treated with the higher concentrations of DTT. On the basis of their apparent molecular weights, the three bands appear to be dimers composed of subunits as follows: 2*+7, 2*+9, and 5+10. The results of this study are consistent with a "block" model of the molecular structure of glutenin.

Cereal Chem. 69(4):452–455

The contribution of the glutenin fraction of wheat storage proteins to the breadmaking potential of flour is well documented in the literature and has been reviewed by Bushuk and Tkachuk (1991). The presence or absence of certain high molecular weight (HMW) subunits in the polymeric (molecular) glutenin has been shown to be strongly correlated with breadmaking quality (Payne et al 1984, Ng and Bushuk 1988). In the continuing search for a structure-functionality relationship, several models have been proposed for the structure of glutenin (Kasarda et al 1976, Khan and Bushuk 1979, Graveland et al 1985, Ewart 1988 and references therein, Kasarda 1989, Ng et al 1991). However, the fine details of the structure remain a challenging subject of research.

The marked effect of disulfide reducing agents on the rheological properties of dough in the farinograph is also well documented in the literature (Bloksma and Bushuk 1988). This effect has been generally explained on the basis of reduction of "rheologically effective" disulfide bonds of gluten proteins. In a recent report, Ng and coworkers (1991) postulated that the effective disulfide bonds in the glutenin molecules are those that link "partially reduced glutenin oligomers" into polymeric glutenin. Each partially reduced oligomer comprises most or all of the glutenin subunits linked by disulfide bonds that are less rheologically effective. The exact structure of the partially reduced glutenin oligomers is yet to be elucidated.

In the present study, we have extended the work of Ng et al (1991) by examining fractions of partially reduced glutenin by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

Wheat Flour

The straight-grade flour used in this study was milled on a Buhler pneumatic laboratory mill from a pure variety of Canada Western Red Spring wheat (cv. Katepwa). The protein and ash contents of the flour were 14.5 and 0.54% (14% moisture basis), respectively.

Reagents

Dithiothreitol (DTT), a disulfide reducing agent, and *N*-ethylmaleimide (NEMI), a sulfhydryl blocking agent, were obtained from Sigma Chemical Company (St. Louis, MO). All other chemicals used were of reagent grade.

Farinograph Mixing

Mixing under air. Fifty grams of flour and 32.3 ml of water were mixed into a dough with 0 (control), 20, 80, 500, and 3,000 μmol of DTT. Immediately after mixing for 20 min, subsamples of the doughs were collected, frozen, freeze-dried, and ground manually with a mortar and pestle. The ground samples were stored in sealed containers in a refrigerator.

Mixing under nitrogen (N_2). Fifty grams of flour and 3,000 μmol of DTT were placed in a farinograph mixing bowl, gently flushed with nitrogen, and stirred frequently for 20 min. Water (32.3 ml) was added, and mixing was continued under nitrogen. Analogous doughs were prepared to which 500 or 2,000 μmol of NEMI was added after 3 min of mixing with 3,000 μmol of DTT. Doughs with or without NEMI were collected immediately, sealed in a container that had been flushed with N_2, frozen, and freeze-dried. After freeze-drying, the freeze-drier was brought to atmospheric pressure by adding nitrogen through the bleeding valve. Samples were sealed, removed from the freeze-drier, and stored in a freezer.

Electrophoresis

SDS-PAGE was used according to Ng and Bushuk (1987), with or without 2-mercaptoethanol (ME) in the SDS-PAGE extracting buffer.

For the determination of subunit composition of the dimeric bands, the region containing the bands was excised with reference to a flanking lane that had been previously cut out and stained as in the conventional SDS-PAGE procedure. The gel piece con-

[1]Publication 204 of the Department of Food Science, University of Manitoba, Winnipeg, MB R3T 2N2 Canada.

taining the bands was subsequently ground in a mortar and pestle, extracted with SDS-PAGE extracting buffer (with ME), and centrifuged at 20,000 × *g* for 30 min. The supernatant was analyzed by SDS-PAGE.

For the two-step electrophoresis, the desired lane of gel was excised after the first step and prepared for the second step as described by Singh and Shepherd (1985).

RESULTS AND DISCUSSION

Dough Mixing in Farinograph

From the farinograms in Figure 1, it can be seen that a small amount of disulfide reducing agent (e.g., 20 μmol of DTT) caused a drastic reduction in dough consistency. The effect on dough consistency seemed to be saturated at about 80 μmol of DTT. The farinograms for 500 μmol of DTT (not shown) were essentially the same as those for doughs treated with 80 μmol of DTT. This observation is consistent with those of Jones and Carnegie (1969), who used the farinograph to determine the proportion of the rheologically effective disulfide groups in wheat flour.

For the doughs treated with 3,000 μmol of DTT and mixed under N_2, the change in consistency was similar to that for analogous doughs mixed under air, except that there was a gradual, although small, increase in consistency beyond 5 min of mixing (results not shown), probably due to the loss of dough moisture caused by N_2 flushing. NEMI, a sulfhydryl blocking agent, had no discernible effect on the farinograms of doughs containing 3,000 μmol of DTT and mixed under nitrogen (results not shown).

Electrophoretic Results for Reduced Doughs

The electrophoretograms for the extracts of the partially reduced doughs (Fig. 2) showed that no HMW glutenin subunits were released, in spite of the drastic drop in farinograph consistency, upon addition of 20 μmol of DTT. This observation is consistent with the earlier findings of Ng et al (1991).

For the samples mixed with higher levels of DTT, the HMW glutenin subunits appeared gradually at differential rates with increasing DTT concentration. The pattern and the band intensity of the HMW glutenin subunits of the partially reduced doughs were quite different from those of the completely reduced sample. Difference in susceptibility of glutenin disulfides to DTT suggests variability in spacial arrangement of subunits in the microenvironment of the polymeric glutenin molecules.

At higher levels of DTT (80–3,000 μmol), we observed at least three closely migrating bands, with mobilities slightly lower than that of the largest HMW glutenin subunit (2* for cv. Katepwa). The nature of these bands is discussed below.

It is noteworthy that the closely migrating low-mobility bands migrated into the gel under conditions where a large amount of protein was retained at the front of the separating gel. This suggests that the low-mobility bands are intermediate products of further reduction of partially reduced glutenin oligomers upon exposure to additional DTT.

Experiments were undertaken to exclude oxygen and to block the free sulfhydryl groups that were produced upon addition of DTT during mixing to investigate the possibility of the low-mobility bands being formed during mixing by oxidation or by reassociation of glutenin subunits produced by reduction with DTT. The dough samples were analyzed for these closely migrating low-mobility bands. As shown in Figure 2 (lanes 7–10), these bands appeared in the electrophoretograms of doughs mixed in the presence of DTT with or without the sulfhydryl blocking agent (NEMI). Accordingly, it was concluded that the low-mobility bands exist as part of the structure of the glutenin polymer and are not an experimental artifact.

SDS-PAGE of Low-Mobility Bands

To determine the subunit composition of the low-mobility bands, the bands were analyzed by SDS-PAGE after their complete reduction with ME (Fig. 3). The results showed that these bands contained all five HMW subunits and no low molecular weight (LMW) subunits. Two-step electrophoresis (results not shown) produced similar evidence.

On the basis of the relative molecular weights (by SDS-PAGE) of the low-mobility bands and the HMW glutenin subunits, it was estimated that the following dimeric combinations of the HMW subunits fit the molecular weights of the three low-mobility bands: 2*+7, 2*+9, and 5+10. These combinations remain to be verified by other analytical methods.

Lawrence and Payne (1983) reported analogous closely migrating bands, named "O bands," which resulted from partial reduction of ground wheat endosperm before electrophoresis. Whether or not the bands described here, obtained by a different procedure, are the same as the O bands remains to be established.

Also illustrated in our result (Fig. 3, lane 3), derived from the gel piece containing the dimeric bands, is a band with a slightly lower mobility than that of the largest HMW glutenin subunit

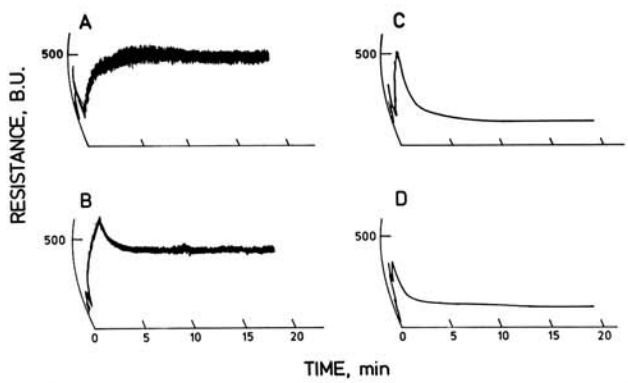

Fig. 1. Farinograph curves for partially reduced doughs. **A,** Flour (50 g) and water (control); **B,** control + 20 μmol of dithiothreitol (DTT); **C,** control + 80 μmol of DTT; **D,** control + 3,000 μmol of DTT.

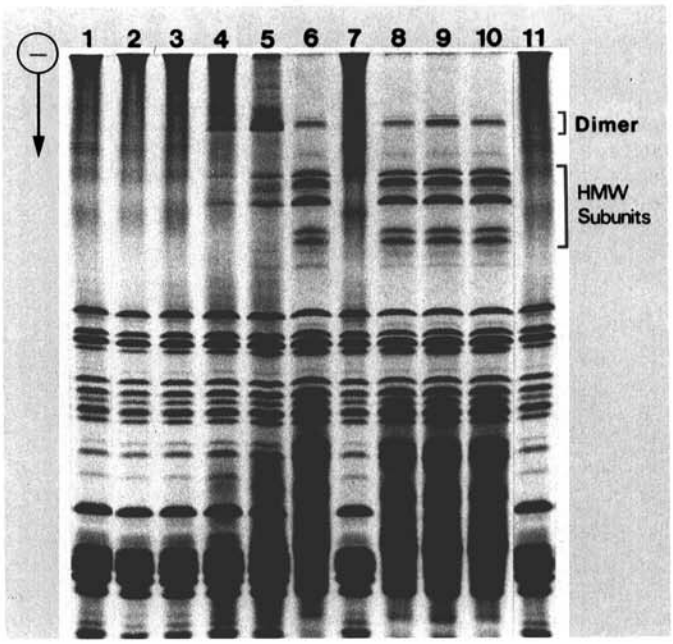

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of the partially reduced doughs mixed under air (lanes 2–6) or nitrogen (lanes 7–10). Lanes 1 and 11, control flour; 2, flour and water (control dough); 3, control dough + 20 μmol of dithiothreitol (DTT); 4, control dough + 80 μmol of DTT; 5, control dough + 500 μmol of DTT; 6, control dough + 3,000 μmol of DTT; 7, control dough + 500 μmol of *N*-ethylmaleimide (NEMI); 8, control dough + 3,000 μmol of DTT; 9, control dough + 500 μmol of NEMI and 3,000 μmol of DTT; 10, control dough + 2,000 μmol of NEMI and 3,000 μmol of DTT. Dimer = Position indicating the region with dimeric bands (see text); HMW = high molecular weight.

(2*) but with a similar band intensity in the electrophoretogram, suggesting its close association with the HMW subunits and/or with their dimers. On the basis of mobility in SDS-PAGE, this band may be one of the E-type bands reported by Gupta and Shepherd (1987). The nature of its association with the HMW glutenin subunits is not known.

It is interesting to note that the release and accumulation of the dimeric bands as shown by SDS-PAGE seem to be related to the small drop in farinograph consistency beyond the marked decrease upon first addition of a small amount of DTT. At a low level of DTT, the dimers were not seen. After the consistency reached a plateau at about 80 μmol of DTT, the bands appeared and were present at all higher levels of DTT. This result suggests that the disulfide bonds that are reduced to produce dimeric bands are of the rheologically ineffective type.

Results of Two-Step SDS-PAGE

The bottom part of the two-step gel for the dough mixed with 500 μmol of DTT (Fig. 4) shows a few faint, but clearly visible, bands corresponding in mobility to the region of the LMW glutenin subunits. These bands are not artifacts because parallel experiments for doughs that were reduced with 3,000 μmol of DTT and analyzed under the same conditions repeatedly showed that only the dough reduced with 500 μmol of DTT showed these bands. This result may explain why Graveland et al (1985), who used a higher concentration of reducing agent, did not detect any LMW glutenin subunits.

Besides the LMW glutenin subunits, the two-step gel also showed bands corresponding to the HMW glutenin subunits. The bands in the gel of the first step, with relative molecular weights larger than those of the dimeric bands, appear to be oligomers composed predominantly of HMW glutenin subunits. The production of these oligomers suggests that the disulfide bonds that link HMW glutenin subunits in polymeric glutenin are less susceptible to reduction than the disulfides between the LMW and HMW glutenin subunits or between the LMW glutenin subunits. Differential susceptibility of disulfides to reduction would lead to a differential release of LMW glutenin subunits with increasing concentration of reducing agent.

Additionally, the region between the dimeric bands and the HMW subunits in the first-step gel showed bands of oligomers comprising LMW glutenin subunits and a single HMW glutenin subunit. Obviously, some LMW glutenin subunits appear to be tightly linked to a single HMW subunit in the polymeric structure of glutenin.

CONCLUSIONS

SDS-PAGE results for doughs reduced with low concentrations of DTT (20 μmol of DTT/50 g of flour) showed that no breakdown products can be visualized by this analytical method. However, farinograph results for the same doughs showed a marked drop in dough consistency, suggesting a marked decrease in molecular size of the component(s) responsible for the consistency of dough. The key component in this regard is presumed to be glutenin.

At higher concentrations of DTT (80 or more μmol/50 g of flour), a group of dimeric bands was observed of known HMW glutenin subunits and another polypeptide that may be equivalent to one of the E bands reported by Gupta and Shepherd (1987); the concomitant additional drop in farinograph consistency was small.

The observation that the steady-state farinograph consistency leveled off to a constant minimum at a concentration of DTT equivalent to only a fraction of the total disulfide content in dough supports the hypothesis on the presence in gluten (dough)

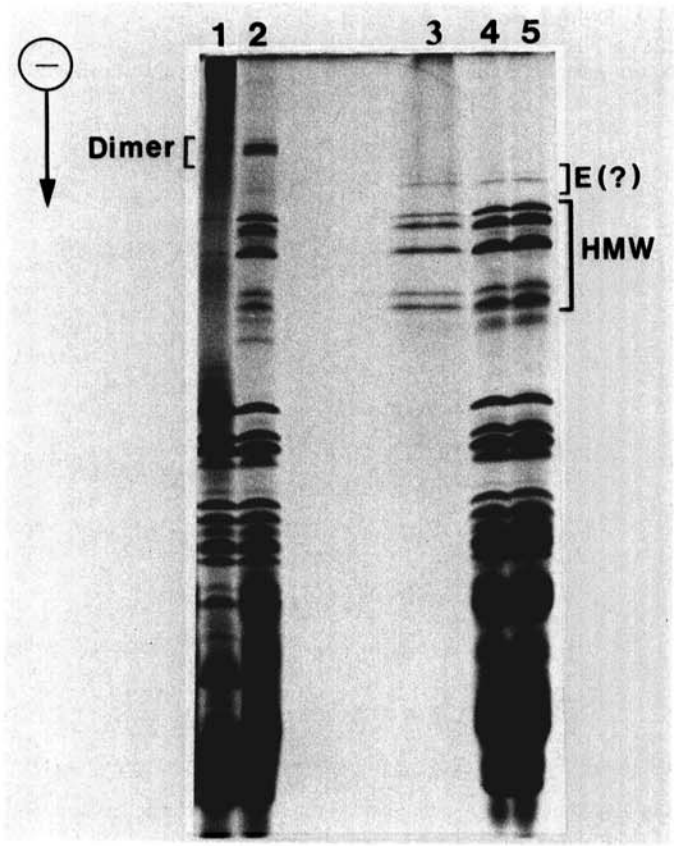

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of the dimeric bands. 1, Control flour; 2, dough with 3,000 μmol of dithiothreitol; 3, extract of gel portion containing dimeric bands; 4, dough mixed with 3,000 μmol of dithiothreitol and 2-mercaptoethanol in extracting buffer; 5, control flour with 2-mercaptoethanol in extracting buffer. Dimer = Position indicating the region with dimeric bands (see text); E(?) = position indicating the region with E bands as reported by Gupta and Shepherd (1987); HMW = high molecular weight.

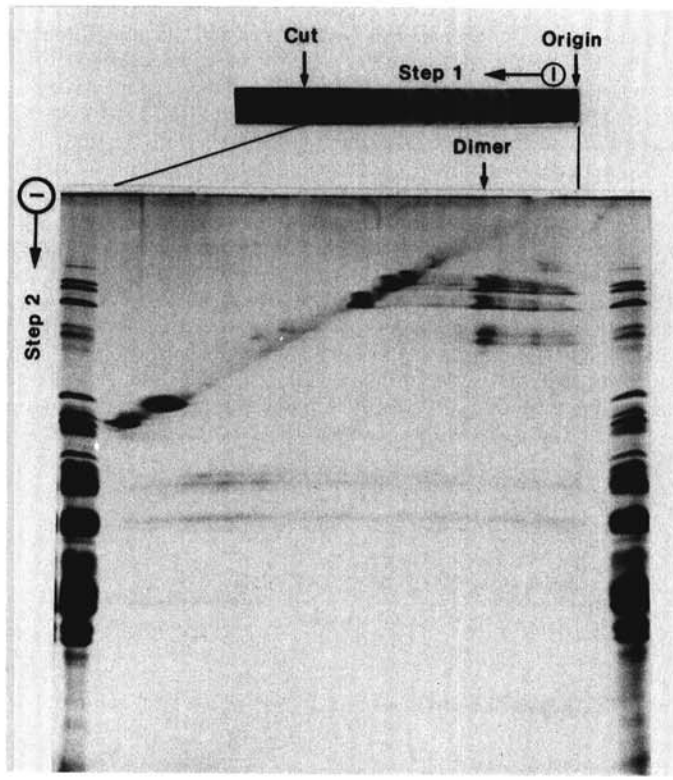

Fig. 4. Two-step electrophoretic pattern for partially reduced dough with 500 μmol of dithiothreitol. Origin = Position indicating the front of the separating gel in the first step; Dimer = position indicating the closely migrating dimeric bands in the first step. The two flanking lanes are for control flour (with 2-mercaptoethanol).

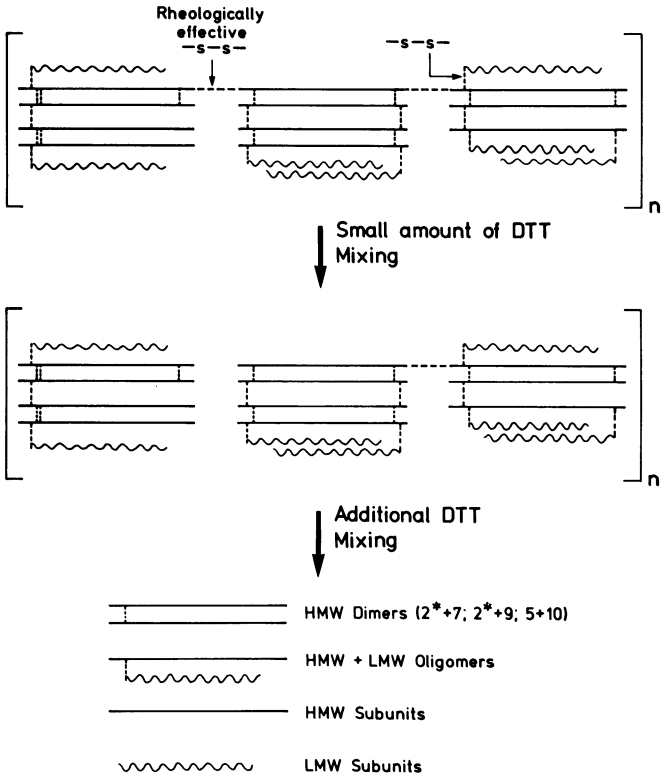

Fig. 5. A hypothetical model for the polymeric structure of glutenin and its breakdown upon exposure to increasing amounts of dithiothreitol (DTT). HMW and LMW = High and low molecular weights, respectively.

of rheologically effective disulfide bonds. We postulate that these bonds join unique structural blocks (partially reduced oligomers) of glutenin subunits forming polymeric (molecular) glutenin. The release of dimeric bands with further addition of DTT suggests that these bands result from reduction of rheologically ineffective disulfide bonds within the structural blocks. The depolymerization of the structural blocks did not contribute substantially to the rheological properties as measured on the farinograph.

The accumulation of the dimeric bands occurred in much higher concentrations than that of other oligomers that entered the SDS-PAGE gel. This differential appearance of breakdown oligomers suggests that there are, in polymeric glutenin, relatively stronger linkages between the two specific HMW glutenin subunits that appeared as dimers. The fact that the HMW glutenin subunit dimers are linked in certain combinations in polymeric glutenin is further confirmed by Werner et al (in press).

The two-step electrophoresis (Fig. 4) showed the presence of two other oligomers: 1) those composed predominantly of HMW subunits and 2) those of LMW subunits and a single HMW subunit.

Results of the present study suggest that polymeric glutenin comprises unique submolecular structural units that can be identified as distinct partially reduced glutenin oligomers. The type of the oligomer produced apparently depends on the concentration of reducing agent added. The first group of distinct oligomers is produced by the reduction of the rheologically effective disulfide groups. These are HMW oligomers and contain all or most of the subunits of total glutenin. Further reduction, at higher concentrations of DTT, produced other unique oligomers of gradually decreasing molecular weight. The oligomers that have been identified in the present study include dimers of specific HMW subunits, oligomers of all five HMW glutenin subunits, and oligomers of several LMW subunits and only one HMW subunit. The oligomers of all five HMW subunits may contain all five subunits linked together or a mixture of oligomers that as a whole contain all the HMW subunits.

The new evidence presented in this paper is incorporated into the modified model of polymeric glutenin shown in Figure 5, in which the HMW glutenin subunits are linked by disulfide bonds at the ends as suggested by the known evidence that the cysteine residues are mostly located on the ends of the subunits (see Kasarda 1989 and references therein). This model is somewhat different from that of Graveland et al (1985). It can better explain 1) the differential release of HMW glutenin subunits and production of glutenin oligomers and 2) the relationship of the glutenin structure to rheological properties as measured with a farinograph, especially the marked drop in consistency upon addition of small amounts of DTT without production of glutenin (monomeric) subunits. Further work is required to determine the precise details of the proposed model at the molecular level of structure.

ACKNOWLEDGMENTS

We thank D. D. Kasarda for discussions after the presentation of the content at the AACC 76th Annual Meeting. Financial assistance from the Natural Sciences and Engineering Research Council of Canada, and the University of Manitoba in the form of a postgraduate fellowship to L. Gao, is gratefully acknowledged.

LITERATURE CITED

BLOKSMA, A. H., and BUSHUK, W. 1988. Rheology and chemistry of dough. Pages 131-217 in: Wheat: Chemistry and Technology. Vol. 2. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

BUSHUK, W., and TKACHUK, R., eds. 1991. Gluten Proteins 1990. Am. Assoc. Cereal Chem.: St. Paul, MN.

EWART, J. A. D. 1988. Studies on disulfide bonds in glutenin. Cereal Chem. 65:95-100.

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 glutenins from wheat flour. J. Cereal Sci. 3:1-16.

GUPTA, R. B., and SHEPHERD, K. W. 1987. Interaction between genes controlling a new group of glutenin subunits in bread wheat. Theor. Appl. Genet. 74:459-465.

JONES, I. K., and CARNEGIE, P. R. 1969. Rheological activity of peptides, simple disulphides, and simple thiols in wheaten dough. J. Sci. Food Agric. 20:60-64.

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.

KASARDA, D. D., BERNARDIN, J. E., and NIMMO, C. C. 1976. Wheat proteins. Pages 158-236 in: Advances in Cereal Science and Technology. Vol. 1. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

KHAN, K., and BUSHUK, W. 1979. Studies of Glutenin. XII. Comparison by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of unreduced and reduced glutenin from various isolation and purification procedures. Cereal Chem. 56:63-68.

LAWRENCE, G. J., and PAYNE, P. I. 1983. Detection by electrophoresis of oligomers formed by the association of high-molecular-weight glutenin protein subunits of wheat endosperm. J. Exp. Bot. 34:254-267.

NG, P. K. W., and BUSHUK, W. 1987. Glutenin of Marquis wheat as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 64:324-327.

NG, P. K. W., and BUSHUK, W. 1988. Statistical relationship between high molecular weight subunits of glutenin and breadmaking quality of Canadian-grown wheats. Cereal Chem. 65:408-413.

NG, P. K. W., XU, C., and BUSHUK, W. 1991. Model of glutenin structure based on farinograph and electrophoretic results. Cereal Chem. 68:321-322.

PAYNE, P. I., HOLT, L. M., JACKSON, E. A., and LAW, C. N. 1984. Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 304:359-371.

SINGH, N. K., and SHEPHERD, K. W. 1985. The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. Theor. Appl. Genet. 71:79-92.

WERNER, W. E., ADALSTEINS, A. E., and KASARDA, D. D. In press. Composition of HMW-glutenin subunit dimers formed by partial reduction of residue glutenin. Cereal Chem.

[Received October 17, 1991. Revision received February 24, 1992. Accepted February 25, 1992.]