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

To the Editor:

The marked effects of disulfide reducing agents and sulfhydryl blocking agents on the properties of dough as reflected by the farinograph test are well documented in the literature (Bushuk 1961, Bloksma 1972, Bloksma and Bushuk 1988). The effect of reducing agents has been explained on the basis of the reduction of the so-called rheologically effective disulfide bonds of gluten proteins (Bloksma and Bushuk 1988). Although these bonds are generally presumed to be the interpolypeptide bonds of glutenin, their precise nature has not been determined. In this context, we analyzed by electrophoresis the proteins of doughs that had been mixed in the farinograph in the absence and presence of disulfide reducing agents and sulfhydryl blocking agents. The main aim of this experiment was to determine whether the reduction, reflected by a drastic drop in dough consistency, produces high molecular weight (HMW) subunits of glutenin. This communication reports the results obtained.

The straight-grade flour used in this experiment was milled on a Buhler pneumatic laboratory mill from the grain of a pure variety (cv. Katepwa) of Canadian hard red spring wheat. The flour yield was 71%; its ash and protein contents, respectively, were 0.54 and 14.5% (14% mb).

Fifty grams of flour and 32.3 ml of water were mixed into a dough in a farinograph mixer in air for 20 min. Five doughs were prepared: A, flour and water (control); B, control plus 100 μmol of *N*-ethylmaleimide (NEMI, a sulfhydryl blocking agent obtained from Sigma Chemical Co., St. Louis, MO) added at 15 min; C, B plus 20 μmol of dithiothreitol (DTT, a disulfide reducing agent obtained from Sigma) at 0 min; D, B plus 40 μmol of DTT at 0 min; and E, B plus 80 μmol of DTT at 0 min. An analogous set of doughs was mixed, in which 2-mercaptoethanol (Sigma), another disulfide reducing agent, was used instead of DTT, at the same molar concentration levels.

Subsamples of the doughs were frozen, freeze-dried, and ground manually with a mortar and pestle into a fine, flourlike powder. Proteins of the samples were analyzed by polyacrylamide gel electrophoresis (PAGE), according to Sapirstein and Bushuk (1985), and by sodium dodecyl sulfate (SDS)-PAGE with or without reduction by 2-mercaptoethanol before electrophoretic analysis, according to Ng and Bushuk (1987). The SDS-PAGE analyses were terminated when the tracking dye reached the bottom of the gels to ensure that the protein components of low molecular weight remained in the gel. The original flour was analyzed along with the dough samples.

Farinograph results (Fig. 1) showed the well-known effects of NEMI and DTT on mixing properties. The farinograph and electrophoresis results for 2-mercaptoethanol treatments were the same as those for DTT and hence will not be considered further. The mechanism by which NEMI affects dough consistency in a way similar to that of DTT is not completely understood. One would expect that when a single bond, linked between two oligomers, has been broken in the middle of the polymer, the viscosity of the polymer would be reduced by roughly half. The farinograph results (Fig. 1, C and D), which indicate that 20 and 40 μmol of DTT added to the dough system produced roughly 400 and 200 BU, respectively, after 5 min of mixing, are consistent with this prediction.

PAGE patterns (not shown) for the five dough samples and the original flour were the same. Accordingly, the changes that lead to the drastic change in farinograph dough consistency do not involve changes in the gliadin proteins that are resolved by the PAGE electrophoregrams.

Electrophoregrams obtained by the SDS-PAGE procedure in which the 2-mercaptoethanol was omitted (Fig. 2, unreduced), clearly did not show the presence of HMW subunits of glutenin in any of the doughs examined, including those treated with 80 μmol of DTT. It was not possible to determine whether any low molecular weight subunits of glutenin were present in the electrophoregrams, since their presence would be masked by gliadins that migrate to the same region.

The results presented here suggest that the drastic change in dough consistency caused by the addition of disulfide reducing agents does not involve reduction of glutenin to the polypeptide subunit level of structure. Therefore, we postulate that changes in rheological properties of dough within the range encountered in practical breadmaking involve a structural level that is intermediate between polymeric molecules and the single-chain polypeptide subunits, which are resolved by the standard (with reduction) SDS-PAGE technique. The intermediate structural units of glutenin will be referred to as "partially reduced glutenin oligomers" (C. J. Brock, *personal communication*).

According to this hypothesis, each partially reduced glutenin oligomer contains most (perhaps all) of the polypeptide subunits of native polymeric glutenin. This modified hypothesis on the structure of glutenin and the changes in the structure on partial reduction, which causes a decrease in dough consistency in the

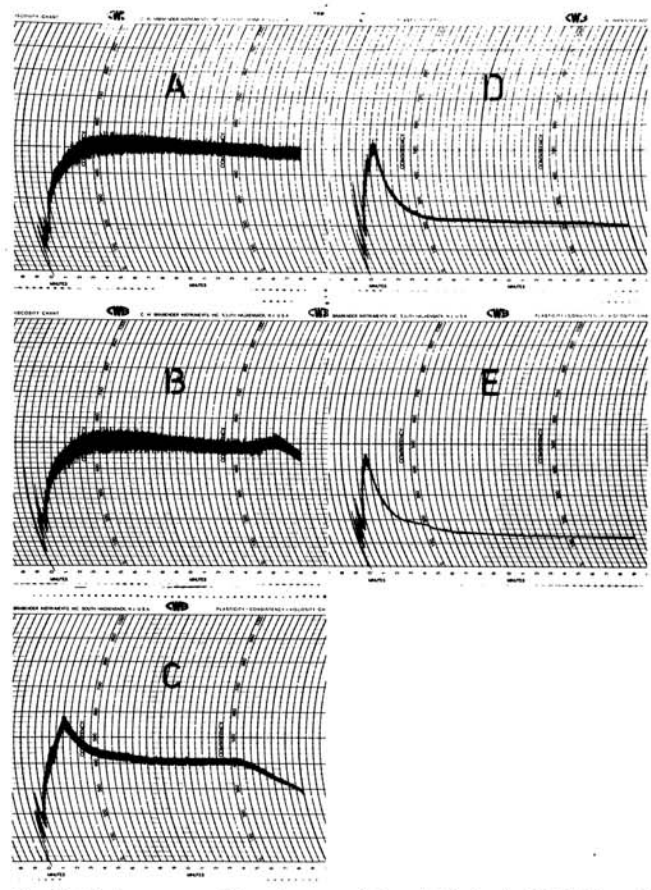

Fig. 1. Farinograms of doughs containing dithiothreitol (DTT) and N-ethylmaleimide (NEMI). A = flour and water (control), B = control plus 100 μmol of NEMI added at 15 min, C = B plus 20 μmol of DTT at 0 min, D = B plus 40 μmol of DTT at 0 min, E = B plus 80 μmol of DTT at 0 min.

[1]Publication 181 of the Food Science Department, University of Manitoba.

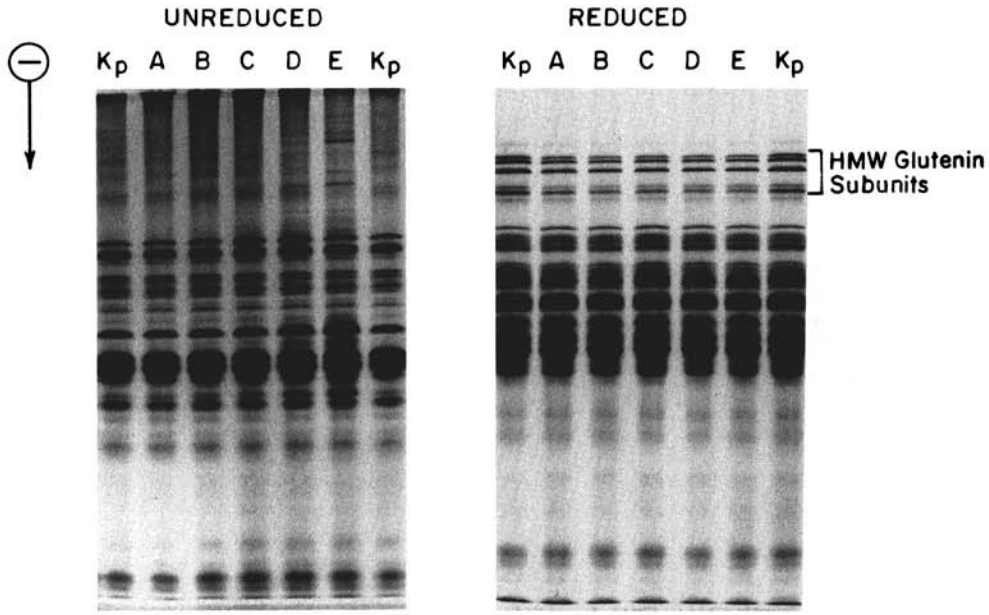

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of dough extracts analyzed under unreduced and reduced conditions. Lanes represent dough samples as follows: Kp = Katepwa flour, A = flour and water (control), B = control plus 100 μmol of NEMI added at 15 min, C = B plus 20 μmol of DTT at 0 min, D = B plus 40 μmol of DTT at 0 min, E = B plus 80 μmol of DTT at 0 min, HMW = high molecular weight.

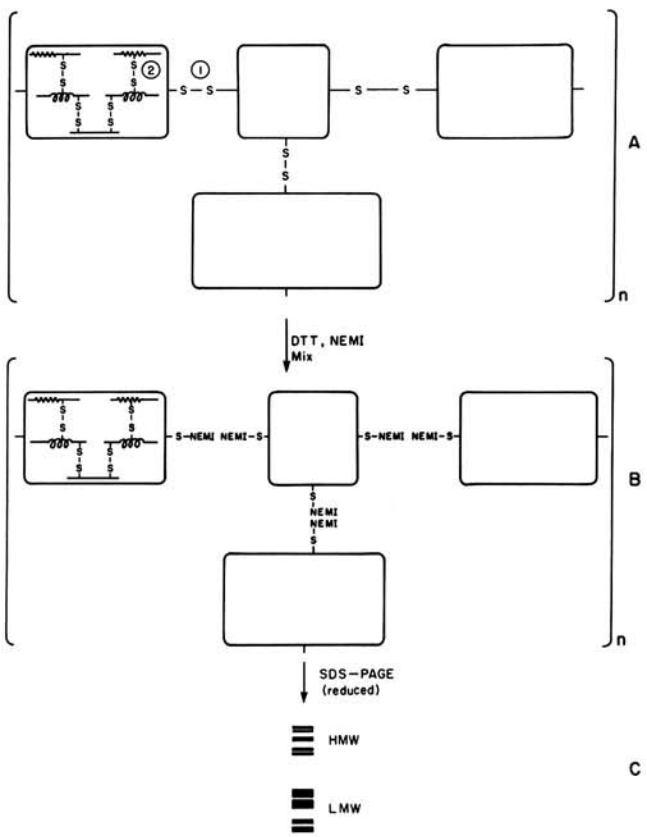

Fig. 3. A simple model of glutenin structure: A = glutenin oligomer, B = partially reduced glutenin oligomers, C = glutenin subunits, 1 = rheologically active disulfides, 2 = rheologically inactive disulfides, DTT = dithiothreitol, NEMI = N-ethylmaleimide, HMW = high molecular weight, LMW = low molecular weight.

range relevant to breadmaking technology, are shown schematically in Figure 3. The model for glutenin structure postulated here differs somewhat from those published by Ewart (1979), Khan and Bushuk (1979), and Graveland et al (1985). The model is offered in this preliminary publication in the hope that it will stimulate further research on the structure of functional glutenin.

ACKNOWLEDGMENTS

We thank C. J. Brock (Flour Milling and Baking Research Association, Chorleywood, England) for helpful suggestions for revising the manuscript. Financial assistance from the Natural Sciences and Engineering Research Council of Canada and the United Grain Growers Ltd. is gratefully acknowledged.

P. K. W. NG, C. XU, and W. BUSHUK
Food Science Department
University of Manitoba
Winnipeg, MB, Canada R3T 2N2

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[Received October 9, 1990. Accepted February 12, 1991.]