### COMMUNICATION TO THE EDITOR

## A New Perspective on Glutenin Structure Based on Fractionation by Free-Flow Preparative Isoelectric Focusing<sup>1</sup>

### To the Editor:

It is generally accepted that it is the collective properties of the proteins present in wheat flour that dictate its suitability for processing into bread. There is now substantial evidence that most of the intervarietal differences in breadmaking potential of wheat cultivars result from quantitative and qualitative differences in glutenin proteins (Orth and Bushuk 1972, Payne et al 1987, Ng and Bushuk 1988). However, little is known about the molecular (multi-subunit) structure of glutenin except that it incorporates, inter alia, many polypeptides (subunits) cross-linked by disulfide bonds. The extremely low solubility of glutenin complicates the isolation, fractionation, and characterization of its polymers by available techniques. Accordingly new techniques need to be developed.

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Recently we were introduced to a new free-flow isoelectric focusing (IEF) technique, based on the Rotofor apparatus (Bio-Rad Laboratories Limited, Mississauga, ON, Canada), to fractionate flour proteins in a free-solution pH gradient. The apparatus consists of a cylindrical focusing chamber with 20 compartments divided by membrane screens that maintain the separation when power is switched off prior to collection of the fractionated samples. This communication reports some preliminary results on structural relationships between the high molecular weight (HMW) glutenin subunits and disulfide bonds based on the fractionation by Rotofor apparatus.

Flour of cultivar Neepawa was used. Five grams of flour was dispersed into 55 ml of sample buffer solution, which consisted of 0.01M Tris-HCl at pH 8, 6M urea, 2% (w/v) sodium dodecyl sulfate (SDS), 2% (v/v) Nonidet P-40, and 2.5% (v/v) of Pharmalyte, pH 3-10. The mixture was stirred for 4 hr at room temperature and centrifuged at  $3,000 \times g$  for 20 min at room temperature. All the supernatant was then loaded into the Rotofor

# REDUCED UNREDUCED 8 9 10 12 14 15 Np Np 7 8 9 10 12 14 15 HMW **GLUTENIN** SUBUNITS GLIADINS + LMW GLUTENIN SUBUNITS + NON-STORAGE **PROTEINS**

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of reduced and unreduced Neepawa protein fractions obtained from free-flow preparative isoelectric focusing fractionation. Numbers along the top are the fraction numbers and Np is the total flour protein of Neepawa. The high molecular weight (HMW) glutenin subunits identified by numbers are according to Payne and Lawrence (1983). The arrow heads under unreduced fractions 8 and 9 indicate HMW proteins. LMW = low molecular weight.

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cell according to the manufacturer's manual. The anode and cathode solutions were  $0.1M~H_3PO_4$  and 0.1M~NaOH, respectively. The sample was focused overnight (about 17 hr) at a constant power of 18 W. Ethylene glycol was used as a coolant at  $-10^{\circ}$  C. After focusing, 20 fractions were collected according to the manufacturer's manual. Fractions 1 and 20 were at the anode and cathode terminals, respectively. The pH of the fractions was measured and ranged from 2.12 to 12.65. The low and high pH values at terminal fractions were most likely due to some diffusion of electrode buffers through the membranes into the Rotofor cell during the focusing (personal communication, B. Storeshaw). The fractions were freeze-dried.

The freeze-dried materials were first dispersed in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) buffer as described by Ng and Bushuk (1987) except without 2-mercaptoethanol. An aliquot of this dispersion was analyzed by SDS-PAGE under unreduced conditions. To another aliquot of the mixture, 2-mercaptoethanol was added to 5% (v/v), and the reduced proteins were analyzed by SDS-PAGE (Ng and Bushuk 1987).

SDS-PAGE patterns for fractions 7-10, 12, 14, and 15, and total protein of Neepawa flour (Np, used as the standard reference) are shown in Figure 1. Fractions 1-5 produced SDS-PAGE patterns that were essentially the same as those of total protein extracts of flour. Fraction 6 contained very little protein. Fraction 11 was a mixture of fractions 10 and 12, and similarly, fraction 13 was a mixture of fractions 12 and 14. Fractions 16-20 contained essentially no protein. Accordingly, SDS-PAGE results for fractions 1-5, 6, and 16-20 are not reported.

Our results (Fig. 1) showed that the HMW subunits of glutenin form several different polymers that have different isoelectric points (pI) and hence can be fractionated by isoelectric focusing. While there is some overlap in the composition of neighboring fractions, fractions with distinctly different subunit compositions have been isolated. For example, fractions 8, 9, and 10, with acidic pI ranging from 5.02 to 6.25, contained primarily subunits 2\* and 5 (nomenclature of Payne and Lawrence 1983), whereas fraction 15, with basic pI 8.10, contained mainly polymers of subunits 7, 9, and 10 and only trace amounts of polymers with subunits 2\* and 5.

SDS-PAGE patterns for the same fractions separated without reduction of disulfide bonds showed that flour protein also contains small quantities of certain HMW proteins in single polypeptide form. Fractions 8 and 9 contained bands (Fig. 1, arrow heads) that have similar mobilities as subunits 2\* and 5 in the

respective fractions of reduced glutenin.

Our results suggest that molecules of glutenin, as well as being widely polydisperse in molecular weight, are heterogeneous in composition (i.e., number and type of subunits). In addition to polymeric molecules comprising different numbers and different types of subunits, some HMW proteins exist in flour in single polypeptide form. The extent to which these structural features are genetically controlled and regulated and how they are related to the breadmaking potential of a wheat variety remain to be elucidated. Finally, our results showed that the Rotofor apparatus can be used effectively for fractionation of wheat storage proteins including the glutenins.

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