

cell according to the manufacturer's manual. The anode and cathode solutions were 0.1M H₃PO₄ 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°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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