

Influence of Gliadin-Rich Subfractions of Glenlea Wheat on the Mixing Characteristics of Wheat Flour^{1,2}

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ABSTRACT

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The effect of gliadin-rich subfractions of extra-strong wheat on the mixing properties of Canada Prairie Spring (CPS) wheats and Canada Western Extra Strong Red Spring wheat (CWES) cv. Glenlea was determined by the 2-g mixograph. Thirteen subfractions isolated from the single ethanol extract of Glenlea showed differences in their SDS-PAGE patterns of total proteins, low molecular weight glutenin subunits, the ω -gliadin component, and acid-PAGE electrophoregrams. High molecular weight glutenin subunits were found only in one subfraction isolated by increasing the concentration of ethanol. Subfractions that remained solubilized in the water phase after removal of ethanol from the extract were deficient in ω -gliadins and contained a number of fast-moving protein bands. These fractions caused a significant delay (from 2.64 to 5.41 min and from 5.75 to 8.16 min) in the mixograph peak development of CPS and Glenlea flours, respectively. On the contrary, the water-insoluble subfractions reduced the mixing time requirement (from 2.6 to 1.08 min

and from 5.8 to 1.7 min for CPS and Glenlea flours, respectively) and caused a rapid decline in the dough stability as the mixing continued. Both base flours showed an increase in peak height with the addition of ethanol-extractable protein subfractions. Mixograph development time and energy to peak increased with the addition of water-soluble subfractions but decreased with water-insoluble subfractions of the 70% ethanol extract. The band width at peak increased when water-soluble subfraction 6.5 was added to CPS flour but decreased when it was added to Glenlea flour. Removal of ethanol-extractable components from flours resulted in loss of viscoelasticity. Adding subfraction 1.5 back to the flour residue caused a return of this physicochemical attribute. Addition of a non-water-dispersible subfraction (1.5) to CPS flour or CPS flour residue caused a significant increase in the formation of gluten. Approximately 35–42% of the added gliadins were incorporated into the gluten network of CPS flour and 34–52% into the flour residue.

It is generally accepted that the viscoelastic properties of wheat flour dough depend primarily on the dough's protein constituents (MacRitchie 1992), and prolamines are recognized as the most important functional proteins (Weegels et al 1996). During the preparation of gluten, removal of water-extractable components, including starch, from the dough system causes a further increase in the viscoelastic properties. The water-insoluble network commonly known as gluten is a complex chemical system predominantly containing glutenin and gliadin proteins (Bietz and Wall 1972). The glutenin is known to provide elasticity, whereas gliadin provides viscosity and extensibility in a dough system (Wall 1979, Mills et al 1990, Ciaffi et al 1996).

Dough properties play a significant role in the breadmaking process. During processing of flour to the end product, many physicochemical changes take place in the protein matrix as a consequence of hydration, conformational changes, interactions, and biochemical reactions. The identification of key ingredients influencing dough properties remains important for the improvement of end-product quality.

Some researchers believe that the overall functionality of proteins in obtaining desirable end-use qualities results mainly from the glutenin component (Payne et al 1979, Chakraborty and Khan 1988, Lawrence et al 1988, Weegels et al 1994) and that gliadin merely acts as a dilutant. Qualitative variation in gliadin is considered to have an insignificant effect on dough rheology. Others find gliadin important in governing the end-use quality because glutenin alone cannot explain the differences in the end-use quality of wheats (Hoseney et al 1969, Preston et al 1975, Branlard and Dardevet 1985, van Lonkhuijsen et al 1992, Hussain and Lukow 1993, Hou et al 1996). Gliadin proteins have the tendency to aggregate through noncovalent bonds (hydrophobic and/or ionic

bonds) and, depending on the physical conditions, a gliadin-rich alcohol extract can yield a number of subfractions. These subfractions, dissimilar in their polypeptide composition and physical attributes, may assert variable influences on dough mixing properties when added to a base flour (Huebner and Bietz 1993).

Recent research on the interactions of protein fractions is of interest because of the introduction of sensitive and computerized techniques (Weipert 1990, Bekes and Gras 1992, Gupta and MacRitchie 1994, Weegels et al 1994) and the accessibility of specific proteins. With these efficient microtechniques, it has become easier to identify changes in dough properties resulting from the addition of individual protein fractions to the reference flour.

Most of the earlier work on estimating the contribution of specific gliadins was based on statistical comparisons (Branlard and Dardevet 1985, Hou et al 1996). Reconstitution experiments with purified gliadins are needed to substantiate any relationship.

This study was made with the following objectives: 1) to separate the 70% alcohol extract into various subfractions and characterize their chemical composition and/or functionality; 2) to determine, by fortification, the functional contribution of each subfraction to the dough-mixing properties of selected base flours, and 3) to examine the role of gliadins in the formation of the gluten network.

MATERIALS AND METHODS

Flour Samples

Canada Western Extra Strong red spring (CWES) wheat, cv. Glenlea, was grown at Portage la Prairie, Manitoba, Canada, in 1995 and was milled into straight-grade flour using a Buhler experimental mill. The protein content was 13.7% (14% mb). This flour was used for gliadin extraction and as a base flour for the addition of gliadin subfractions. The second base flour was produced from a Canadian Prairie Spring (CPS) wheat composite with 10.7% protein (14% mb).

Protein Extraction and Fractionation

Preparation of 70% ethanol extracts. Glenlea flour (200 g) was shaken gently with three volumes of petroleum ether (bp 40–60°C) for 5–10 min at room temperature (22 ± 2°C). The excess

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solvent was removed, and the residue flour was air dried overnight in the fume hood. The defatted flour was extracted at ambient temperature with five volumes of 70% aqueous ethanol. The flour-ethanol mixture was stirred for 4 hr and was then filtered through an ethanol-washed (twice) paper towel. The filtrate was centrifuged at $8,500 \times g$ for 10 min at room temperature. The clear supernatant thus obtained was chosen as the starting material for further fractionation. All operations were done at room temperature except where indicated. To prevent denaturation of proteins, none of the extracts or protein subfractions was subjected to high temperatures. The fractionation procedure was performed in duplicate.

Fractionation of ethanol extract. Although the flour protein extraction by neutral 70% ethanol does appear to separate proteins into fractions differing markedly in average M_r , the separation is not clear cut and some degree of overlapping occurs. By altering critical temperatures and the balance of water and alcohol, the crude ethanol extract of Glenlea flour was made to generate 13 subfractions that differed in their polypeptide composition. The possible interaction forces facilitating precipitation could be hydrogen bonding, electrostatic interactions, and hydrophobic

interactions (association forces) between protein molecules or between solvent(s) and proteins (Holme and Briggs 1959). The schematic outlined in Fig. 1 was employed to obtain various subfractions. Six different procedures were used to study various factors affecting chemical and physical interactions among ethanol-extracted constituents. Wherever necessary, the soluble constituents present in the solution were recovered by vacuum concentration at 40°C using a rotary evaporator (Buchi, Rotavapor R110, Lab. Technik AG, Switzerland). The soluble and insoluble proteins were frozen, lyophilized, and ground to a fine powder using a mortar and pestle. Subsequently, the ground samples were passed through a $40\text{-}\mu\text{m}$ mesh to ensure uniformity of particle size. The protein content was determined by the micro-Kjeldahl procedure according to AACC method 46-12 (AACC 1995) modified by Williams (1993) using the Kjeltac System I (Tecator, AB Hoganas, Sweden). A nitrogen-to-protein factor of 5.7 was used to calculate total protein content.

Protein Separation

SDS-PAGE. SDS-PAGE of the Glenlea flour sample and the ethanol-extractable subfractions was done under reduced and

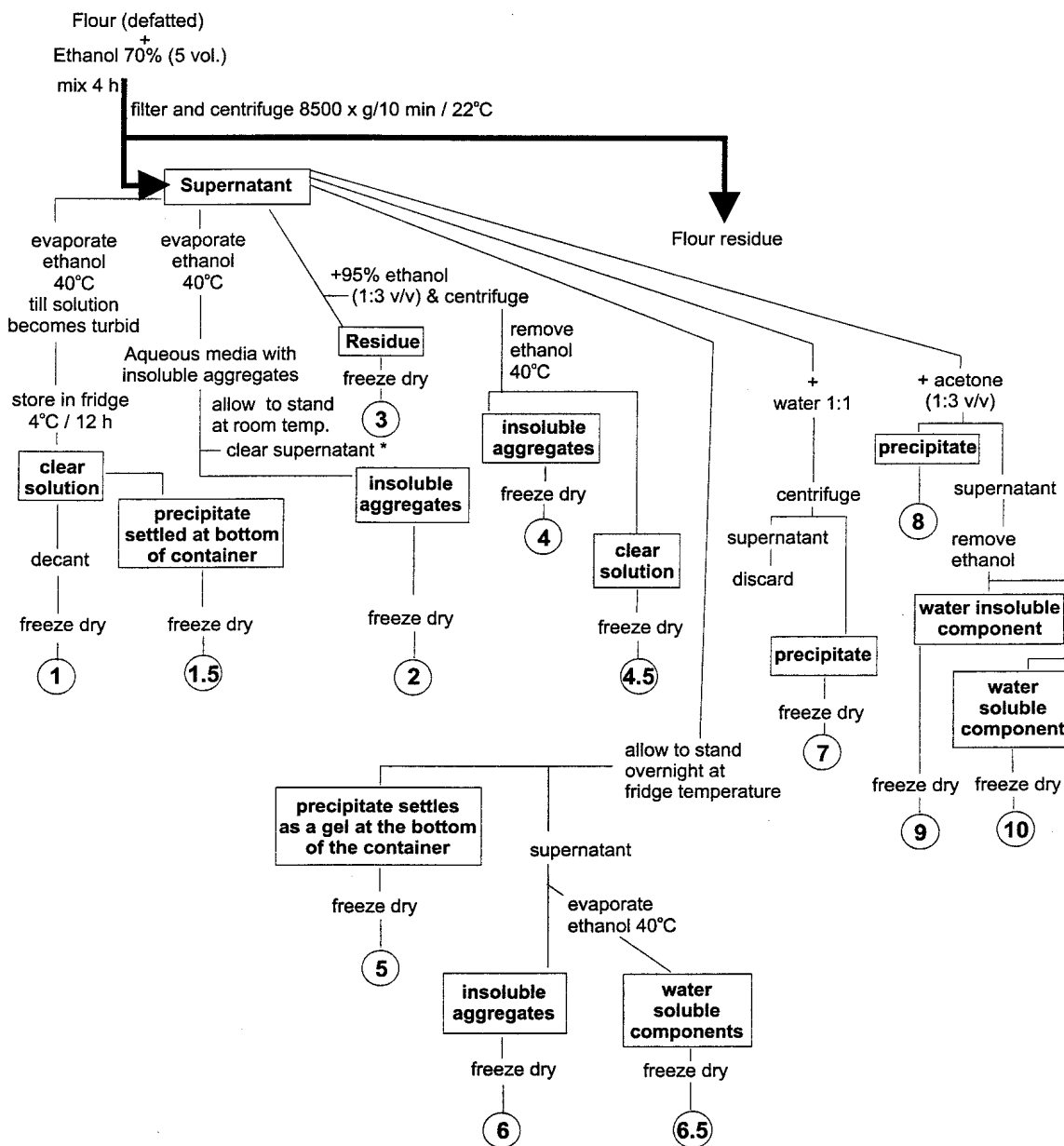


Fig. 1. Procedure for extraction and separation of various subfractions from the 70% ethanol extract of Glenlea endosperm proteins.

unreduced conditions in a 10% polyacrylamide gel (Hussain and Lukow 1993). The acrylamide gel solution contained sucrose (6.5%), acrylamide (9.85%), bis-acrylamide (0.15%), SDS (0.1%), and Tris HCl, pH 8.8 (37.6%, v/v). The final volume of the gel solution was adjusted with distilled deionized water. Freeze-dried protein samples (2 mg/mL) were dissolved in SDS-sample buffer containing 2-mercaptoethanol. Reducing agent in the sample buffer was omitted for the unreduced samples. Depending on the protein content of the dissolved subfraction, 5–15 μ L was applied to the acrylamide gel for electrophoresis.

Acid-PAGE. The gliadin composition of subfractions selected on basis of dissimilarities of SDS-PAGE patterns was also determined at pH 3.1 in the presence of aluminum lactate, as described elsewhere (Sapirstein and Bushuk 1985). Glycerol (10%) was added to the gel solution to improve protein band quality (Hussain and Bushuk 1991). A locally constructed electrophoresis unit (Hussain et al 1988) was used to cast and run 3-mm thick acid-PAGE gels. A Neepawa standard was used to designate various subgroups of gliadins (Bushuk and Sapirstein 1991).

Electrophoresis of ω -gliadin and low molecular weight glutenin subunits. D-zone ω -gliadin and low molecular weight (LMW) glutenin subunits were separated according to Hussain and Lukow (1993) and Lukow et al (1994), respectively. In this methodology, the freeze-dried subfractions were sequentially extracted with

50% propanol and 50% propanol containing a bond-breaking reagent (dithiothreitol). The 50%-propanol extract was used to separate ω -gliadins, whereas the extract containing dithiothreitol was used for separation of LMW glutenin subunits. Both monomeric and polymeric proteins were separated in 10% polyacrylamide gel by SDS-PAGE.

Gliadin Fortification and Mixing Effects

Changes in the dough-mixing properties of the base flours were determined using a direct-drive 2-g computer-assisted mixograph (National Manufacturing Div., TMCO, Lincoln, NE). The two base flours were supplemented with the specific amount of subfractions required to increase protein content by 10% (MacRitchie

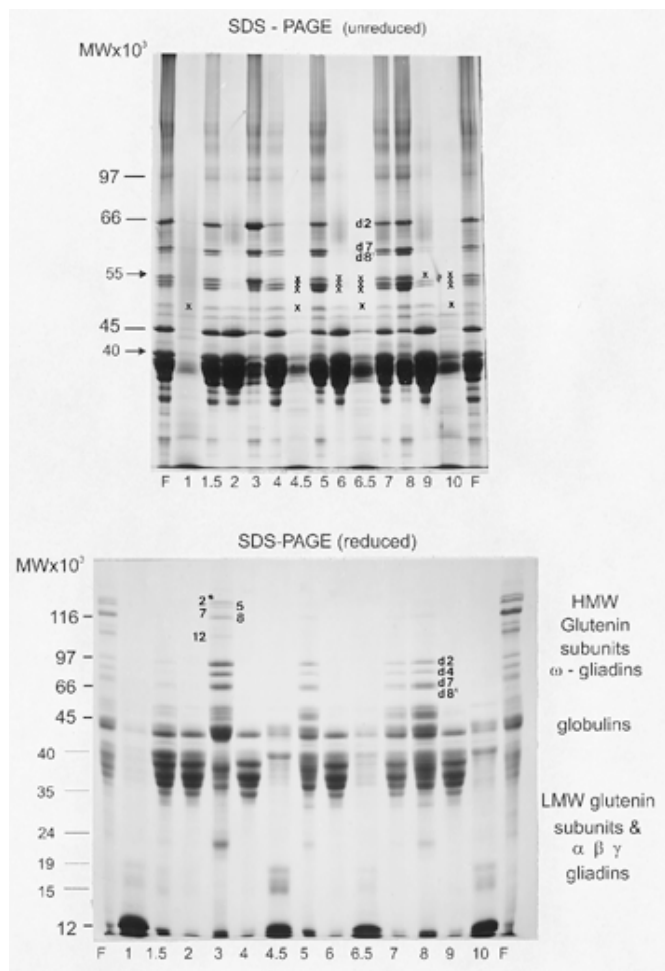


Fig. 2. SDS-PAGE patterns of 13 unreduced and (2-mercaptoethanol) reduced subfractions of 70% ethanol extract of Glenlea flour. From left, lanes 1 and 15 represent Glenlea-flour proteins (F) and lanes 2–14 represent subfractions 1, 1.5, 2, 3, 4, 4.5, 5, 6, 6.5, 7, 8, 9, and 10. Fraction numbers correspond to those of Fig. 1. Unreduced protein bands missing from electrophoregrams are represented by x. MW = molecular weight; HMW = high molecular weight; d2, d4, d7, and d8' = ω -gliadin.

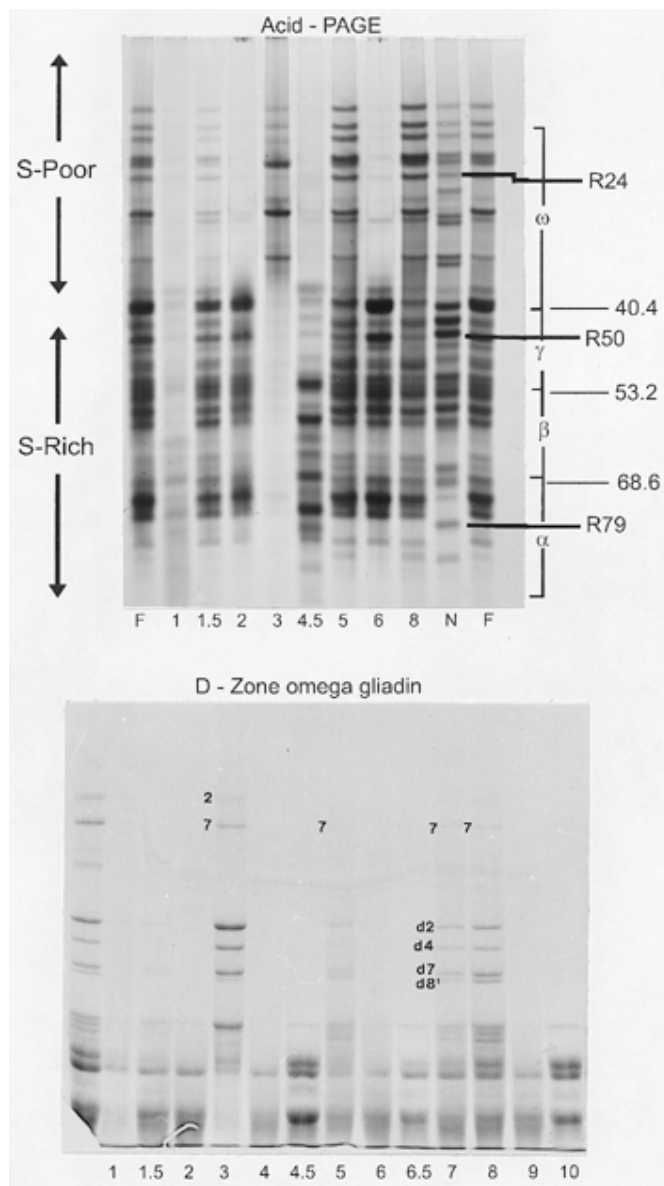


Fig. 3. Subfractions separated by acid-PAGE (top) and SDS-PAGE (bottom). **Top**, lanes 1 and 11 (labeled F) contain Glenlea flour; lanes 2–9, subfraction 1, 1.5, 2, 3, 4.5, 5, 6, and 8; and lane 10, Neepawa standard. Mobilities of gliadin subgroups are indicated by ω , γ , β , and α . The numbers given for protein bands mark boundaries between these subgroups. R indicates the Neepawa bands defined as having moved 24, 50, and 79 units into the (pH 3.1) polyacrylamide gel. **Bottom**, D-zone ω -gliadins identified as d2, d4, d7 and d8'. Note the high molecular weight glutenin subunit(s) coextracted with ω -gliadins in subfractions 3, 5, 7, and 8. Lane 1 (far left) represents Glenlea flour and lanes 2–14, various subfractions. Fraction numbers correspond to those of Figure 1.

1985). Since the protein content of the subfractions varied from 65 to 82%, the addition of freeze-dried subfractions ranged between 0.24 and 0.30 g of the 2-g mixograph sample.

In a separate experiment, water absorptions of the gliadin-fortified flours were determined using method 54-40A (AACC 1995), with water additions increased in samples that contained the additional proteins (fraction 1.5). An additional 1 mL of water was required for every 2% increase in the added protein in the 100-g flour sample. Therefore, dough water was increased from 1.24 to 1.34 mL to compensate for the protein increase in the 2-g mixograph (Gras et al 1990). This dough water absorption (67%) was kept constant for all test samples fortified with the gliadin subfractions. All results reported were the means of duplicate batches.

In mixograph studies requiring protein fortification, the lyophilized protein subfractions were allowed to swell in a predetermined amount of distilled water for 5 min in the mixograph bowl (temperature = 30°C) before the addition of base flour for dough mixing.

To determine the denaturing effect of 70% ethanol on the functional properties, Glenlea base flour was stirred for 4 hr with ethanol, and the resultant ethanol-flour suspension was air dried for 2 hr in the fume hood (without removing the alcohol extract). A corresponding set of flour samples was prepared in which 70% ethanol was removed by filtration and centrifugation. The two flours were frozen, lyophilized, ground, and sieved through 40-mesh screen before being subjected to the mixograph analyses. All mixing tests were performed in duplicate, and only the means were plotted for comparison.

Addition of Gliadins and Gluten Recovery

To determine the role of added gliadins in the formation of gluten, experiments were made on 2-g batches of CPS flour and CPS residue flour (retained after removal of ethanol extract). Fraction 1.5 was selected for this experiment mainly because it produced a mixograph curve with the shortest mix time, because it gave a higher yield, and because it could be easily prepared by thermally induced precipitation. Flour samples with and without the addition of 5, 10, 25, and 50% (w/w) gliadin fraction 1.5 were made into dough by gentle mixing with a spatula after the addition of distilled water. The amount of water was adjusted to account for the increased protein content of the gliadin-fortified blend. The flour residue samples and their blends with gliadins required less water (0.75 ml) to make dough similar in consistency to that made with the normal flour. The dough was rested for 30 min and then washed under running tap water to remove water-washable components, including starch. Wet gluten was centrifuged for 5 min at $8,500 \times g$ to remove unbound water. The wet gluten was weighed and freeze-dried. The amount of gliadin incorporated to form a gluten network was determined by calculating gain in dry gluten versus the added amount of subfractions.

RESULTS AND DISCUSSION

Characterisation of Subfractions

SDS-PAGE comparison of 13 reduced and unreduced subfractions demonstrate that most subfractions differed in their protein band pattern (Fig. 2). Analysis of the electrophoregrams revealed that polypeptides ranging between M_r 45 and 35 kDa were the major constituents of all subfractions of the 70% ethanol extract. All those fractions that remained in the solution after ethanol was evaporated from the extract (fractions 1, 4.5, 6.5, and 10) contained: 1) a fast moving band, closer to the dye front ($M_r < 12$ kDa) and 2) a set of three bands (M_r 19, 17, and 15 kDa). These subfractions also contained faint bands (Fig. 2) in the electrophoretic mobility region usually assigned to γ - and/or β -gliadin (M_r 40–30 kDa). Since the 70% ethanol extract was prepared by the direct extraction of flour, the water-soluble fractions would include

albumins and some globulins. However, the quantities of these proteins compared to gliadins were negligible. Preextraction of flour with water was avoided because a considerable proportion of ω -gliadins would have been lost in the water extract (Hussain and Lukow 1994). The SDS-PAGE patterns of reduced subfractions 4, 6, and 9 were similar (Fig. 2). However under nonreducing conditions (Fig. 2), a set of three bands (M_r 53–55 kDa) were absent in the patterns of subfraction 6 and one of these bands in subfraction 9. A set of these bands (M_r 53–55 kDa) and an additional (M_r 50 kDa) protein band (Fig. 2) were absent in all water-soluble subfractions (1, 4.5, 6.5, and 10). The d4, ω -gliadin band (M_r 68 kDa) observed in the reduced extracts (Khelifi et al 1992) of subfractions 3, 5, 7, and 8 was absent in the corresponding unreduced extracts (Fig. 2). Both reduced and unreduced SDS-PAGE patterns showed a fast-moving band (M_r 24 kDa) associated with the bands of ω -gliadins. The complete set of ω -gliadins was more conspicuous in subfraction 3, which was precipitated from the 70% ethanol extract by addition of 95% ethanol (1:3, v/v). This subfraction was also unique in containing the complete set of high molecular weight (HMW) glutenin subunits, 2*, 7+8, and 5+10 (Fig 2). HMW glutenin subunit 7, which is normally overexpressed in Glenlea, appeared in three subfractions 5, 7, and 8. HMW glutenin subunits other than subunit 7 appeared lightly stained or were absent in these subfractions. Glenlea flour preextracted with 70% ethanol also contained these HMW glutenin subunits (results not shown). Thus, it appears that HMW glutenin exists in different sizes of fragments: 1) smaller fragments that can be easily extracted in ethanol, 2) medium size fragments that are extracted only when glutenin polymer is reduced, and 3) larger fragments that are unextractable by aqueous alcohol or alcohol containing reducing agents. However, the electrophoretic mobility of these subunits in acrylamide gel remains unaltered by the size of polymers. Similar results were obtained by Bietz and Wall (1980).

The gliadin composition of the samples was determined with acid-PAGE, pH 3.1, to ascertain that subfraction 3 had a unique gliadin composition (Fig. 3); α -, β -, and γ -gliadins were absent in this subfraction. All other subfractions analyzed by the acid-PAGE system contained varying amounts of γ -, β -, and α -gliadins. The acid-PAGE patterns of water-soluble fractions 1 and 4.5 showed the absence of ω -gliadin bands. However, subfraction 4.5 contained lightly stained bands in the γ -gliadin region compared to the intensely stained bands of β - and α -gliadins (Fig. 3). The two fractions (1 and 4.5) differed in the staining intensities of their polypeptides. On the other hand, the water-insoluble subfractions 5 and 8 contained all four gliadins of staining intensities comparable to those shown by the Glenlea flour (first and last lanes). Visual evaluation of the SDS-PAGE of the D-zone ω -gliadins (Fig. 3) shows that the staining intensity of protein band d8' was much lower than the intensities of protein bands, d2, d4, and d7.

LMW glutenin electrophoregrams of the 13 subfractions are not presented because small amounts of the LMW glutenin subunits were extracted in 70% ethanol, and that produced very faint bands. However, subfractions 1.5, 3, 5, and 6 contained this group of proteins. Subfraction 3 contained only band 50 of the LMW glutenin subunits; other subunits of LMW glutenin unique to Glenlea (viz. 30, 20, 10, and 8) (Lukow and Townley-Smith 1996) were missing in this fraction. However the presence of d2, d4, and d7 ω -gliadins were quite noticeable in this particular subfraction, even after preliminary washing with 50% propanol. Ethanol 70% (v/v) predominantly extracts gliadins, but certain nongliadin components are coextracted. Therefore, it is likely that some of these subfractions may contain certain nongliadin components.

The overall distribution of the various polypeptides in the subfractions prepared from 70% ethanol extract of Glenlea flour is summarized in Table I. The results reported above consistently point to the unique polypeptide composition of subfraction 3. These results were in agreement with those of Meredith (1965),

who reported loss of solubility of ω -gliadins with increased concentration of alcohol. All preparations except subfraction 3 contained α -gliadin. With the exception of fractions 1 and 3, all subfractions contained γ - and β -gliadin subgroups. Only four subfractions (3, 5, 7, and 8) contained all bands of the ω -gliadin subgroup. A faint band of ω -gliadin (d7, Fig. 3) can be recognized in water-soluble subfractions 1.5 and 10. The work described here

does not provide quantitative data, as the complete removal of precipitates was not possible. Thus the removal of HMW gliadins (ω -gliadins) and HMW glutenin subunits can be scaled up by increasing the concentration of alcohol in a 70% ethanol extract.

Gliadin Fortification and Dough Mixing

The 2-g mixograph curves of the original base flours and of Glenlea flours supplemented with water-soluble or -insoluble gliadin subfractions are presented in Fig. 4. The addition of water-soluble subfraction 4.5 caused a significant delay (from 5.75 to 8.16 min) in the peak mixograph development time (MDT), whereas the addition of non-water-dispersible (alcohol-soluble) fraction 3 resulted in a shorter mixing requirement to attain maximum resistance (from 5.75 to 1.70 min), followed by a rapid drop in curve height as the mixing continued. Similar results were obtained with the CPS base flour supplemented with subfractions 1 and 1.5 (results not presented). Variation in the rate of dough development could be largely due to the differences in the polypeptide composition of the gliadins and/or their interaction with the base flour components (Dong et al 1992).

The differential mixing effects of individual subfractions on MDT using CPS and Glenlea base flours is summarized in Fig. 5. Only the water-soluble subfractions (1, 4.5, 6.5, and 10) showed an increase in mixing time. The time difference resulted mainly from the long lag time for fraction 4.5. In contrast, addition of water-insoluble subfractions (1.5 and 2-9) resulted in the opposite effects (shorter mix time followed by a rapid breakdown). The water-soluble subfractions contained S-rich α -, β -, and γ subgroups of gliadins in addition to globulins and albumins, while water-insoluble subfractions contained S-poor ω -gliadins (Shewry et al 1986, Muller and Wieser 1995) in addition to α -, β -, and γ -gliadins. Subfraction 1 in CPS blends and subfraction 4.5 in Glenlea blends were most effective in delaying mixograph development time from 2.64 to 5.41 and from 5.75 to 8.16 min, respectively, whereas addition of subfractions 1.5 and 3 to CPS and Glenlea flours were more effective in shortening the mixing requirements from 2.64 to 1.08 min (fraction 1.5) and from 5.75 to 1.70 min (fraction 3). It was interesting to note that the addition of subfraction 1 to CPS and Glenlea base flours produced different MDT and energy to peak (ETP) results compared to those of other water-soluble subfractions (4.5, 6.5, and 10). The two parameters (MDT and ETP) increased significantly when subfraction 1 was blended with CPS base flour, but the addition of subfraction 1 to Glenlea base flour had a minimal effect on its MDT (Fig. 5). Subfraction 1 predominantly contained monomers (M_r 33,000-38,000 kDa), and other protein bands (M_r 18,000-32,000 kDa) were absent compared to other water-soluble fractions (Fig. 2). It is very likely that the interaction between proteins of two different

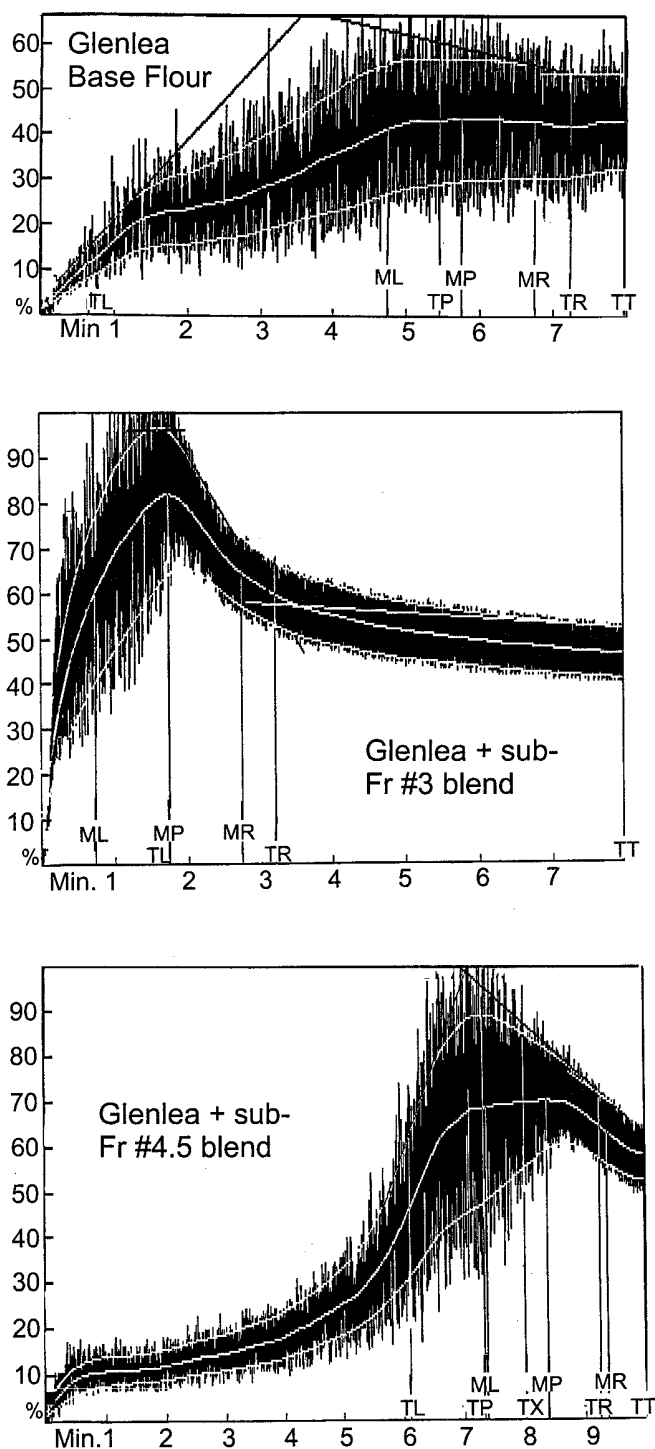


Fig. 4. Comparison of mixograph curves of Glenlea base flour (mixograph development time [MDT] = 5.75 min), with and without addition of water-insoluble subfraction 3 (MDT = 1.70 min) and water-soluble subfraction 4.5 (MDT = 8.16 min). Two-letter codes: 1st letters T and M refer to envelope and midline analysis, respectively; 2nd letters P, L, and R refer to peak, left, and right of peak, respectively; T = curve tail and x = arbitrary time.

TABLE I
Distribution of Various Polypeptides in Subfractions of 70% Ethanol Extract^a

Subfraction	Gliadin			Glutenin		
	α	β	γ	ω	HMW	LMW
1	+++	***	***			
1.5	+++	+++	+++	+		+++
2	+++	+++	+++			
3				+++	+++	+
4	+++	+++	+++			
4.5	+++	+++	+			
5	+++	+++	+++	***	+	+++
6	+++	+++	+++			+++
6.5	++	***	***			
7	***	+++	+++	+++	+	
8	+++	+++	+++	+++	+	
9	+++	+++	+++			
10	+++	+++	+	+		

^a +++ = Present, ++ = present as incomplete set of bands, + = most bands missing, *** = faint bands; HMW and LMW = high and low molecular weight.

sources, i.e., fractionated proteins of Glenlea and flour proteins of CPS, when combined, may behave differently than proteins coming from a single source (Glenlea). Nevertheless, the two base flours were comparable in responding to gliadin fortification ($r = 0.93, P < 0.001$).

The mixograph results reflect the fact that there was some difficulty in forming a continuous phase with the addition of the water-soluble subfractions of 70% ethanol extract of Glenlea wheat. Although the protein contents of the water-soluble and insoluble subfraction blends were similar, the shapes of the mixing curves were different (Fig. 4). Dough supplemented with

water-soluble fraction 4.5 showed a gradual increase in curve height before reaching the peak, whereas dough supplemented with water-insoluble fraction 3 showed an abrupt increase in curve height (Fig. 4). Therefore, flour water absorption may not be a linear function of protein content alone (Finney and Shogren 1972) but may also be influenced by the quality of protein, particularly in reconstituted flours.

The height of the mixograph curve is an indication of water absorption and dough strength (Finney and Shogren 1972), i.e., the ability of its proteins to withstand the stress of mixing. Gliadin fortification, regardless of polypeptide composition (Fig. 5)

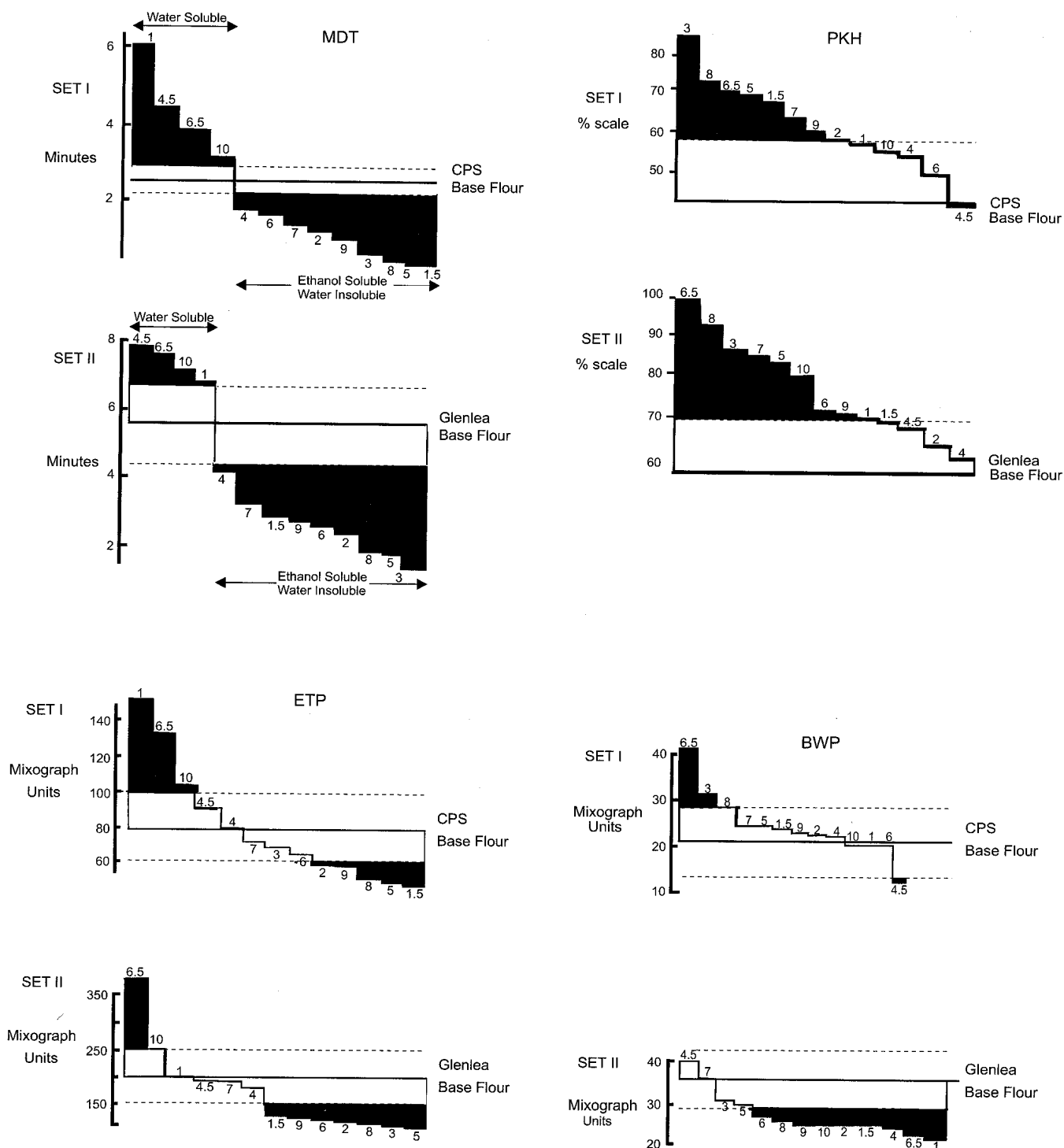


Fig. 5. Effect of gliadin fortification on various parameters of the mixograph curve viz. mixograph development time (MDT) required to attain maximum dough resistance, peak height (PKH), energy to peak (ETP), and band width at peak (BWP) for Canada Prairie Spring (CPS)-based (Set I) and Glenlea-based (set II) flours. Number on each bar correspond to subfractions listed in Figure 1. Dark areas represent significance at $P < 0.05$.

showed an overall increase in peak height (PkH) for both base flours. The maximum increase in PkH was achieved with the addition of subfraction 3 (from 43.8 to 84% of full scale) followed by subfractions 8 (to 73.3%) and 6.5 (to 70.5%) in CPS blends and subfraction 6.5 (from 57.9 to 99.1%) followed by subfractions 8 (to 91.8%) and 3 (to 86.8%) in Glenlea blends. Subfraction 6.5, which did not contain HMW gliadins, gave a similar increase in PkH as that from subfractions 3 and 8, which were deficient in ω -gliadins, suggesting that the presence or absence of HMW gliadin may not be the only criteria for increase in PkH, as reported elsewhere (Preston et al 1975). Nevertheless, the two base flours (CPS and Glenlea) showed parallel changes with respect to PkH ($r = 0.74, P < 0.002$). Subfractions 1, 10, 4, 6, and 4.5 were ineffective in producing significant mixing height increases in CPS flour, as were subfractions 1, 1.5, 4.5, 2, and 4 in Glenlea flour (Fig. 5).

The effects of various gliadin subfractions on the ETP parameter of the mixing curve are also shown in Fig. 5. The results appear to coincide with those of the MDT parameter. CPS flours with water-soluble subfractions 1 and 6.5 and Glenlea flours fortified with subfractions 6.5 and 10 required more ETP compared to water-insoluble subfractions 8, 5, and 1.5 (with CPS) and 1.5, 9, 6, 2, 8, 3, and 5 (with Glenlea). In fact, Glenlea flour supplemented with water-insoluble fractions produced an ETP lower than normally encountered for the original base flour. The two base flours showed a comparable response to the addition of the various subfractions ($r = 0.85, P < 0.001$). However the ETP changes with gliadin addition were greater for Glenlea base flour than for CPS flour.

Changes in the band width at peak (BWP) due to the addition of various subfractions (Fig. 5) indicate that the two base flours behaved differently. A water-soluble subfraction (6.5) added to the CPS flour showed a significant increase in the BWP (from 21.4 to 40.9), whereas the same subfraction produced a narrow BWP when added to the Glenlea flour (from 35.6 to 25). This reduction in band width could be due to the changes in the gliadin-glutenin balance. Most subfractions (1, 1.5, 2, 4, 4.5, 5, 6, 7, 8, 9, and 10) in combination with the CPS flour were ineffective in producing significant changes in BWP. However, subfractions (1, 1.5, 2, 4, 5, 6, 6.5, 8, 9, and 10) mixed with Glenlea base flour showed a significant reduction in BWP.

Experiments with flour residue. The residue obtained by extracting Glenlea flour with 70% ethanol gave a flat mixograph curve showing a total loss of viscoelasticity (Fig. 6). However, when subfraction 1.5 was added back to the flour residue, the mixing properties of the reconstituted residue were restored, and the mixograph curve was comparable to that of the original base flour (Fig. 6). These results clearly indicate the importance of

gliadins in maintaining the normal mixing behavior of the wheat-flour dough. Other subfractions were not tested because of the shortage of sample.

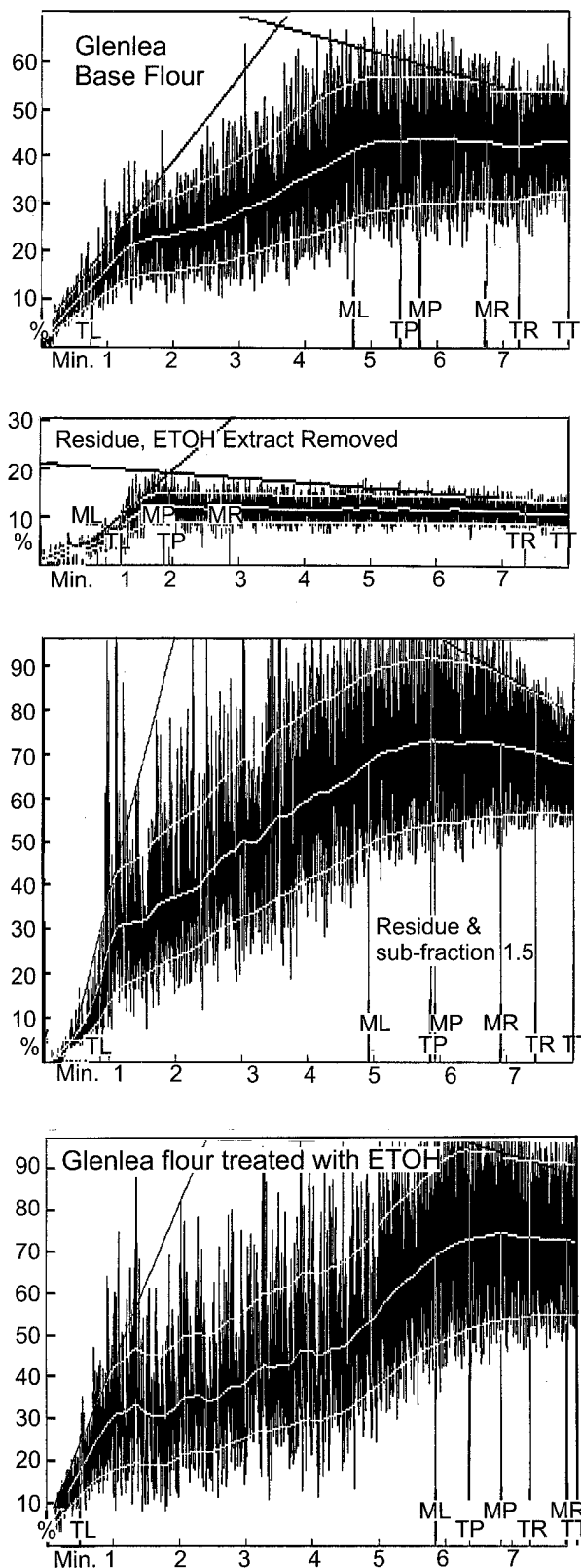


Fig. 6. Mixograph curves comparing Glenlea flour samples, treated/un-treated and extracted/unextracted with 70% ethanol. Note the loss of viscoelastic properties due to removal of ethanol-extractable flour components, regain in viscoelasticity by the addition of subfraction 1.5, and retention of viscoelastic behavior of ethanol-treated flour.

TABLE II
Gluten Recovery (d w) After Addition of Gliadin^a

Base-Flour	Added (%)	Gliadin (mg/2g)	Gluten Content ^b (mg)	Increase in	
				Gluten Recovery (mg)	Incorporation (%)
CPS flour	0	0	135.6e	0	0
	5	100	176.9d	41.3	41.3
	10	200	205.6c	70.0	35.0
	25	500	312.8b	177.2	35.4
	50	1,000	561.9a	426.3	42.6
Flour residue	0	0	4.6e	0	0
	5	100	38.8d	34.2	34.2
	10	200	76.9c	72.7	36.3
	25	500	279.3b	274.7	54.9
	50	1,000	530.1a	525.5	52.5

^a Means of duplicate samples.

^b Values with different letters are significantly different at $P < 0.05$.

To determine whether any physicochemical changes took place by the extraction of flour with ethanol, the flour-ethanol slurry was freeze-dried as-is, without removing any ethanol-extractable components. The ethanol-treated Glenlea flour gave a mixing curve with similar MDT and higher PkH than the curve of the corresponding ethanol-untreated samples. The dough prepared from the ethanol-treated and freeze-dried portion produced mixograms with wider variations in band width compared to those of the original flour (Fig. 6).

Gliadins and Formation of Gluten Network

The amount of gluten prepared from CPS flour residue and from the original flour fortified with various percentages of gliadin subfraction 1.5 are presented in Table II. The proportional increase in the amount of gluten recovered from gliadin-supplemented flour residue and unextracted flour demonstrate the involvement of gliadins in the formation of the gluten protein network. However, the gluten recovered from samples containing more than 10% added gliadins was of weaker consistency than that from samples containing less than 10%. Nevertheless, the incorporation of gliadin varied between 35 and 42% of the added amount of subfraction for CPS flour and was 34–52% for CPS flour residue (Table II). The proportionate incorporation of gliadin into flour or residue flour was similar ($r = 0.99$, $P < 0.01$).

CONCLUSIONS

The alcohol-extractable endosperm proteins can be further subdivided into several fractions differing in their polypeptide composition simply by altering the water and alcohol proportions in a binary solvent. The extractability of HMW glutenin subunits in aqueous ethanol suggests that HMW glutenin subunits may exist in varied polymer fragments: 1) small fragments extractable in unreduced aqueous alcohols; 2) larger fragments extractable in aqueous alcohol coupled with reducing agent, and 3) fragments of extra-large dimension that remain unextracted. However, the electrophoretic mobility of these fragments remains unaltered. Increasing the ethanol component in the binary solvent produces a subfraction having a unique polypeptide composition.

Results of this study point to a chemical basis for the mixing characteristics. The two base flours fortified with various subfractions of the 70% ethanol extract showed similar changes in their mixing characteristics for most parameters. The exception was that addition of some subfractions to CPS flour produced greater mixograph BWP compared to that of Glenlea, which had a reduction in band width. These results indicate the possible interactions between proteins of different origins (Schropp and Wieser 1996). Comparison of ethanol-extracted flour residue and ethanol-treated flour suggest that the removal of alcohol-soluble components resulted in loss of dough-forming properties. The residue flour regained its dough behavior upon addition of a fraction rich in gliadin components. The return of viscoelasticity after incorporation of added gliadins into ethanol-extracted flour residue accentuates the involvement of gliadins in the formation of the gluten protein network. Gluten yield may possibly be increased by the addition of Glenlea gliadins. The strength of gluten can also be modified by manipulating the amount of added alcohol-extractable proteins.

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