# Proteins Extracted by Water or Aqueous Ethanol During Refining of Developed Wheat Dough to Vital Wheat Gluten and Crude Starch as Determined by Capillary-Zone Electrophoresis (CZE)

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Cereal Chem. 81(5):673–680

**ABSTRACT**

Fluids applied to large-sale, technical separation of wheat starch and protein also extract soluble proteins. The degree and rate of extraction and the specific components extracted depend on the flour, the flour hydration and development, the starch-displacing fluid composition, the temperature, and the mechanical processing method. This study sought to identify major extracted protein groups using high-performance capillary zone electrophoresis (CZE) applied directly to fluids obtained during laboratory-scale technical separations. A dough-ball or compression separation method was applied using a Glutomatic system and a batter or dispersion method was applied using a a McDuffie mixer and Pharmasep vibratory separator. Process fluids were water at 22°C to model commercial practice and 70 vol% ethanol in water at –13°C to model the cold ethanol process being developed here. Data were referenced to use of 70 vol% ethanol in water at 22°C in the Glutomatic compression method. The dough processed by each method was developed by mixing to a separable state. When flooded with excess water, this dough immediately released starch and water-soluble or albumin proteins. When flooded with excess cold aqueous ethanol, neither the albumin nor gliadin proteins appeared in significant amounts until the bulk of the starch had been displaced, regardless of the mechanical method. Even with extraction and manipulation well beyond that necessary for starch displacement, the net amount of gliadin proteins dissolved was only ≈10% of that available from wet developed dough using 70 vol% ethanol at 22°C. There was more gliadin protein in the fluids at earlier stages of processing when the batter dispersion method was applied using cold ethanol. The most common soluble proteins revealed in the electrophoresis patterns for the batter compression method using cold aqueous ethanol were initially albumins and later γ-gliadins. Albumins not appearing as soluble in cold 70 vol% ethanol were found in the insoluble crude starch, suggesting their precipitation in the dough fluids during the change from free water to cold aqueous ethanol. These results establish that some protein is dissolved during starch displacement by cold aqueous ethanol, but that the amounts may be limited by control of the mechanical working of the dough in the presence of the displacing fluids.

The disassembly of wheat to create value-added, enriched components is a critical reductive step in the biorefining of wheat. In integrated grain biorefining, the disassembly of the grain to crude protein and crude starch initiates steps that lead to both food and biobased platform chemicals and products such as vital gluten, gluten components, refined starch, ethyl alcohol, and pure chemicals (Koutinas et al 2003). Disassembly of wheat grain depends critically on the creation of a separable condition or state for the grain. A common route to a separable state is to 1) mill the grain to flour, 2) mix the flour with water, and then 3) develop the batter mechanically. If properly done, the flour-and-water dough will appear to be unmixed with separate coalesced protein tendons or fibers as well as clusters of starch (Tipples and Kilborn 1975; Robertson et al 2000). The separable state may be exploited by flooding the developed dough with excess water (Grace 1989) or refrigerated ethanol (Robertson and Cao 1998a,b) and simultaneously working the batter to compress, decompress, stretch, and relax it. Alternatively, the batter may be dispersed into excess water (Anderson et al 1960; Weegels et al 1988) or refrigerated ethanol (Robertson and Cao 2003). The excess of each fluid mobilizes the starch and carries it through a porous barrier that retains protein.

At the same time, the solid-starch-displacing fluids extract soluble components and suspend insoluble protein fragments that pass through the separation barrier or screen with the starch. In commercial processing using water, there are ≈1,100 kg of solubles (including albumin protein and hemicellulose) generated per metric ton of vital wheat gluten recovered (Grace 1989). The application of refrigerated ethanol to developed dough, though operationally similar to water in the physical displacement of starch, differs chemically because of differences in grain-component solubility and in particular the potential for removal of aqueous-ethanol-soluble gliadin proteins. Furthermore, during the initial stages of the separation in which the free fluids in the dough change from water to the concentration of the displacing ethanol, there is a possibility for precipitation and reincorporation of water-soluble components.

Ultimately, the compositional changes have potential physicochemical and functional consequences. Compositional changes that were related to selective solubility of grain components may help to explain why wheat gluten produced using aqueous-ethanol has different and, in many instances, enhanced functional properties when compared with wheat gluten produced using water (Robertson and Cao 2001, 2002, 2003).

The primary objectives of this research were to identify protein component groups removed during the processing of wheat to vital gluten and crude starch, and to characterize the kinetics of protein removal processes from wet, developed dough. We sought to characterize the dependence of these factors on 1) the fluid used to displace starch, 2) the intensity of the mechanical energy input during separation, and 3) the duration of the exposure to both the displacement fluid and the input of mechanical energy. The scope was limited to water, the current fluid of commercial practice, and aqueous ethanol, a potential alternative process fluid described above that has been the subject of earlier research (Robertson et al 1998a, 1998b, 1999, 2000, 2001a, 2001b, 2002, 2003). These developmental research efforts emphasize the use of ethanol as an alternate processing fluid because of its increasing availability and intriguing potential as a separating agent or process fluid in integrated grain refining (Krochta et al 1981; Robertson and Pavlath

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Publication no. C-2004-0816-03R.

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1986; Chien et al 1988, 1990; Hojilla-Evangelista 1992), previous use in food applications as an extractant, and as a carrier for coatings, and the availability of well-developed methodologies (distillation, adsorption, etc.) for its regeneration. High-performance capillary zone electrophoresis (CZE) was applied to the task of protein identification. Others have used CZE on aqueous and alcohol extracts of dry wheat flour to characterize cereal storage protein composition and compositional changes such as those that occur during maturation (Bietz and Schmalzried 1995; Lookhart and Bean 1995a,b, 1996; Bean et al 1998; Scholz et al 2002; Bean and Lookhart 2003). There also has been recent interest in the characterization of water-soluble proteins from cereals (Lookhart and Bean 1995b; Bean and Tilley 2003). The present report is the first use of CZE to assess protein molecular changes during wet processing.

## MATERIALS AND METHODS

### Reagents

Unbleached flour with 13.4% protein (moisture free basis, mfb, 5.7 × N) from a commercial supplier (Giusto, San Francisco, CA) was used in all experiments, and reagent-grade, anhydrous ethyl alcohol was diluted with distilled water to the desired concentration. Concentrations of aqueous ethanol are vol% concentrations. We made most use of 70 vol% ethanol; when aqueous ethanol is noted, this is its concentration. Other concentrations are specifically identified. CE phosphate buffer (0.1*M* pH 2.5) was obtained from Bio-Rad (Hercules, CA). This buffer contains a linear polymer modifier (hydroxypropyl methyl cellulose).

### Protein Separation

This laboratory study used two mechanical methods for separation to vital gluten and crude starch, two displacing fluids, and temperatures at ambient and below. One of the mechanical methods was a dough-ball or compression method. Here, the dough was repeatedly compressed and stretched and then allowed to decompress and relax while being exposed to drop-wise flow of starch displacing fluid. The other method was a batter method in which the dough was dispersed and shredded in an excess volume of starch-displacing fluid.

Each laboratory-scale method produced three product streams: 1) wet, enriched insoluble protein or gluten that also contained nonprotein solids and some soluble protein; 2) wet, enriched insoluble starch that also contained some insoluble protein and soluble protein; and 3) spent displacing fluid that contained soluble proteins. The starch displacing fluids were 70% ethanol at 22°C or –13°C, and 100% water at 22°C. The water extraction was used as a base case.

For batter dispersion separation, 700 g of flour (12.6% moisture) was developed with 700 mL of distilled water in a Hobart mixer (model A120, Troy, OH) with a McDuffie bowl pin mixer from National Manufacturing (Lincoln, NE) for 40 min at 60 rpm. The batter was then relaxed for 60 min. In the ethanol process, this step was conducted while chilling the batter in the McDuffie bowl to 5–10°C using a low-temperature refrigeration system (model MC480A1, FTS, Stoneridge, NY). After this relaxation period, the batter was dispersed in 1 L of distilled water at 22°C or in 70% ethanol at –13°C with continuing mixing for 3 min. The concentrated protein fraction was collected on a 213-µm screen using a vibratory separator (Pharmacep PH12 vibratory separator, Sweco, Florence, KY). This step was performed two additional times. With the ethanol process, an additional final dispersion in 2 L of 100% ethanol for 5 min of continuous mixing was added for the removal of water. When necessary, some fluid and starch samples were lyophilized at –20°C and 20 pascal using a freeze-dry system (Freezone 12/79480/77450, Labconco, Kansas City, MO) and milled using a laboratory mill (No. 3100, Perten, Reno, NV). Protein content was determined by combustion with a nitrogen analyzer (Leco Corp., St. Joseph, MI) and was 66 ± 4% protein for gluten from both processes (N × 5.7). The collected starch fractions from each dispersion containing the supernatant were centrifuged at 8,000 rpm in a temperature-controlled centrifuge (model 4239R, ALC Int. Srl., Milan, Italy) to separate the supernatant containing solubles from the settled insoluble starch and proteins.

For the dough compression separation method using the Glutomatic system (Perten Instruments Springfield, IL), batter was developed in a microfarinograph (model 8110, C.W. Brabender, Duisburg, Germany). A 10 g (12.6% moisture) sample was mixed with 10 mL of distilled water for 24 min at 22°C, followed by 30 min of retention time. In the ethanol process, the microfarinograph mixing bowl was chilled to yield a 10°C batter during this retention period. The batter was then transferred to the mixing and washing chamber of the Glutomatic instument (model 2200) containing a modified screen support with open area increased by 135% and a 160-µm polyethylene attached screen (39% open area, mesh 230 µm thick). Water or 70% ethanol at 22°C and –13°C was pumped by the Glutomatic system into the mixing chamber and washed over the batter at a rate of 50 mL/min. The gluten fraction remained on the screen while the passed wash slurry containing mostly starch was collected in a series of weighing boats for the indicated time intervals. Temperature-controlled centrifugation and filtration of supernatant were applied before analysis by capillary electrophoresis.

In a separate experiment, 6.1 g of water gluten was washed with 70% ethanol, and 7.0 g of ethanol gluten (2.36 g, mfb) was washed with distilled water at 22°C. Samples were collected as previously described.

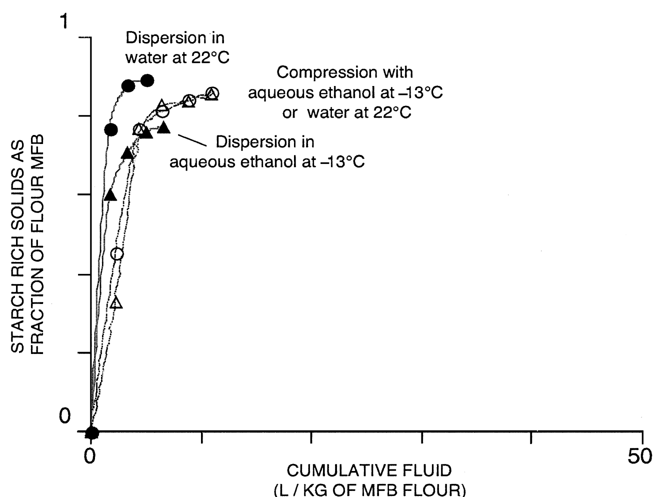


**Fig. 1.** Starch-rich, nonprotein solids recovered by fluid displacement of starch from wheat flour previously hydrated and developed with water to a separable state.

**TABLE I**
**Peak-Area Linear Regressions for Selected Peak Ranges[a]**

| Peaks | Temp. (°C) | Slope | *R* | Range |
|---|---|---|---|---|
| γ : α–β | 22 | 0.43 | 0.989 | 0–320s |
| γ : α–β | –13 | 0.26 | 0.977 | 0–220s[b] |
| | –13 | 0.34 | 0.687 | 0–320s[b] |
| γ : albumin | 22 | 13.5 | 0.876 | 0–320s |
| γ : albumin | –13 | 0.76 | –0.338 | 0–320s |

[a] Obtained for use of 70 vol% ethyl alcohol in water as a starch-displacing fluid applied to wet wheat flour dough with mechanical manipulation. See Fig. 1 for the regions considered.

[b] Two of the last three points of 14 total points were >2× the correlation value and were excluded from the first correlation row at this condition and are included in the second row.

**Capillary Electrophoresis**

A capillary electrophoresis system was used for all capillary electrophoretic (CE) separations (G1602A, Agilent Technologies, Wilmington, DE). Uncoated fused-silica capillaries from Agilent were 50 µm diameter with a 24.5 cm effective length. Proteins were detected by UV absorbance at 200 nm.

For CZE, we used phosphate buffer. CZE analytical runs were at 30°C and 15 kV for 30–50 min. Samples were pressure-injected at 50 mbar for 10 sec (dispersion experiments) and 45 sec (compression experiments). The times were selected to balance separation quality, detect the principal protein groups, detect minor protein amounts, injection-to-injection comparability between samples, and to eliminate the need to adjust protein concentration (dilution for concentrated samples, concentration for dilute ones) or to tailor injection times. This "one size fits all" approach overloaded high concentration samples but clearly distinguished albumin and gliadin proteins and prominent gliadin subgroups. Capillary cleaning protocol was 3 min with 0.1*N* HCl, 5 min with 0.1*N* NaOH, 1 min with deionized water, and 2 min with phosphate buffer. This method substitutes nitric with hydrochloric acid but is otherwise like that of Bietz and Schmalzreid (1995). By routine use of this procedure, no diminishment of separation was observed.

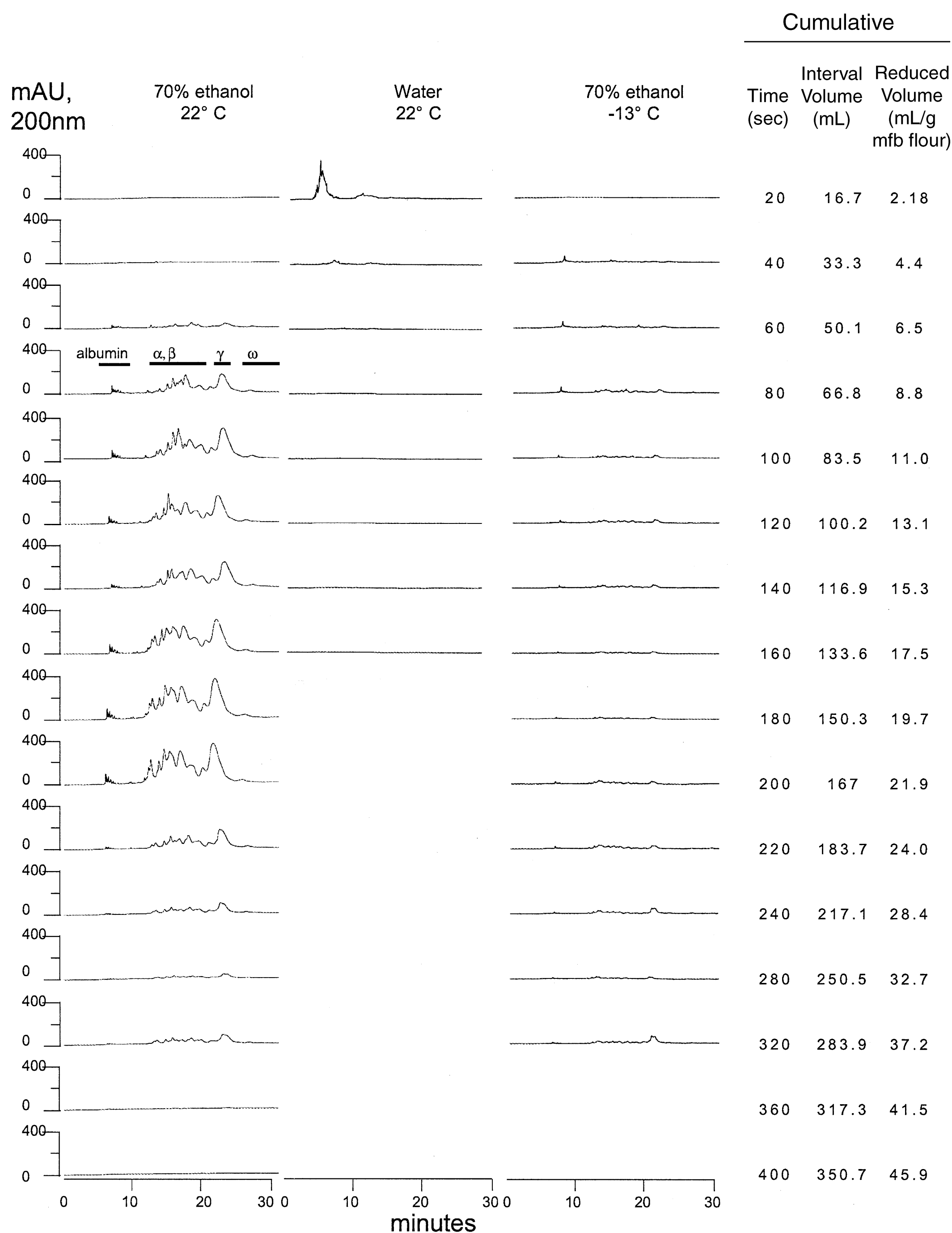


**Fig. 2.** Soluble protein electrophoresis patterns (all reported using the same absorbance vertical scale) for consecutive fractions collected during doughball or compression using the Glutomatic system. Basis is equal fluid volume. Time scale is from top to bottom with each trace representing proteins collected in a 20- or 40-sec periods. Starch displacement fluid is indicated at the top of each column. Cumulative volume applied per unit of flour in the batter is shown in the last column.

## RESULTS AND DISCUSSION

### Crude Starch

We found that starch removal by the compression or dispersion method was very rapid and most crude starch was displaced in the first two intervals of either method (Fig. 1). However, the compression and dispersion separation methods do this in significantly different ways. If the dough matrix is visualized as a flexible sponge soaked in and containing a starch suspension, then the compression method is analogous to repeated squeezing and rewetting the sponge so that the starch suspension is gently washed, convected, or pumped away. In contrast, the dispersion method pulls the sponge apart in excess fluid to create a suspension that is filtered. We interpreted the compression as gentle or benign and the dispersion as aggressive initially because of the physical appearance of the crude protein product. Compression-generated crude gluten was undivided and cohesive from water, or spongy and curd-like from ethanol at –13°C (Robertson and Cao 1998). By contrast, dispersion-generated crude gluten was extended, webbed, and semicohesive from water, or noncohesive, shredded, and fibrous from ethanol solutions at –13°C. There was a high level of fluid turbulence in the dispersion fluids but not in the

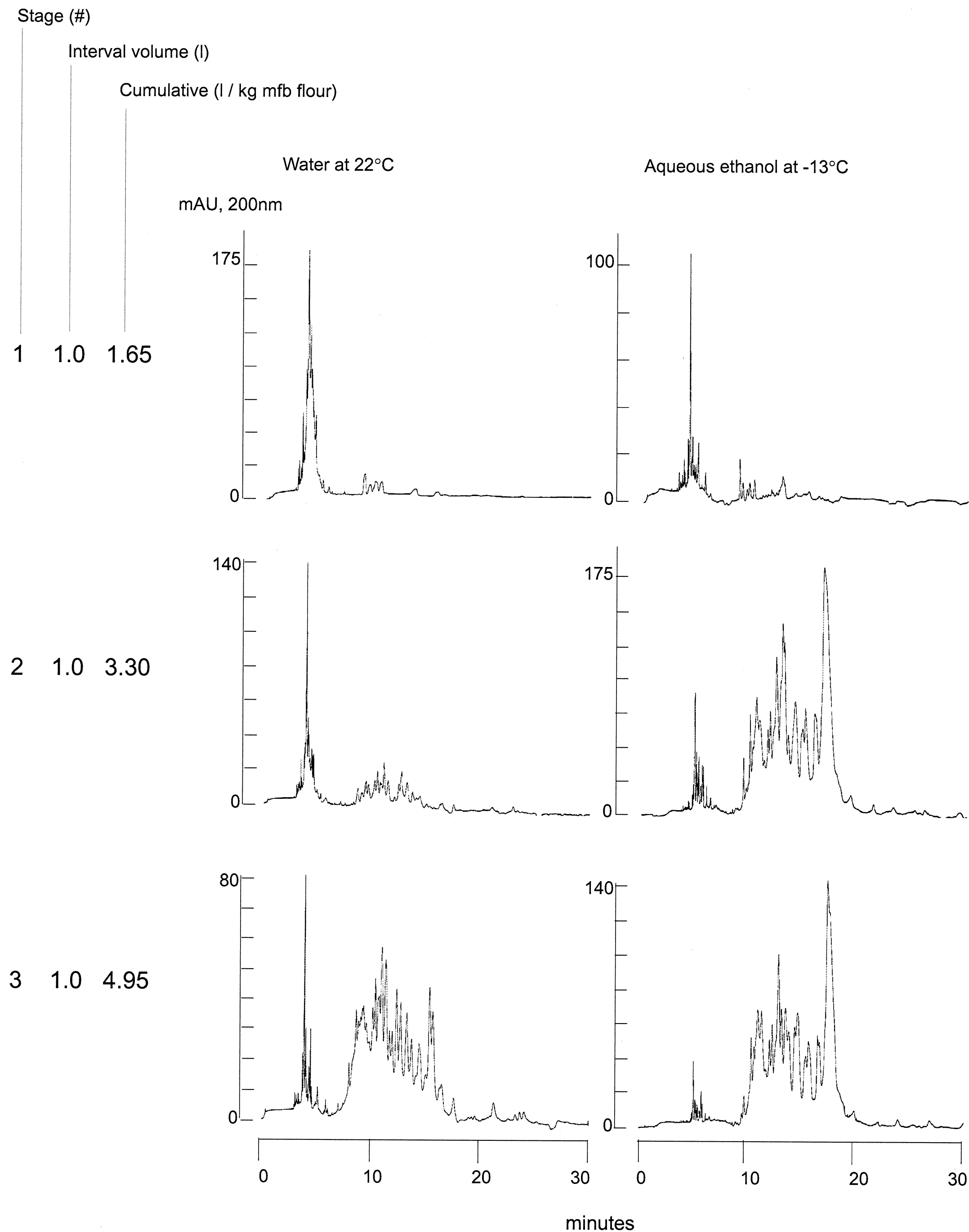


**Fig. 3.** Soluble protein electrophoresis patterns (all reported using the same vertical absorbance scale) for consecutive fractions collected during dough batter or dispersions performed using the Hobart mixer and McDuffie mixing bowl. Basis is equal fluid volume. Event scale is from top to bottom with each trace representing proteins collected in a dispersion event. Starch displacement fluid was water at 22°C and 70% ethanol at –13°C. Cumulative volume applied per unit of flour in the batter is shown to the left of the electrophoresis traces.

compression fluids that flowed dropwise over and through the dough mass.

Additionally, the basis of comparison that we employ in reporting the data is the cumulative volume applied divided by the moisture-free mass of flour subjected to the exposure. In the compression method, there was continuous fluid application but batch-fluid collection. By contrast, the dispersion method used batch fluid application and batch collection. Each collection interval during compression used 2.18 L/kg and each stage of dispersion used 1.65 L/kg. However, there were a total of 300 sec of continuous mechanical energy input in each stage of dispersion, but only 20 sec and <40 discrete events of compressive input in a collection interval. There are, at most, two compression events per revolution in the Glutomatic system because the impeller does not always successully engage the dough mass. This difference in separation work reinforced the interpretation of the respective methods as benign or aggressive.

As reference to the discussion of protein extraction by aqueous ethanol (70 vol%) at –13°C or water at 22°C, we subjected a wet, developed dough to extraction by aqueous ethanol at 22°C. Ethanol at this temperature and concentration is well known for its ability to dissolve nonglutenin proteins of wheat. Both albumin and gliadin proteins were observed in the electrophoresis patterns when aqueous ethanol was applied with compression in the Glutomatic system (Fig. 2). The patterns were similar to those reported for the non-Osbourne extraction of dry wheat flour by aqueous ethanol (Lookhart and Bean 1995b), where proteins elute in CZE generally in the order of increasing molecular weight: albumins and globulins, α- and β-gliadins, γ-gliadins, and finally ω-gliadins. There was, however, a delay in the appearance of these proteins until after the first two extraction intervals, and extraction continued until the 35–40 L/kg interval or a total of 320 sec. The delay, high-solvent requirement, and slow protein removal at this reference condition results from resistance to extraction of the developed and hydrated protein but also imperfect mixing and slow concentration change within the dough matrix.

We noted that the time of appearance and amount of albumin protein appearing as soluble protein using aqueous ethanol at 22°C was linked to the time of appearance and amount of the HMW gliadin. For instance, a linear correlation of $R = 0.9$ (Table I) existed between albumin-group peak areas and γ-gliadin peak areas. This correlation suggested that the extraction of albumin-like proteins by aqueous ethanol at 22°C from wet, developed dough depends on dissolution of the gliadin and the resulting disruption of the gliadin-glutenin complex.

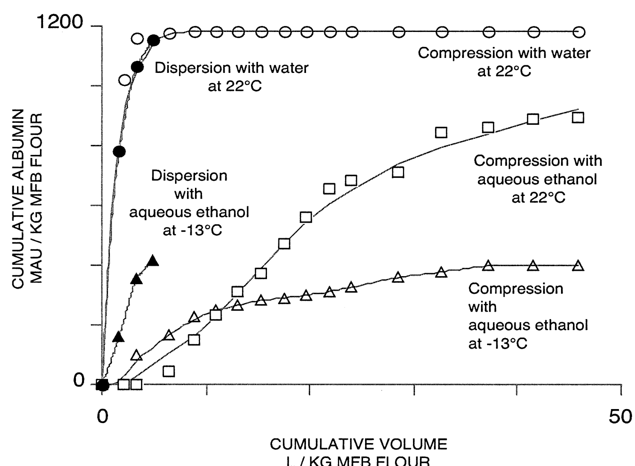


**Fig. 4.** Cumulative albumins and albumin-group protein as soluble components of fluids produced by compression and dispersion methods using aqueous ethanol and water with temperatures as noted.

## Albumin Proteins

When we subjected a wet, developed dough to extraction by water at 22°C, there was immediate release of albumin protein in the first intervals of extraction, whether the compression method (Fig. 2) or dispersion method (Fig. 3) was applied. We found that the amounts of water-soluble albumin proteins were greater than those found in aqueous ethanol at 22°C. Furthermore, the release of albumin protein closely followed the pattern of fluid displacement of the crude starch (Fig. 1). No albumin proteins were observed at the longer elution times. This suggests that at the end of development and before the separation events, the free water in the dough that surrounds the insoluble starch already contains the dissolved albumins, and that both starch and albumin are displaced from the matrix by bulk fluid movement. The electrophoresis pattern for this group is similar to published patterns for extraction from dry flour (Lookhart and Bean 1995b; Bean and Tilley 2003).

When aqueous ethanol at –13°C was applied to the displacement, albumin-group proteins were detected, but both the total amount in the process fluid and the rate of extraction were substantially less than the levels observed for either water or aqueous ethanol at 22°C (Figs. 2–4). This reflects process conditions that were applied intentionally to minimize protein solubility (Robertson and Cao 1998a,b). Furthermore, there was no correlation between the albumin-group peak area and the γ-gliadin peak area, suggesting that the albumins extracted in this way represent a subset of albumin-group proteins that is loosely associated with or weakly entrapped by the undissolved protein matrix in the batter.

Reduced detection of albumins in aqueous ethanol suggest that these proteins either were not extracted but remained as insoluble solids in the protein matrix, or were displaced as insoluble, precipitated solids with the crude starch. We tested these hypotheses first by exposing freshly prepared, dispersion-method crude gluten that was still wet with aqueous ethanol at –13°C to extraction by water at 22°C using the compression method. This revealed rapidly

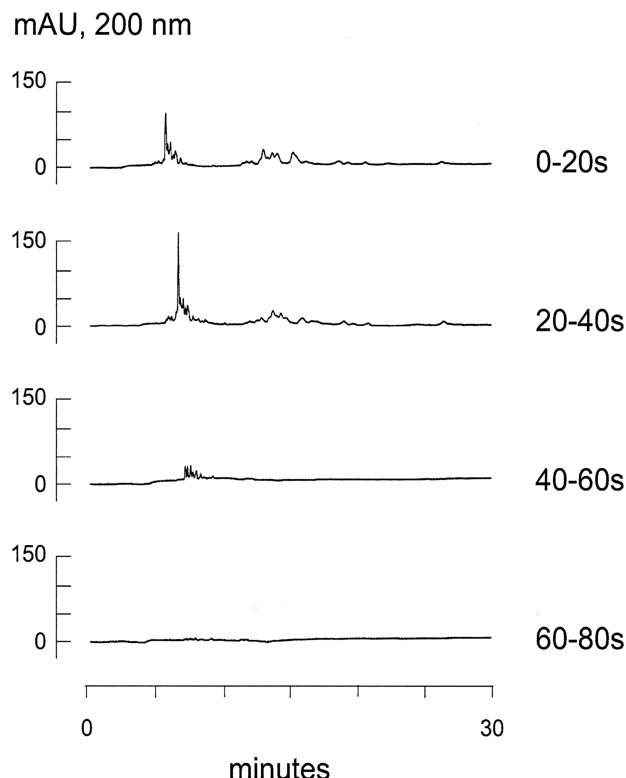


**Fig. 5.** Electrophoresis pattern for extraction of albumin proteins by water at 22°C applied to gluten protein produced by fluid displacement with aqueous ethanol at –13°C. Basis is equal fluid volume. Traces are annotated with the interval collection time.

eluting and rapidly extracted allbumin group protein (Fig. 5). However, the cumulative amount recovered in this way was only ≈10% of the albumins extracted by water. We then used water to extract lyophilized, crude starch that had been produced by the dispersion method and found albumin proteins in a typical electrophoresis pattern for a water extract (not shown). This amount of albumin protein accounted for the missing soluble protein in aqueous ethanol at –13°C fluid.

Electrophoresis patterns suggest compositional differences for the albumin proteins extracted by water at 22°C and aqueous ethanol at 22°C or –13°C. As shown in Fig. 6, there are differences in the definition and relative heights of the peaks obtained, and the elution times. In our experience with CZE, peak positions and definition are dependent on the concentration and composition of the sample. To clarify the interpretation of these traces, we focused attention on the unfiltered supernatant fluids obtained from the dispersion method using water at 22°C by first decanting after starch settling, freezing, and lyophilizing. These lyophilized solids contained both soluble protein and possibly insoluble, suspended protein. We applied CZE to extracts of these solids using water and aqueous ethanol solutions of 10–90 vol% at 22°C (Fig. 7). The extraction with water revealed the characteristic albumin group pattern (Fig. 6) with a prominent central peak. However, as the ethanol content of the fluid increased, there was 1) overall progressive reduction in the extraction of all albumins, 2) selective, and more complete reduction of extraction of the shoulder peaks in the albumin group, and 3) a shift of all peaks in the group to higher elution times. Only traces of the albumin group proteins were observed when 90 vol% ethanol was applied. These data support the interpretation that the albumins extracted have different compositions, but also that the prominent central peak is the same protein. The peak migration is attributed to a lower overall concentration and to the effect of the sample ethanol on the subsequent protein interactions in the electrophoresis capillary. The reader will note the appearance of a peak in the gliadin group with a retention time corresponding to γ-gliadin that showed most strongly for 60–70 vol% ethanol. This may have its source in small insoluble protein fragments included in this fraction.

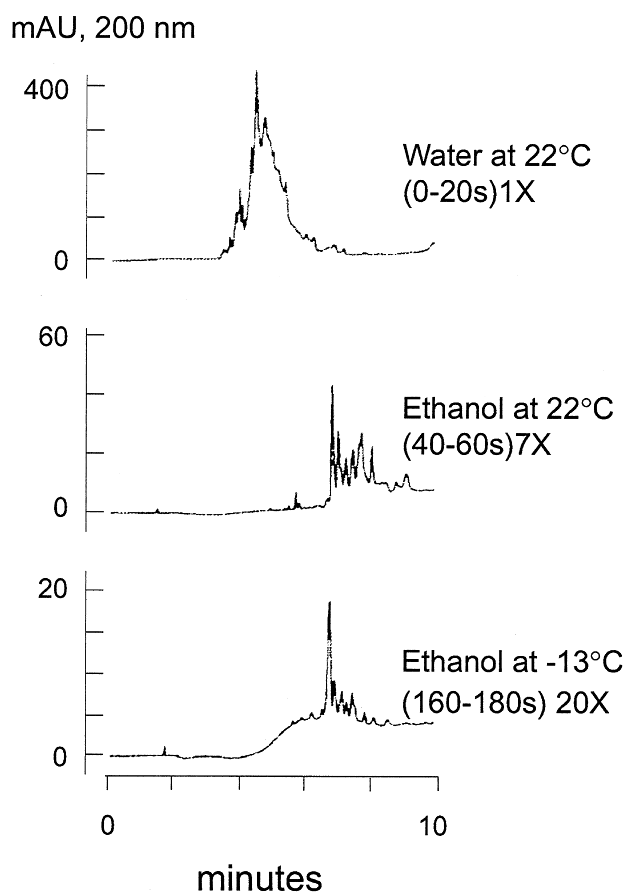


**Fig. 6.** Rapidly eluting proteins in the characteristic albumin range electrophoresis patterns (reported using normalized vertical scale). Basis is equal volume.

**Gliadin Proteins**

Unlike the albumin group, whose rate of removal by water closely followed that for the displacement removal of starch, gliadin protein extraction by aqueous ethanol at –13°C was independent

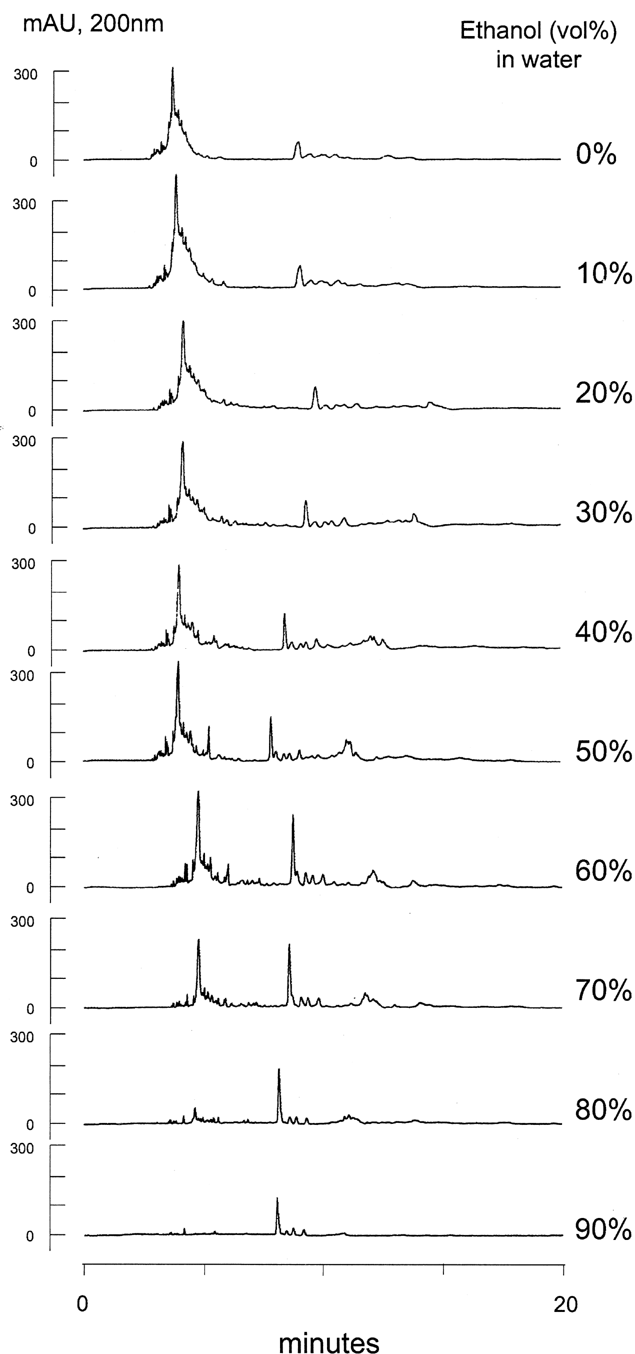


**Fig. 7.** Electrophoresis patterns for aqueous ethanol extracted proteins in freeze-dried, water-soluble protein initially prepared using the dispersion method and water at 22°C. Traces are annotated with ethanol percentage (v/v%). Basis is equal solids/equal extraction volume/equal fluid sample volume.

of the starch displacement and was very much slower with high solvent and mechanical input required (Fig. 2, 3, and 8). This was attributable to high mass transfer resistance presented by the large component tendons making up the developed protein matrix, chemical resistance of the developed protein to solvent action, and reduced solubility.

When the compression method was used, the net gliadin protein removed by extraction using aqueous ethanol at –13°C was ≈10% of that extracted at the reference aqueous ethanol at 22°C. An observation important to the use of the method for starch displacement was that negligible amounts of gliadin protein were extracted during the period of starch displacement (<10 L/kg). In contrast, the more aggressive mechanical conditions of the dispersion method led to earlier extraction of gliadin group proteins by aqueous ethanol at –13°C. Unexpectedly large amounts of gliadin range protein also were observed for the dispersion method in water (Fig. 3 and 8). Excess mechanical energy input to dough reportedly has altered molecular weight and solubility of gluten proteins (Tanaka and Bushuk 1973; Danno and Hoseney 1982; Aussenec et al 2000). Disaggregation of protein lipid complexes (Bekes et al 1983) may also be involved.

As noted above and in Fig. 2, the alternative refining method employing compression and aqueous ethanol at –13°C dissolves far less protein than at 22°C. However, when the electrophoresis patterns for cold ethanol use in compression (right electrophoresis column of Fig. 2) are expanded using a reporting ordinate based on the largest protein peak (examples in Fig. 9), details of the extraction are revealed.

Albumin extraction dominates early results. Albumins, α-, β-, and γ-gliadins contribute more or less equally during mid-experiment, and γ-gliadins dominate toward the end. For the period up to 220 sec of exposure, but not afterward, we observed good linear correlation (Table I) between the α- and β-gliadin peak areas and the γ-gliadin peak areas ($R$ = 0.98). These early extraction results imply nonselective or random release of these gliadin subgroups from the gluten network. Similar but less complete progressions of protein composition also appear in the electrophoreses-dispersion separations using water or cold ethanol (Fig. 3). These patterns of increasing molecular weight as extraction proceeds reflect progressive weakening of the resistance to extraction by the dough as well as progressive increase in the ethanol concentration of the extracting fluid as the water in the dough is displaced with ethanol. The ratio of γ-gliadin to α- and β-gliadin areas (compression method) was ≈0.3 for aqueous ethanol at –13°C and 0.4 at 22°C. This suggests lower solubility at –13°C for the higher molecular weight γ-form of gliadin than for the lower molecular weight α and β forms of gliadin.

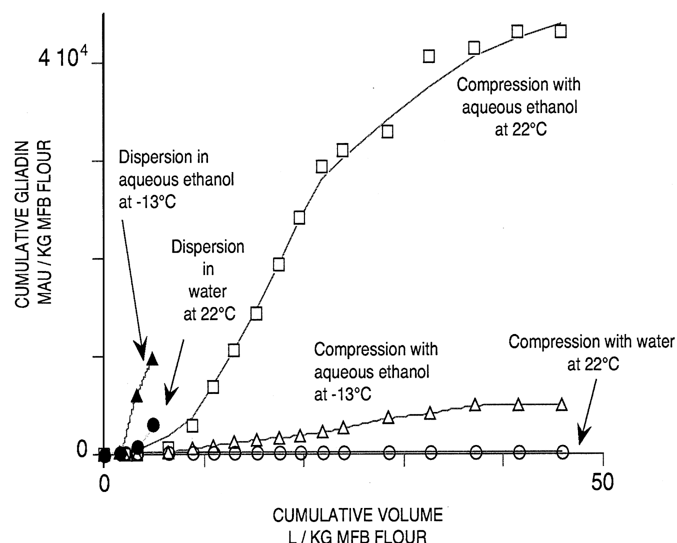


**Fig. 8.** Cumulative extraction of gliadin proteins from hydrated wheat dough by compression or dispersion and using water or aqueous ethanol.

## CONCLUSIONS

We have identified differences in the protein compositions of fluids used to prepare wheat vital gluten produced by displacement of starch with water or cold 70 vol% ethanol. These were identified by capillary zone electrophoresis of the soluble protein content of the fluids used in processing or by redissolving the after drying. Rapidly eluted albumin proteins were found in the extracts using either water or aqueous ethanol, but the amounts released as soluble in ethanol are much less than those soluble in water and the electrophoresis patterns are dissimilar. Water-soluble and ethanol-insoluble albumins were identified in the crude starch fraction. It is important to note that negligible amounts of gliadin protein are released during the critical period in which starch is displaced by either method.

The mechanical extension and tearing that occur during dispersion reduce the protection afforded by the developed dough or batter state improved solvent access and increased extraction of soluble protein that may be otherwise unavailable or entangled in the protein matrix. Conversely, a temperature of –13°C and short exposure times to mechanical work limit the extent and rate of extraction of both albumin group and gliadin group proteins.

Dough structure that is created to enhance separability of starch by fluid displacement (a separable state), reduces the rate of extraction of albumins and gliadins by 70% ethanol at 22°C and –13°C,

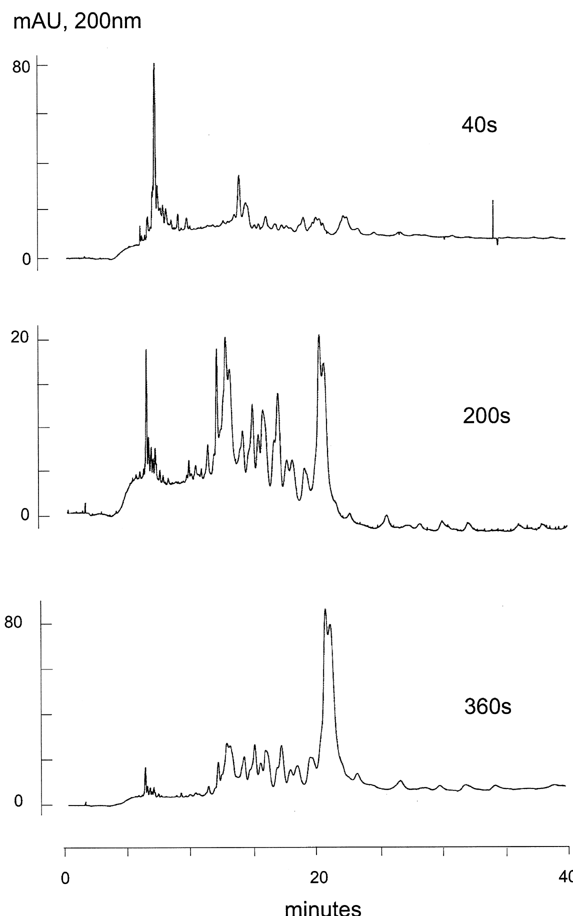


**Fig. 9.** Changes in extracted soluble proteins during compression and displacement with 70 vol% ethanol in water at –13°C. Electrophoresis patterns obtained for direct analysis of fluids collected for the intervals indicated.

but not albumins by water at 22°C. A complex separable dough structure, reduced protein solubility, and limited mechanical distortion of dough structure during starch displacement create a synergy that makes the cold-ethanol method of starch separation and protein enrichment technically feasible.

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[Received January 30, 2004. Accepted May 17, 2004.]