

# Farinograph Responses for Wheat Flour Dough Fortified with Wheat Gluten Produced by Cold-Ethanol or Water Displacement of Starch

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## ABSTRACT

Cereal Chem. 78(5):538–542

The objective of this research was to identify and define mixing characteristics of gluten-fortified flours attributable to differences in the method for producing the gluten. In these studies, a wheat gluten concentrate (W-gluten) was produced using a conventional process model. This model applied physical water displacement of starch (dispersion and screening steps), freeze-drying, and milling. W-gluten was the reference or "vital" gluten in this report. An experimental W-concentrate was produced using a new process model. The new model applied cold-ethanol (CE) displacement of starch (dispersion and screening steps), freeze-drying, and milling. Freeze-drying was used to eliminate thermal denaturation and thereby focus on functional changes due only to the separation method. The dry gluten concentrates were blended with a weak,

low-protein (9.2%), soft wheat flour and developed with water in a microfarinograph. We found that both water and cold-ethanol processed gluten successfully increased the stability (St) and improved mixing tolerance index (MTI) to create in the blended flour the appearance of a breadbaking flour. Notably, in the tested range of 9–15% protein, the St for CE-gluten was always higher than the St for W-gluten. Furthermore, the marginal increase in St (slope of the linear St vs. protein concentration) for the CE-gluten was ≈57% greater than that for the W-gluten. The slope of the MTI vs. protein data was lower for the CE-gluten by 24%. Flour fortified with CE-gluten exhibited higher water absorption (up to 1.8% units at 13.5% P) than flour fortified with W-gluten.

The major use of enriched wheat gluten (70–80% protein) is as "vital" gluten for the fortification of weak or low-protein flour to improve baking performance (Magnuson 1985). In the laboratory, a reference vital gluten is usually produced from wet, developed wheat flour as a concentrate by water displacement of starch, freezing, and then vacuum drying. In industry, vital gluten is processed similarly to laboratory vital gluten but is not frozen and is exposed to high-temperature air for drying. The quality of commercial vital gluten for breadmaking is well known for process and source-related variability (Booth et al 1980; Stenvert et al 1981; McDermott 1985; Grace 1989; Wadhawan and Bushuk 1989a,b). The quality of vital gluten for breadmaking has been measured by a variety of physical, thermophysical, and chemical means (Czuchajowska and Pomeranz 1990).

The separation process producing gluten from wheat grain presents many opportunities for changes in protein vitality. These opportunities include mechanical milling of grain, mechanical development of dough, fluid starch displacement from the dough, freezing (laboratory scale) of wet gluten, the method of drying of gluten, and, finally, milling of the gluten. Of these, drying has received the most credit and experimental attention for loss of vitality. The loss of vitality during drying has been attributed to the temperature, moisture, and time of exposure at drying conditions. These have been examined for laboratory conditions but not for commercial driers. Laboratory studies have employed vital gluten produced manually by water displacement, frozen, lyophilized, and then rehydrated, mixed, heated, and tested, or blended with flour and tested. The susceptibility of the gluten to temperature is dependent on the water concentration (activity). Wheat gluten concentrate (W-gluten) containing <10% water can be heated to 90°C for ≤1 hr with neither loss of baked loaf volume nor change in protein solubility.

However, when the water content is raised, thermochemical instability occurs and results in a large reduction in baking quality and protein solubility. For instance, if there is >20% moisture and the gluten is heated for 30 min at 80°C, there are losses of baked bread volume, extensibility, maximum extensibility resistance, protein extractability in 1.5% SDS, and hydrophobicity. There are also increases in disulfide (SS) bonding and irreversible conformational changes leading to increased apparent dough viscosity, lowered gluten compressibility, and increased gluten firmness. The glutenin proteins are particularly susceptible to thermally induced changes. Gluten derived from good breadmaking flours suffers greater changes than that from poor breadmaking flours. (Pence et al 1953; Booth et al 1980; Jeanjean et al 1980; Schofield et al 1983; Weegels and Hamer 1992; Weegels et al 1994a,b). Commercially produced W-gluten, also known as gum gluten, contains ≈60% moisture but only 10–14% when dry. W-gluten is exposed during drying to temperatures that vary from the drier air outlet at 60°C to the air inlet at 100–150°C. The combination of high air-supply temperatures and the gluten moisture levels expose W-gluten to considerable and unavoidable risk of thermochemical change.

The use of the cold-ethanol (CE) method for physical starch displacement (Robertson and Cao 1998a,b; Robertson et al 1999, 2000) produces a gluten state that may be less susceptible to drying-related vitality loss. Properties of ethanol such as low boiling point, high vapor pressure, and low surface tension support the expectation of lowered thermal susceptibility during drying. Furthermore, during drying there is significantly less structural collapse and the porous CE-gluten mass (Robertson et al 2000) allows rapid evolution and removal of vapor. Unpublished laboratory drying times for W-gluten samples are regularly at least 10 times greater than those for a comparable sample of CE-gluten.

However, the use of ethanol and the sensitivity of dough functional properties to minor component changes may create performance-in-use issues because of differential solubilities. Component solubility has been studied for solvent extraction of dry flour or gluten. For instance, ethanol with ≥30% water is a known protein solvent at room temperature (Meredith 1965; Robertson et al 1999) and a protein coagulant in sedimentation tests. Ethanol is an effective grain oil solvent, especially at elevated temperatures and low water content (Rao and Arnold 1956). Hot ethanol removes lipids and lipoproteins from wheat flour leading to a gluten viscosity that is 25% that of a similar formulation using native gluten (Nierle et al 1998) and dried gluten (Dill 1925). It has been suggested that 70% ethanol extraction within the classic fractionation sequence leads to

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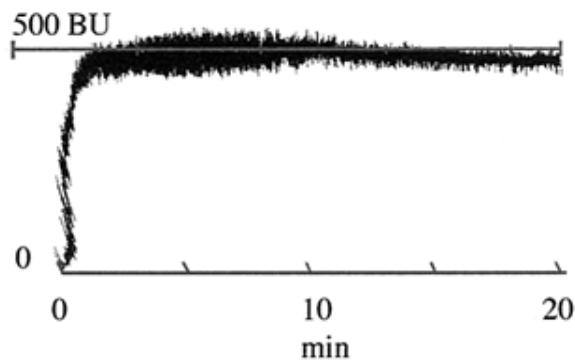
lipid redistribution from the gliadins to the glutenin fraction (Ponte et al 1967). The ability to make inferences from these data is limited because, in the present case, the solvent contact is with a developed, water-containing dough that may resist solvent action (Finney et al 1976) and not with dried or defatted flour or flour components, as is common in the literature cited. Finally, aqueous ethanol has been studied in detail as a denaturant for defatted soy protein (indexed by solubility changes) and is essentially inert at <math>30^{\circ}\text{C}</math> (Fukushima 1969).

The effects identified above suggested the need to assess functional properties of CE-gluten to establish the magnitude and direction of changes in functionality. For its major use to improve weak flours, properties of importance include dough development characteristics and baking performance. We report laboratory micro-farinograph data for dough development using CE-gluten and W-gluten blended with a “weak” flour. These gluten types were prepared using a batterlike method and differ only in the use of a water or CE method for displacement removal of starch. The freshly prepared gluten samples were freeze-dried to minimize thermal effects and to isolate and assess base changes attributable only to the method used for starch displacement. The method was applied to a batch of commercial, blended high-performance bread flour to simulate commercial practice.

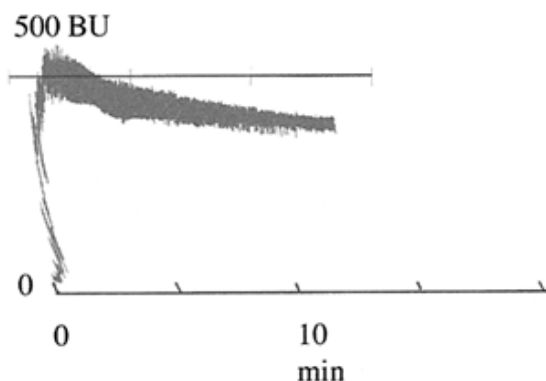
## MATERIALS AND METHODS

### Materials

Unbleached flour with 13.5% moisture-free basis (mfb) protein ( $N \times 5.7$ ) was obtained from a commercial supplier (Giusto, San Francisco, CA) and stored at  $-30^{\circ}\text{C}$  to be used as the source flour for gluten recovery. This flour is a blend of dark northern spring wheat and hard red winter wheat from Montana and is unfortified.



**Fig. 1.** Consistency or development farinogram of blended flour subjected to physical displacement removal of starch to produce wheat gluten or cold-ethanol displacement wheat gluten concentrates.



**Fig. 2.** Consistency or development farinogram of typical “weak” flour for breadmaking, cv. Moro.

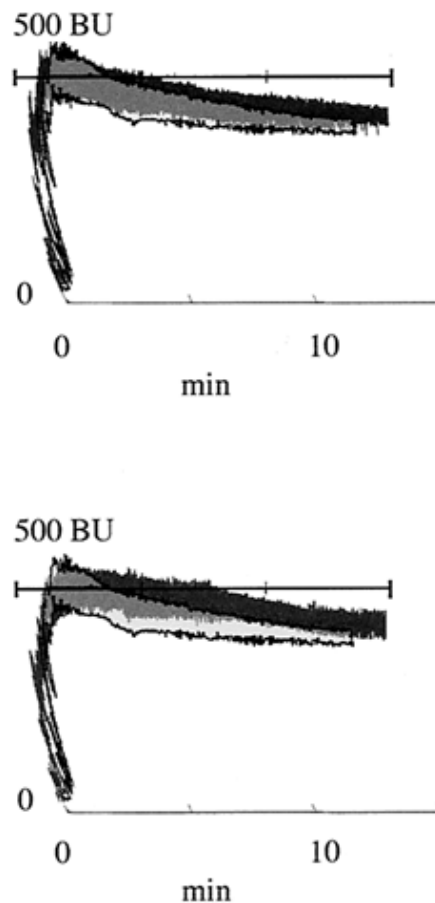
Flour produced from cv. Moro was provided by the Pullman Wheat Quality Laboratory of the USDA-ARS, Pullman, WA, and stored at  $-30^{\circ}\text{C}$ . Moro flour contained 9.2% mfb protein ( $N \times 5.7$ ). Flour moisture content was measured at  $11.4 \pm 0.4\%$  wb (Giusto) and  $12.1 \pm 0.4\%$  (Moro) using a moisture analyzer (IR-100, Denver Instrument Co., Arvada, CO). Undenatured, 200-proof ethyl alcohol was diluted with distilled water as necessary to obtain the desired concentration

### Gluten Preparation Using Aqueous Ethyl Alcohol

A batter was prepared by mixing 150 g of flour and 150 mL of distilled water in a commercial stand mixer (KitchenAide, St. Joseph, MI). The batter was mixed and developed for 19 min at  $22^{\circ}\text{C}$  followed by 40 min of relaxation at  $10^{\circ}\text{C}$ . The batter ( $10^{\circ}\text{C}$ ) was then dispersed with 400 mL of 70% ethyl alcohol with continuous mixing for 5 min in a stand mixer at a controlled temperature of  $-13 \pm 3^{\circ}\text{C}$ . The starch-rich fraction was separated from the glutenrich fraction by screening using 300- $\mu\text{m}$  (48 mesh) and 108- $\mu\text{m}$  (115 mesh) sieves. The gluten-rich fraction retained on the screen was suspended and screened a second time (same conditions as first). A third (and final) suspension was performed using 600 mL of 200-proof ethyl alcohol and mixing for 6 min, then screening.

### Gluten Preparation Using Water

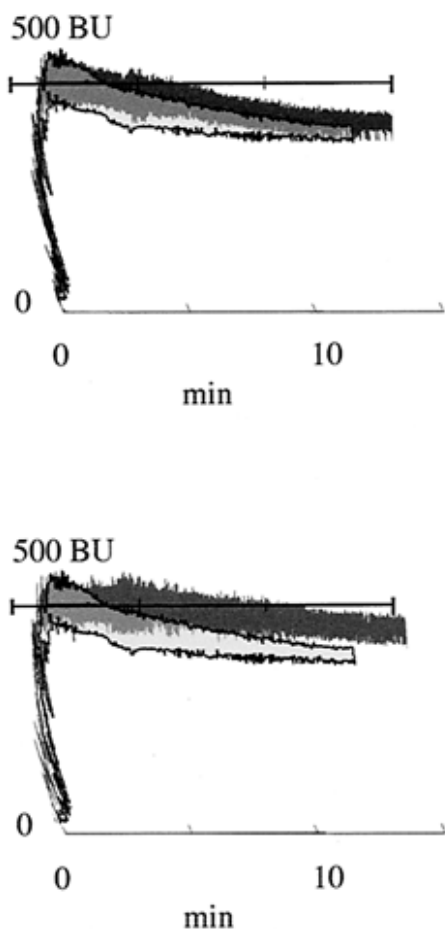
Batter preparation and separation with water was as described above except that the batter was relaxed at  $22^{\circ}\text{C}$  and water at  $22^{\circ}\text{C}$  was the displacing fluid. Three batter suspensions with 400 mL of water followed by screening were performed.



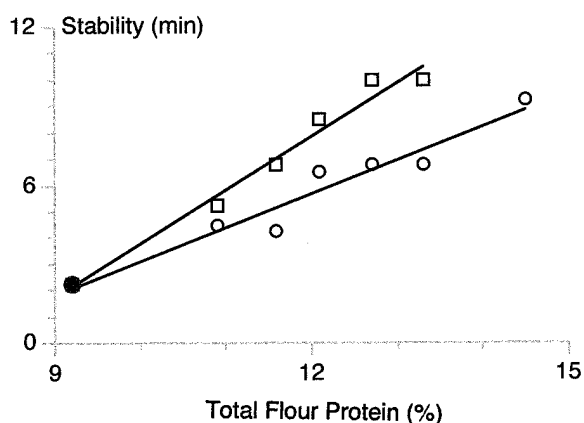
**Fig. 3.** Consistency or development farinogram of cv. Moro flour fortified with dried wheat gluten (produced by water displacement of starch, dried at low temperature and high vacuum). Blended protein content of flour tested was 10.9% protein (top) and 13.3% protein (bottom). Unfortified or base condition is included as an outlined, semitransparent overlay (Fig. 2).

### Gluten Drying

Gluten samples at 70% mfb protein ( $N \times 5.7$ ) were lyophilized for the experiments. Samples were lyophilized at  $-20^{\circ}\text{C}$  and  $200 \times 10^{-3}$  mBar using a freeze-dry system (Labconco, Kansas City, MO). The dried samples from six displacement runs were combined and milled (Perten Laboratory Mill 3100, Reno, NV).



**Fig. 4.** Consistency or development farinogram of cv. Moro flour (9.2% protein) fortified with dried experimental wheat gluten (produced by cold ethanol displacement of starch, dried at low temperature and high vacuum). Blended protein content of flour tested was 10.9% protein (top) and 13.3% protein (bottom). Unfortified or base condition is included as an outlined, semitransparent overlay (Fig. 2).

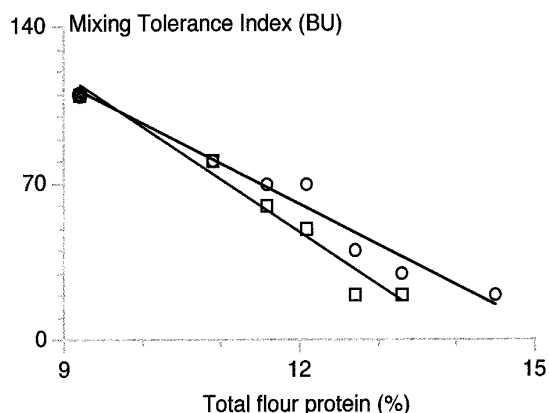


**Fig. 5.** Stability (min) for cv. Moro flour (9.2% protein) blended with added wheat gluten concentrates. Gluten concentrates produced from a developed batter by cold-ethanol displacement ( $\square$ ) or by water displacement ( $\circ$ ). Solid symbols indicate stability of the base, unfortified flour.

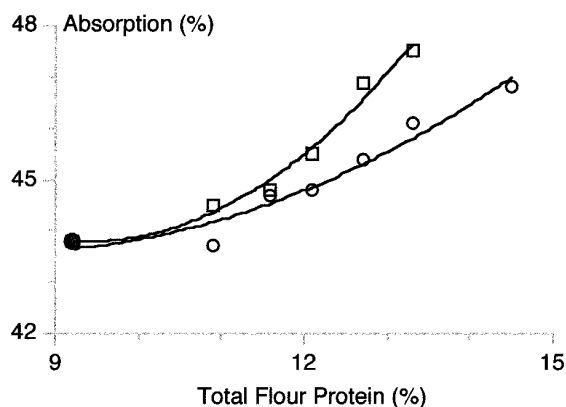
### Flour Fortification and Dough Development

In fortification experiments, Moro flour was used as the base flour supplemented with laboratory-processed gluten to the desired protein level. Dough was developed in a Brabender microfarinograph (model 8110; C. W. Brabender, Duisburg, Germany). Before each reported run, a 10-g sample of flour at 12.1% moisture was placed in the farinograph chamber, the development initiated, and the amount of distilled water needed to produce a response centered on  $500 \pm 10$  BU was determined by standard titrations of the flour with dropwise addition of distilled water. In the reported run, the sample was placed in the farinograph and the previously determined amount of water was added quickly at a rate of 20 mL/min from the farinograph burette. Consistency farinograms were determined in this way. The amount of water absorbed to give a value of 500 BU was reported on a 14% moisture basis.

The standard method (for a 50- or 300-g farinograph) uses an expected standard deviation of 0.5 and 1.5 min for the departure and arrival times used in the calculation of the stability. This was tested using the microfarinograph in a series of eight farinograms of Moro flour fortified to 10.55 mfb with freeze-dried CE-gluten. The procedure followed the Approved Method 54-21 (AACC 2000) modified for the sample amounts appropriate to the 10-g microfarinograph. As applied here, standard deviation was 0 for arrival times and 0.3 min for departure times. These were for eight separate farinograms applied to the same fortified flour (12.1% moisture, 10.5% protein, 56% water absorption).



**Fig. 6.** Mixing tolerance index in Brabender units for cv. Moro flour (9.2% protein) blended with added wheat gluten concentrates. Gluten concentrates produced from a developed batter by cold-ethanol displacement ( $\square$ ) or by water displacement ( $\circ$ ). Solid symbols indicate mixing tolerance index of the base, unfortified flour.



**Fig. 7.** Absorption of water (%) for cv. Moro flour (9.2% protein) blended with added wheat gluten concentrates. Gluten concentrates produced from a developed batter by cold-ethanol displacement ( $\square$ ) or by water displacement ( $\circ$ ). Solid symbols indicate absorption of the base, unfortified flour.

## RESULTS AND DISCUSSION

A farinogram for the source flour from which gluten was separated by displacement removal of starch is shown in Fig. 1. This flour was most like flour exhibiting a typical type III or type IV pattern shown by the majority of bakery bread flour usually from hard red and hard red spring wheat (D'Appolonia and Kunerth 1984.). The source flour had a protein content of 13.5% mfb. A farinogram for the target flour of club wheat cv. Moro (Fig. 2) was most like a type I flour, typical of cake and cookie flours usually from soft wheat. The target flour had a protein content of 9.2%. This flour was subsequently fortified with laboratory-produced gluten and used in dough development studies.

We followed the usual practice of low-temperature vacuum drying as a method to produce a standard or control vital gluten with the least thermal degradation. Thus, both W-gluten and CE-gluten were exposed to low temperature and high vacuum. During the preparation and freezing of the samples for vacuum drying, we noted that the W-gluten was solid at  $-20^{\circ}\text{C}$  and that the wet CE-gluten was not solid but remained pliable. The lack of solidification was expected in view of the low water content and the  $-114.5^{\circ}\text{C}$  freezing temperature of ethanol.

Development farinograms were determined for Moro flour fortified using W-gluten<sub>at  $-20^{\circ}\text{C}/\text{V}$</sub>  and CE-gluten<sub>at  $-20^{\circ}\text{C}/\text{V}$</sub> . Figures 3 and 4 show farinograms with a semitransparent overlay of the farinogram for the base or target flour and objectively for the full data set in Figs. 5–7. In these figures, increasing the level of fortification (and the total protein content) changed the development farinogram from a type I toward a type III/IV pattern. This effect is consistent with studies of flours with increasing amounts of added protein (Aitken and Geddes 1939; Booth et al 1980; He and Hosney 1992). Notably, the similarity of the fortified flour to bread flour was greater for the CE-gluten<sub>at  $-20^{\circ}\text{C}/\text{V}$</sub>  than for the W-gluten<sub>at  $-20^{\circ}\text{C}/\text{V}$</sub> .

The differences between the gluten types was even more apparent in values for the stability (St) derived from the complete data set (Fig. 5) and for the mixing tolerance index (MTI). At every level of fortification, the CE-gluten had a greater St than the W-gluten. Furthermore, the increase in the St was directly proportional to the protein concentration in the range of fortification. The marginal change in the St per unit of protein was 1.28 ( $R = 0.968$ ) for W-gluten and 2.05 ( $R = 0.987$ ) for CE-gluten. This is a 57% increase in the direction of a stronger flour. Generally, a larger value of St is sought in large-scale bakery mixing operations.

Differences between the gluten types were revealed in MTI values (Fig. 6). Here the decrease in MTI was also directly proportional to protein concentration in the range of fortification. The marginal change in MTI per unit of protein was  $-18.03$  ( $R = 0.977$ ) for W-gluten and  $-23.63$  ( $R = 0.982$ ) for CE-gluten. This corresponds to a 24% change in the direction of a stronger flour.

Differences between the gluten types were also revealed in dough water absorption (Fig. 7). CE-gluten absorbed more water than W-gluten at all concentrations. These data can be represented by second-order polynomials with derivatives proportional to the amount of protein. For CE-gluten, the relationship for the slope was  $-4.81 + 0.50 P$  ( $R = 0.978$ ) and for W-gluten  $-1.49 + 0.178 P$  ( $R = 0.947$ ), where  $P$  is total flour protein (%).

Our intent in this study was to quantify functional differences between W-gluten and CE-gluten to establish incentives or disincentives for the alternate technologies. The result favoring freeze-dried CE-gluten over freeze-dried W-gluten was unexpected. At this time, we can only speculate on the causative factors. Contributing chemical factors may include 1) lipid and lipoprotein interactions resulting from concentration, homogeneity, and distribution differences; 2) gliadin concentrations; 3) concentration of water-soluble polysaccharides or pentosans; and 4) concentration of water-soluble proteins. However, we note our use of conditions, in particular low temperature and high ethanol content during displacement, to minimize but not necessarily eliminate lipid and protein

solubility phenomena (Rao and Arnold 1956; Robertson and Cao 1998a,b, 1999).

It should be noted that striking physical differences between W-gluten and CE-gluten may also have a bearing on functional differences. Predominant among these is the difference in level of gluten compactness or condensation. In previous use of a Martin-like method or current use of a batter-like method, W-gluten was produced as a highly condensed, gum-like matrix; whereas, the CE-gluten was respectively spongy (curdlike) or fibrous (Robertson and Cao 1998; Robertson et al 2000). The lower degree of condensation of the CE-gluten was associated with observations of relatively greater bulk or apparent volume after separation (but before drying) and of relatively greater friability after drying. The functional advantages for CE-gluten may arise because of a greater capacity for new interactions when recombined with and diluted by flour and water and redeveloped as a component of a dough matrix.

## CONCLUSIONS

Gluten produced by the CE displacement method has a greater ability on a unit of protein basis than gluten produced by aqueous displacement to effect increases in stability, improve mixing tolerance index, and increase water absorption. Furthermore, the low temperature employed in the production of CE-gluten appears to successfully minimize protein and lipoprotein solubility. Greater definition of interactions between compositional and material or functional properties of gluten concentrates is needed.

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[Received September 13, 2000. Accepted April 19, 2001.]