

Effect of Processing on Functional Properties of Wheat Gluten Prepared by Cold-Ethanol Displacement of Starch

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ABSTRACT

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Functional properties of gluten prepared from wheat flour are altered by separation and drying. Gluten was separated and concentrated by batterlike laboratory methods: development with water, dispersion of the batter with the displacing fluid, and screening to collect the gluten. Two displacing fluids were applied, water or cold ethanol (70% vol or greater, -13°C). Both the water-displaced gluten (W-gluten) and ethanol-displaced-gluten (CE-gluten) were freeze-dried at -20°C as a reference. Samples were dried at temperatures up to 100°C using a laboratory, fluidized-bed drier. Tests of functionality included 1) mixing in a mixograph, 2) mixing in a farinograph, and 3) the baked gluten ball test. Dough-mixing functionality was assessed for Moro flour (9.2% protein) that was fortified up to 16% total protein with dried gluten. In the mixograph, CE-gluten (70°C) produced improved dough performance but

W-gluten (70°C) degraded dough performance in proportion to the amount added in fortification. In the microfarinograph, there was a desirable and protein-proportional increase in stability time for CE-gluten (70°C) but no effect on stability for W-gluten (70°C). Baking was evaluated using the baked gluten ball test and the percentage increase in the baked ball volume relative to the unbaked gluten volume (PIBV). PIBV values were as high as 1,310% for freeze-dried CE-gluten and as low as 620% for W-gluten dried at 70°C . PIBV for CE-gluten was reduced to 77% of the freeze-dried control by fluid-bed drying at 70°C . Exposure of CE-gluten to 100°C air gave a PIBV that was 59% of the reference, but this expansion was still greater than that of W-gluten dried at 70°C . The highest values of PIBV occurred at the same mixing times as the peak mixograph resistance.

Loss of vitality or denaturing of wheat gluten protein may be identified physically by reduced baked bread volume, dough extensibility, capacity for water absorption, the maximum dough extensibility resistance, and apparent dough viscosity. At a chemical level, there may be increased protein hydrophobicity, disulfide bonding, solubility in 1.5% SDS, and solubility in acetic or lactic acid. Irreversible conformational changes of the proteins have been reported. The denaturing process is initiated at 65°C or higher, provided that the water content is $>10\%$. Peak rates of change occur at 40% moisture. If $<10\%$ moisture, 1 hr at 90°C does not denature the gluten (Pence et al 1953; Booth et al 1980; Weegels and Hamer 1992; Weegels et al 1994a,b). The combination of air temperature and gluten moisture during drying presents a clear risk for thermally induced denaturation to gluten when it is dried.

Wheat gluten is commercially produced as a concentrate by aqueous displacement of starch and contains $\approx 60\%$ moisture before drying. This is reduced to 10–14% by drying in hot air in a ring or flash drier. In a ring-type drier, process air enters at 100 – 150°C and exits at $\approx 60^{\circ}\text{C}$ (Grace 1989; Weegels and Hamer 1992). Flash-drying may expose the gluten to even higher temperatures. In a laboratory flash-drying pilot study temperatures of 230 – 330°C produced gluten that had a five- to sixfold increase in viscoelasticity, as indexed by the Brabender Glutograph shear time (Meuser et al 2002).

We have reported an alternate method for concentrating wheat gluten that uses cold ethanol (-13°C) to physically displace starch from a hydrated dough or batter. At the same time that the starch is displaced, liquid water in the dough matrix is displaced and dissolved, leaving a gluten concentrate that is wet with ethanol (Robertson and Cao 1998a,b, 2001a,b; Robertson et al 1999, 2000). A

vapor pressure more than twice that of water suggests the possibility for ethanol removal at $<60^{\circ}\text{C}$, where gluten is much less sensitive to denaturation. Because the cold-ethanol method must recycle the used ethanol, water from ethanol separation is an additional need. However, because of the low surface tension, density, and heat of vaporization of ethanol, and the relatively high efficiency of separation methods for removal of water from ethanol, the energy for removal of ethanol from the gluten solids and water from the used ethanol is expected to be less than direct water removal from the gluten solids (Krochta 1981).

This new solvent-based technology creates equipment needs different from those of current methods. Implementation would require significant investment. Furthermore, the functional properties of gluten from the cold-ethanol method (CE-gluten) are not well described, and ethanol may induce changes to the vital gluten complex of gliadin protein, glutenin protein, and lipid in spite of efforts to manage the process to minimize differential solubility.

It was deemed necessary to assess properties of gluten produced by the alternate methods to determine whether the cold-ethanol methodology would be credited or debited for changed product value. This assessment considered the gluten concentration process as separation of the components (step 1) followed by removal of water or ethanol from the gluten (step 2). Initial studies of the gluten at the end of step 1 used freeze-dried and milled gluten. The dry gluten was hydrated and tested as concentrate or added as a functional supplement to weak flour and then developed with water in the farinograph (Robertson and Cao 2001a) and mixograph (Robertson and Cao 2001b). We concluded on the basis of these mixing-rheological studies, that at the end of step 1, the gluten produced by the cold-ethanol method had improved mixing properties relative to gluten produced by a comparable water-based method. Desirable mixing characteristics such as greater stability time (farinograph) and increased midline peak height (mixograph) were observed for the gluten produced by the new method. These results suggest improved value for vital gluten produced by the new method.

Because the cold ethanol is applied to a water-rich dough or batter, the results may not be directly comparable with prior reports of ethanol effects on dry flour or flour components. For instance, ethanol will extract several flour components, but the amount extracted depends on the processing before the extraction, the temperature of extraction, and the absence of water in the substrate. For instance, dough formation followed by freeze-drying reduced the anhydrous-ethanol extractable matter from the dry material by

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>50% relative to that extracted from flour (Wooton 1966). When dry flour was extracted at ambient temperature with 95 or 90% ethanol and then prepared as a dough, peak mixing development times in a four-pin planetary mixer were increased by 2.5-fold to sixfold (MacRitchie and Gras 1973). This was attributed to changes in the gluten but can be modified by the moisture absorption used in the mixing experiment (Robertson and Cao 2002).

The present report extends the evaluation of gluten from the cold-ethanol method to include changes to functional properties that occur during drying. Functional mixing properties were assessed by the mixograph or farinograph applied to gluten concentrates or straight and gluten-fortified flour. Baking properties were assessed by the baked gluten ball test applied to gluten concentrates. The gluten ball test measures the ability of the balls to entrap air and water vapor and to expand with the entrapped vapors. The test has been used to predict loaf volume expansion of gluten-fortified flour (Czuchajowska and Pomeranz 1990).

MATERIALS AND METHODS

Unbleached flour obtained from a commercial supplier (Giusto, San Francisco, CA) was stored at -30°C and 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. A proximate analysis (Anresco, San Francisco, CA) for this flour on a dry or moisture-free basis (mfb) was 13.4% protein ($N \times 5.7$) by micro Kjeldahl method (AOAC 960.52), 5.6% lipids (AOAC 922.06), 0.6% ash (AOAC 923.03), 69.1% carbohydrates including fiber by difference, and total solids (AOAC 925.09) (AOAC 2000). Protein was assayed at 13.5% in this laboratory by nitrogen determination (LECO model FP428, St. Joseph, MI). Flour produced from cv. Moro was provided by the Pullman Wheat Quality Laboratory of the USDA/ARS, Pullman, WA. A proximate mfb analysis for this flour (Anresco) was 9.9% protein ($N \times 5.7$), 6.3% lipids, 0.4% ash, and 72.9% carbohydrates including fiber. Protein by nitrogen assay was 9.2% mfb ($N \times 5.7$). Undenatured, 200 proof ethyl

alcohol (AAPER Alcohol and Chemical Co., Shelbyville, KY) was diluted with distilled water as desired.

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

Gluten was also prepared using water displacement. Batter preparation and separation with distilled water was the same as above with the following exceptions: 1) the batter was relaxed at ambient temperature, and 2) the displacing fluid was water at ambient temperature. Three batter suspensions with 400 mL of water followed by screening were performed.

For gluten drying, gluten samples were lyophilized at -20°C and 200×10^{-3} mBar using a freeze-dry system (Freezone 12/79480/77450, Labconco, Kansas City, MO). A sample from each run was analyzed for nitrogen with a range of 65–75% protein ($N \times 5.7$). The dried samples from six displacement runs were combined and milled (laboratory mill 3100, Perten, Reno, NV). The combined six-run sample was analyzed (nitrogen determinator) and yielded protein concentrations with a mean of $70\% \pm 2$ standard deviation ($N \times 5.7$).

When performing side-by-side comparisons in fortification studies, the protein content of the CE-gluten matched that of the W-gluten to within $\pm 1\%$. In addition, for a composite sample representing the experiments, a proximate mfb analysis (Anresco) for CE-gluten was 4.3% lipids and 0.7% ash; and for W-gluten was 5.2% lipids and 0.6% ash.

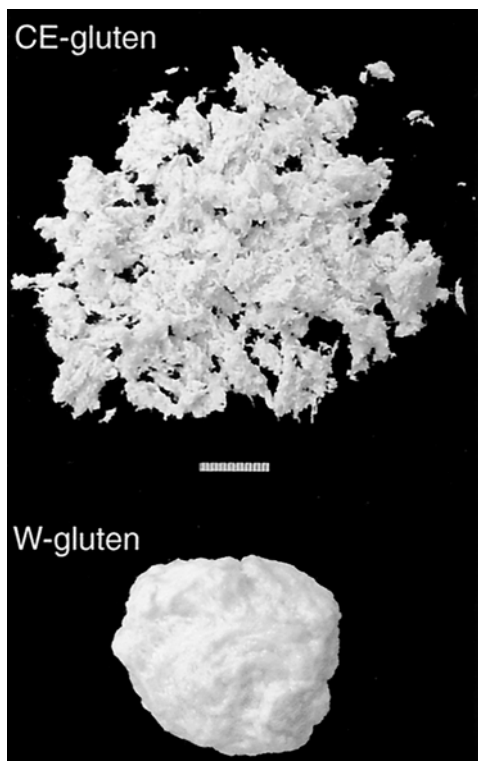


Fig. 1. Wet gluten recovered by batterlike process using cold ethanol (top) or water (bottom). Samples have approximately equal dry mass of 10 g. Scale bar = 2.54 cm.

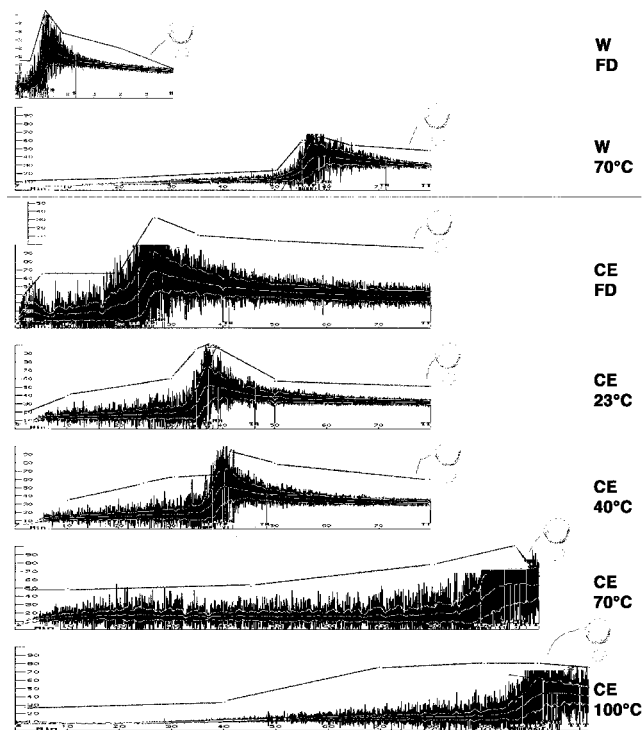


Fig. 2. Mixograph traces and baked gluten ball expansions (actual volume plotted as straight lines and indicated with baked ball icons) for W-gluten and CE-gluten dried in fluid-bed drier and freeze-dried (-20°C). Vertical scale is % torque or final baked ball volume (cm^3).

W-gluten was dried in a fluid-bed dryer (Armfield MK-II, Sherwood Scientific, Cambridge Science Park, Cambridge, England) after the wet gluten was subdivided to pieces 1.5 ± 0.5 g (≈ 1 cm diameter) and then displayed on a screen within the drier chamber. CE-gluten was displayed on a screen in the form it was produced at the end of step 1. For both gluten types, upflowing air at a nominal 1.8 m/sec (14.9 L/sec) was applied in a laboratory fluid-bed drier. Drying was continued until constant weight was achieved. Samples were milled as above.

In flour fortification experiments, Moro flour was used as the base flour and was supplemented with laboratory-processed gluten. The mixograph was a 10-g mixograph and mixing data was collected and analyzed using MixSmart software (National Manufacturing, TMC Division, Lincoln, NB). Gluten-fortified flour absorptions were calculated on a protein basis applying Approved Method 54-40A (AACC 2000). Sample size was 10 g for mixograph analysis of fortified flour and 5 g for gluten analysis.

For farinograph mixing properties, a 10-g microfarinograph (model 8110, C.W. Brabender, Duisburg, Germany) was used. Before each reported run, a 10-g sample of flour at 11.4% moisture was placed in the farinograph chamber, the development initiated, and the amount of water needed to produce a response centered on 500 ± 10 BU was determined by a standard titration of the flour with dropwise addition of water. This was followed with a test in which the previously determined amount of water was added quickly at a rate of 20 mL/min from the farinograph burette.

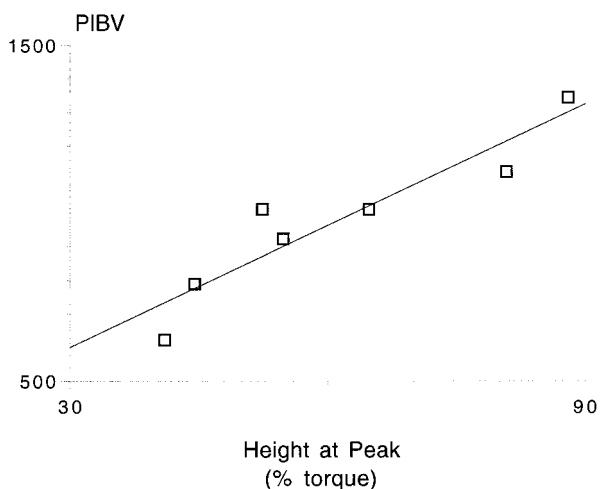


Fig. 3. Correlation between percentage increase in baked gluten ball volume (PIBV) and mixograph midline peak height. Coefficient of linear regression ($y = ax + b$, $a = 12.1$, $b = 240$, and $r = 0.931$).

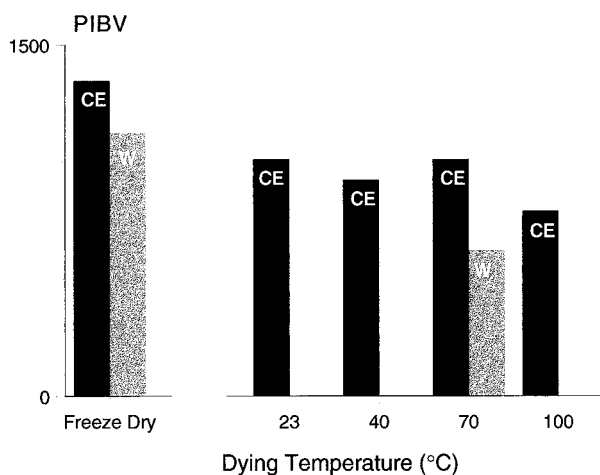


Fig. 4. Changes to baked gluten ball volume (PIBV) for W-gluten and CE-gluten resulting from increased temperature of drying.

For the baked gluten ball test, 5 g of dried and milled gluten (8.9% moisture) was rehydrated to a 164.7% absorption with distilled water and mixed in the 10-g mixograph. The sample was removed from the mixograph and relaxed for 50 min in a closed container. The volume of the ball was measured by volume displacement of distilled water. Each ball was baked at 218°C for 20 min, then 149°C for 30 min in a convection oven (Cuisinart, East Windsor, NJ). After a cool down, the volume of the rigid ball was measured by sand displacement in a graduated cylinder.

RESULTS AND DISCUSSION

Drying

Freshly prepared CE-gluten was dried at 23–100°C in the fluid-bed drier. Three reference points were determined: 1) CE-gluten dried at -20°C in a freeze-drier; 2) W-gluten dried at -20°C in a freeze-drier; and 3) W-gluten dried in the fluid-bed drier at 70°C to elicit heat damage. Drying of CE-gluten was more rapid than that of W-gluten. This was most evident during the first few minutes of drying where the initial rate of weight loss for W-gluten was $<25\%$ of the initial rate for CE-gluten. W-gluten was exposed to the drying temperature for ≈ 2 hr, while the CE-gluten drying was complete in 20–30 min.

Drying differences were attributed to the high ethanol volatility, but may also arise from physical differences between gluten produced by each method (Fig. 1). CE-gluten at the end of separation is fibrous with a large surface area for evaporation of solvent. In

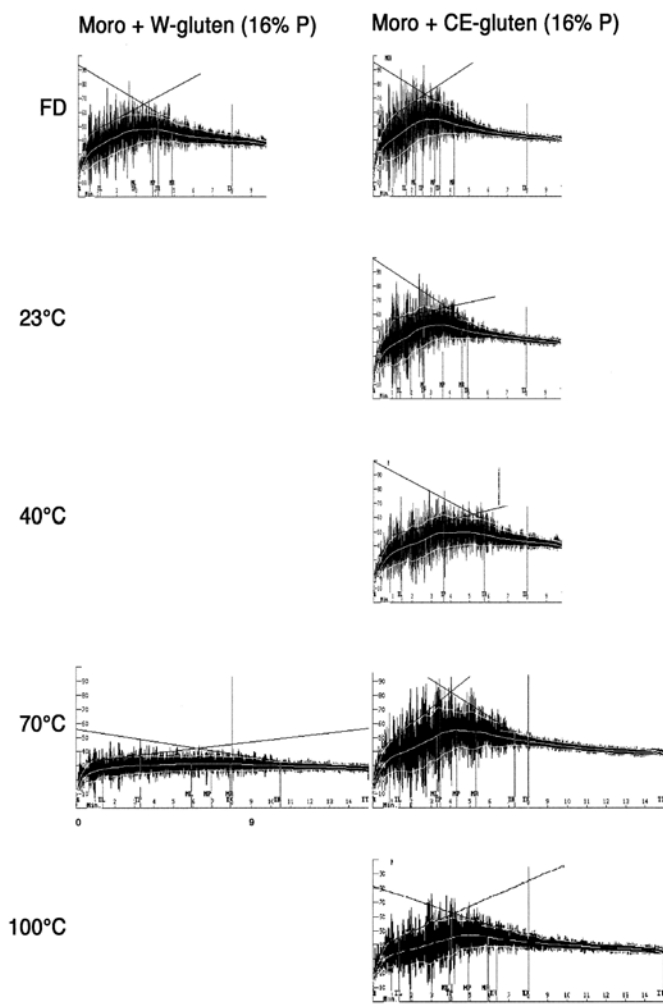


Fig. 5. Mixograph traces for 16% total protein fortification of Moro flour at constant moisture absorption (64%). Vertical scale is % torque and horizontal scale is time (min).

earlier reports using a Martin or dough-ball method, we observed CE-gluten in a curd or spongelike form. W-gluten, produced by the dough-ball or batter method is highly cohesive and gummy, and emerges from separation in a single, relatively dense piece with limited surface area. The W-gluten was subdivided into pieces 1 cm in diameter before drying.

Mixograph and Baked Gluten Ball Test

We have shown that the functional mixing properties of wheat gluten depend not only on the separation process method, but also on the mixing time and evaluation method (farinograph or mixograph) and the mixograph moisture absorption (Robertson and Cao 2001, 2002). In the present study, we found baked ball volume was also dependent on these factors. The baked ball versus mixing time data match the trace of the mixograph. In particular, the times of mixing that produced maximum dough resistance were the same as those that produced maximum ball expansion (Fig. 2). Furthermore, the increase in ball volume (as a percentage increase in volume from the prebaked or PIBV) was directly proportional to midline peak height (maximum midline value) for both methods (Fig. 3). In these tests, the baked balls give the outward appearance of baked bread and contain a number of rigid cells of highly variable dimensions.

The PIBV at the midline peak time was diminished by drying temperatures above those used in freeze drying (Fig. 4). For 23°C drying of CE-gluten, PIBV values were diminished to 77% of the

freeze-dried values, remained constant for gluten dried at up to 70°C. CE-gluten PIBV values were diminished to 59% of the freeze-dried values by drying at 100°C. PIBV for W-gluten was lower than those for CE-gluten at comparable temperatures. For instance, the ratio of PIBV for W-gluten to CE-gluten was 0.8 for freeze drying and 0.6 for 70°C drying. The PIBV ratio comparing W-gluten dried at 70°C to the freeze-dried CE-gluten was 0.4. W-gluten dried at 70°C showed lower expansion in baking than CE-gluten, even when the latter was dried at 100°C. For both gluten types, there was a progressive increase in the midline peak time as the temperature of drying increased.

In view of the complexity of denaturation, dough formation, and baking processes, as well as differences in glutenin-gliadin solubility at > -13°C, drying induced changes to PIBV values were not surprising. However, for most drying temperatures, including denaturing conditions at 70°C, the PIBV was maintained at 85% of the freeze-dried W-gluten PIBV. The reduction of PIBV for W-gluten dried at 70°C was considered a severe condition and was neither intended to be nor should be taken as an indication of commercial practice.

Fortification

Changes to Moro flour by fortification to 16% total protein were monitored on a mixograph. Mixograph traces (Fig. 5) suggest minimal drying temperature effect for CE-gluten dried at ≤70°C (midline peak height of 52.9 ± 2.3 standard deviation compared to 42.7 for the unfortified flour). However, there was a 20% loss of midline peak height when the flour was fortified with CE-gluten that had been dried at 100°C. Moro flour fortified with freeze-dried W-gluten had a lower midline peak height than the comparable CE-gluten fortified flour. However, midline peak height decreased from 55.1 to 31.3% torque for the flour fortified with W-gluten dried at 70°C.

A second evaluation based on flour fortified to final protein concentrations of 11–16% using gluten dried at 70°C (Fig. 6) indicated proportional loss of dough-forming ability for flour with proportionally more W-gluten. At the same time, there was a proportional increase in midline peak height by fortification with CE-gluten. Incremental increases in midline peak height (Fig. 7) were positive for CE-gluten and negative for W-gluten.

A third evaluation of fortified flours in this protein concentration series was made using the microfarinograph (Fig. 8) for gluten dried at 70°C. For this series, there was a near constant stability time at all concentrations for W-gluten but an increasing stability time for CE-gluten (Fig. 9). The stability times for flour fortified with CE-gluten dried at 70°C were greater than that of flour fortified with freeze-dried W-gluten.

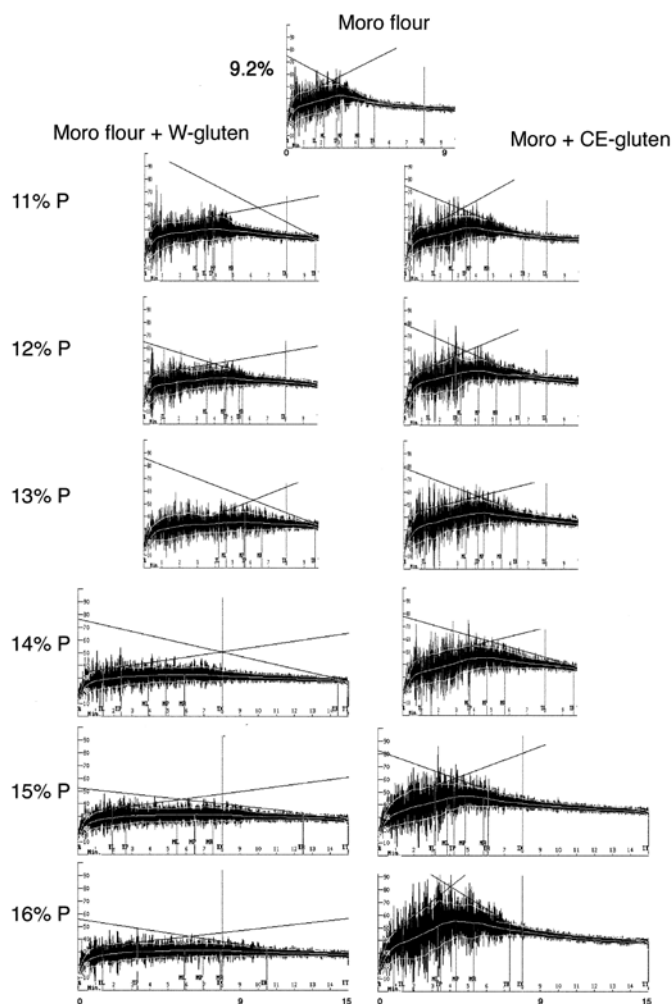


Fig. 6. Fortification level mixing properties revealed in mixograph traces for Moro flour at 9.2% protein and fortified up to 16% protein with CE-gluten and W-gluten dried at 70°C. Vertical scale is % torque and horizontal scale is time (min).

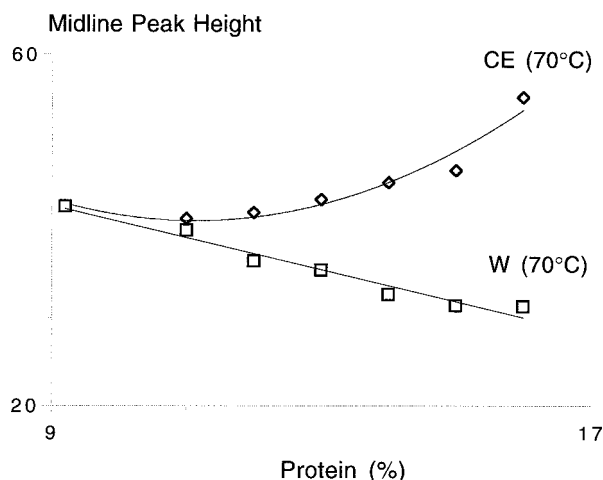


Fig. 7. Changes to mixograph midline peak height for Moro flour fortified up to 16% protein with CE-gluten and W-gluten dried at 70°C.

Conventional Baked Ball Test

The baked gluten ball test was applied to evaluate the baking properties of processed gluten in the absence of other bread dough constituents. The test is rapid. The most recent use of a baked gluten ball test (Czuchajowska and Pomeranz 1990) proposed its use as a method of determining gluten quality. This test suggested a constant mixograph mixing time and excess water for the formation of the gluten ball to be baked. As shown here and earlier (Robertson and Cao 2002), mixograph traces are a function of the gluten tested. Furthermore, the characteristic mixing time at peak and midline peak heights are also dependent on the water absorption. Hence, a constant mixing time test using maximum absorption may create a favorable bias for gluten with times-to-peak that coincide with the test mixing time and a negative bias for gluten with times-to-peak that do not coincide with the test mixing time.

However, because short mixing times would be attractive in a practical test, we used the baked ball method with constant mixing time to compare volumetric expansions of gluten after 5 min of the mixograph. This mixing time was less than the midline peak time (Fig. 2) for any of the gluten tested earlier. The test results suggested equivalence in PIBV for both W- and CE-gluten at the freeze-dried reference, the absence of change with drying temperature for CE-gluten, and a large reduction of the PIBV for W-gluten

dried at 70°C (Fig. 10). Note that the greatest PIBV (600%) reported using this test was less than half of the greatest PIBV (1,344%) measured at the midline peak time (Figs. 2 and 4).

CONCLUSIONS

Gluten prepared by the cold-ethanol method (CE-gluten) is resistant to thermal denaturing at drying temperatures during which water-processed gluten (W-gluten) is denatured. The greatest volume expansion of gluten on baking of rehydrated concentrated gluten occurs at the peak mixograph mixing time. This is true for gluten from either method. Even heat-damaged W-gluten will expand substantially if mixed to its peak mixing time. Comparison of gluten baking expansion at the peak mixing time suggests some drying diminishment of the mixing and baking properties of CE-gluten relative to freeze-dried CE gluten. However, CE-gluten exposed to drying temperatures of 70°C still increased mixing height and increased stability of Moro flour with poor baking quality, while W-gluten dried at this temperature had no effect (farinograph stability time) or a deleterious effect (mixograph mixing peak height). These results suggest impeded ability of W-gluten (70°C) to interact with native proteins of the Moro flour and to develop under the mixing conditions used for bread dough.

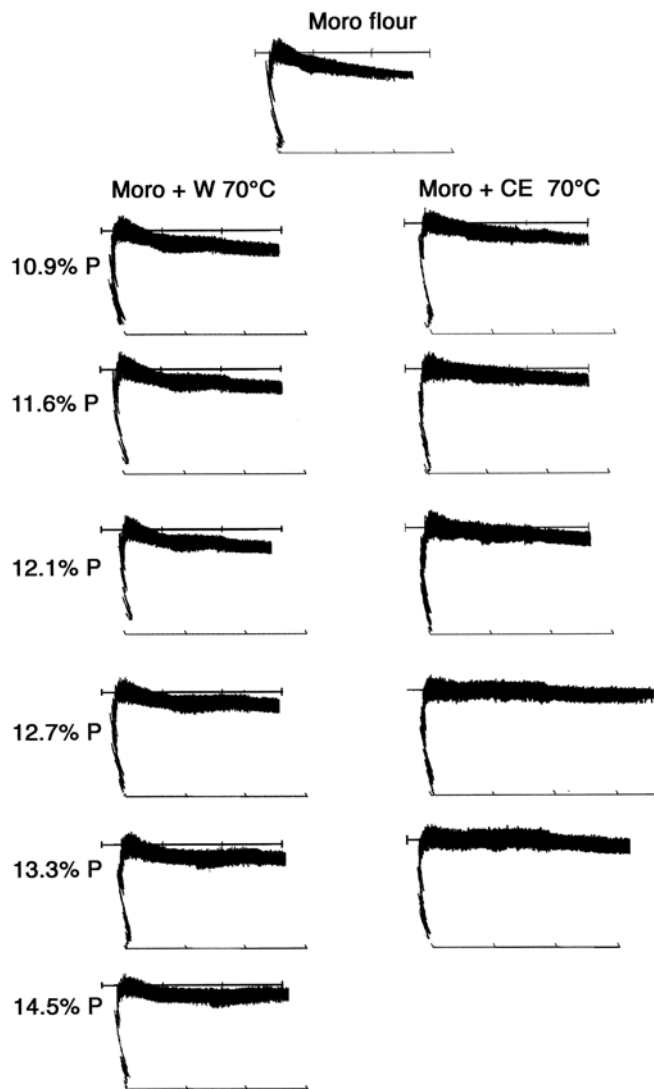


Fig. 8. Fortification level modification of mixing properties revealed in farinograph traces for Moro flour at 9.2% protein and fortified up to 14.5% protein with CE-gluten and W-gluten dried at 70°C. Vertical scale is consistency (BU) and horizontal scale is time (min).

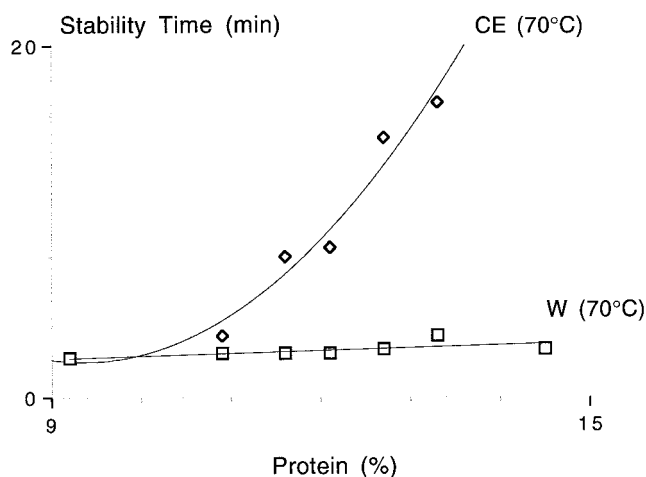


Fig. 9. Changes to farinograph stability time for Moro flour fortified with CE-gluten and W-gluten dried at 70°C.

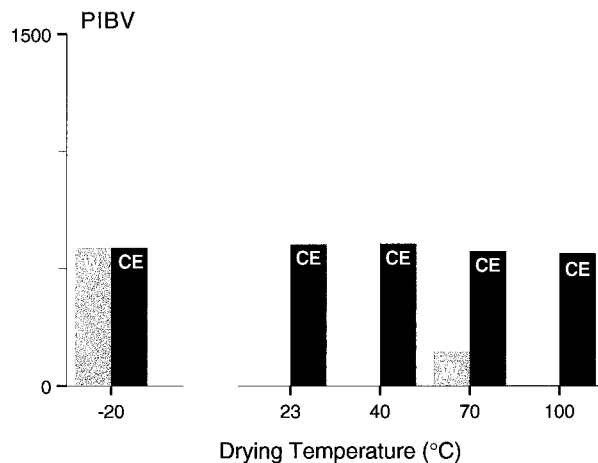


Fig. 10. Percentage increase in baked gluten ball volume (PIBV) using constant 5 min mixograph mixing and 160% water absorption for W-gluten and CE-gluten dried in fluid-bed drier with heated air and freeze dried.

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