

Mixograph Responses of Gluten and Gluten-Fortified Flour for Gluten Produced by Cold-Ethanol or Water Displacement of Starch from Wheat Flour

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ABSTRACT

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The total protein of gluten obtained by the cold-ethanol displacement of starch from developed wheat flour dough matches that made by water displacement, but functional properties revealed by mixing are altered. This report characterizes mixing properties in a 10-g mixograph for cold-ethanol-processed wheat gluten concentrates (CE-gluten) and those for the water-process concentrates (W-gluten). Gluten concentrates were produced at a laboratory scale using batter-like technology: development with water as a batter, dispersion with the displacement fluid, and screening. The displacing fluid was water for W-gluten and cold ethanol ($\geq 70\%$ vol, -12°C) for CE-gluten. Both gluten types were freeze-dried at -10°C and then milled. Mixograms were obtained for 1) straight gluten concen-

trates hydrated to absorptions of 123–234%, or 2) gluten blended with a low protein (9.2% protein) soft wheat flour to obtain up to 16.2% total protein. The mixograms for gluten or gluten-fortified flour were qualitatively and quantitatively distinguishable. We found differences in the mixogram parameters that would lead to the conclusion of greater stability and strength for CE-gluten than for W-Gluten. Differences between the mixograms for these gluten types could be markedly exaggerated by increasing the amount of water to the 167–234% range. Mixograms for evaluation of gluten have not been previously reported in this hydration range. Mixograms for fortification suggest that less CE-gluten than W-gluten would be required for the same effect.

Enrichment of protein to $>70\%$ without simultaneous attenuation of protein mixing and baking properties is a primary goal for commercial vital wheat gluten processing. Attainment of these objectives enables wheat gluten protein to satisfy the needs of its principal market, the fortification of low protein flour for bread manufacture. The unique functional properties of wheat may also lead to unique nonfood uses (Magnuson 1985; Bietz and Lookhart 1996). The enrichment goal may be achieved through a number of technologies. However, full conservation of mixing and baking properties often is not achieved universally because of chemical changes to the gluten components that are initiated during enrichment and extended by exposure to high-temperature drying (Weegels and Hamer 1992). Relatively long exposure to drying temperatures is needed because of the gummy form of the wet protein and severe case or surface hardening of the drying particles.

We have reported on a method for concentrating wheat gluten that displaces starch from a hydrated dough or batter using cold ethanol (Robertson and Cao 1998a,b, 1999; Robertson et al 2000). We recently compared farinograph mixing properties of gluten prepared by this new method to those of conventionally produced gluten and found that the new method accentuates functional properties (Robertson and Cao 2001). The goal was to identify changes up to the end of the enrichment. Therefore, freeze-drying the gluten before rehydration and testing was used to minimize changes anticipated for high-temperature drying. We note that even though used at times as a reference standard (Dreese et al 1988), freeze-dried gluten may differ from native gluten in some respects (Webb et al 1971). The farinograph was employed in the reported testing program because it is often recommended for use with low-protein flour and we wanted to document mixing property changes to a soft wheat, low protein flour as a function of added protein concentrate. We found that the farinograph documented improved mixing properties for gluten produced by the cold-ethanol method (CE-gluten) relative to gluten produced by the conventional water-displacement

method (W-gluten) in fortifications of 9–13% total flour protein. The effect was comparable to that determined for farinograms of mixtures of hard and soft wheat flour with respectively high and low protein content (Kunerth and D'Appolonia 1985).

Both the farinograph and the mixograph are in regular use for testing flour development properties and are especially used to assess relative differences between wheat cultivars, native wheat protein content, or flour formulations. These devices employ different mechanical actions: the mixograph uses counterrotating pins to fold, twist, pull, and stretch the dough, and the farinograph uses counterrotating sigmoid blades to press, shear, and stretch the dough. (Mani et al 1992; Ingelin 1997; Shogren 1997). Each device also has been used to evaluate gluten properties. Farinograms of gluten diluted with starch have been reported (Bushuk 1963). An alteration of a farinograph (50-g bowl using 300-g bowl linkage) has been used to account for the very high resistance of gluten for direct testing of gluten. Direct testing of gluten concentrates that are not diluted with starch is desirable to focus the response on protein-protein interactions but exposes the farinograph mixer to very high mechanical stress (Miller and Hoseney 1996). The mixograph was judged better suited to test the development of undiluted gluten (Bushuk 1963).

The present report extends the evaluation of W-gluten and CE-gluten to include the use of the mixograph. The report compares gluten prepared from the same flour used by us in earlier studies. Freshly prepared gluten was frozen, freeze-dried, and milled before testing. In the present study, we applied the mixograph to evaluate mixing properties of 1) straight or "pure" gluten made by rehydrating W-gluten or CE-gluten; and 2) flour dough made with a soft, low protein wheat flour fortified to higher protein levels with either W-gluten or CE-gluten.

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 for this flour on a dry or moisture-free basis (mfb) (Anresco, San Francisco, CA) was 13.4 % protein ($N \times 5.7$) by micro Kjeldahl (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 also assayed at 13.5% using nitrogen determination (model FP428, Leco Corp., 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)

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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 (DSP-KY-417, AAPER Alcohol and Chemical Co., Shelbyville, KY) was diluted with distilled water as necessary for the desired concentration.

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 (model KSM50-PWH, KitchenAid Corp., St. Joseph, MI). 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 controlled temperature of $-13^\circ\text{C} \pm 3$. 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 and mixing as above for 6 min, and then screening.

Water Displacement

Batter preparation and separation with distilled water was the same as described 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.

Gluten Drying and Analysis

Gluten samples from each run 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 at 65–75% P ($N \times 5.7$). Dried samples from six

displacement runs were combined and milled (3100, Perten, Reno, NV). The combined six-run sample was analyzed (nitrogen determinator) and yielded protein concentrations with a mean of 70% ± 2 SD ($N \times 5.7$). When performing side-by-side comparisons, the protein content of the CE-gluten matched that of the W-gluten to $\pm 1\%$. In addition, for a composite sample representing the experiments, a proximate mfb analysis (Anresco) yielded 4.3% lipids and 0.7% ash for CE-gluten; and 5.2% lipids and 0.6% ash for W-gluten.

Flour Fortification and Dough Development

In fortification experiments, Moro flour was used as the base flour and supplemented with laboratory-processed gluten. The 10-g mixograph was used with mixing data collected and analyzed using MixSmart software (National Mfg., TCMCO, Lincoln, NB). For straight gluten, absorptions of 123–234% were used. This range of absorptions bracketed measurable mixograph responses. 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.

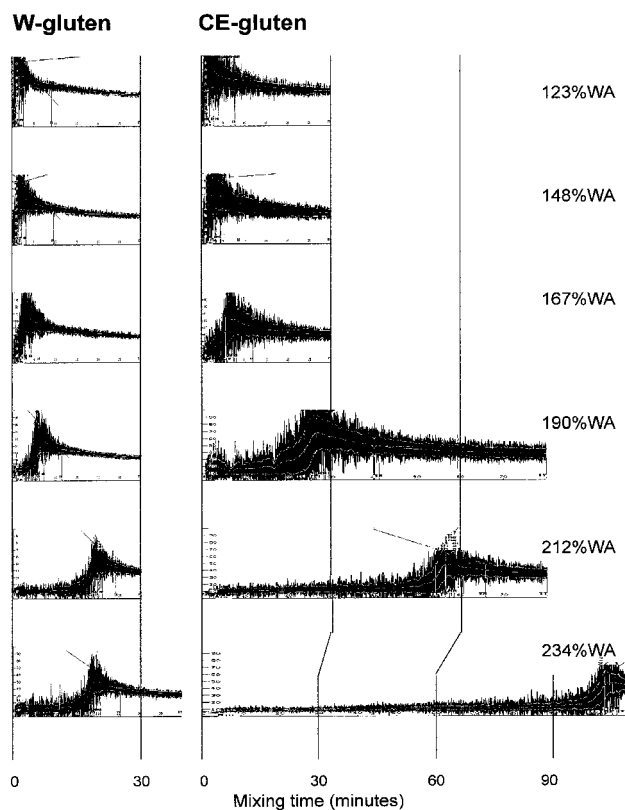


Fig. 1. Mixograms for water processed gluten (W-gluten, on the left) and cold-ethanol processed gluten (CE-gluten, on the right) demonstrating mixing or development characteristics influenced by water absorption (WA). Ordinate units in each frame are 0–100% torque.

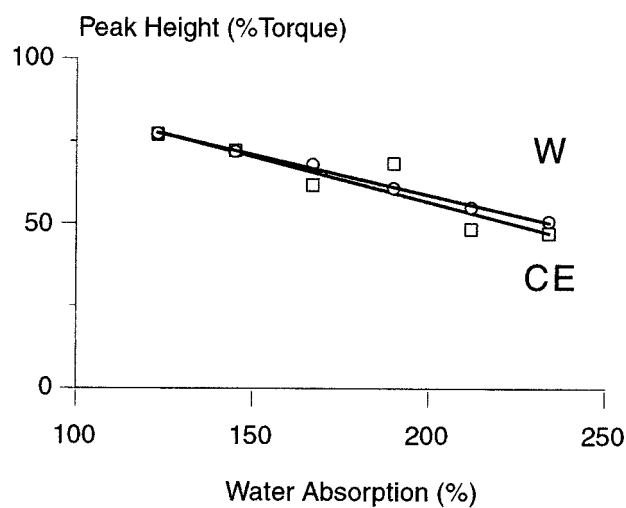


Fig. 2. Height at peak as a function of water absorption or water content from mixograms (Fig. 1) for water processed gluten (W-gluten, circles) and cold-ethanol processed gluten (CE-gluten, squares). Linear regression coefficients for W-gluten were $a = -0.245$, $b = 108$, and $r = 0.998$. Linear regression coefficients for CE-gluten were $a = -0.275$, $b = 112$, and $r = 0.919$.

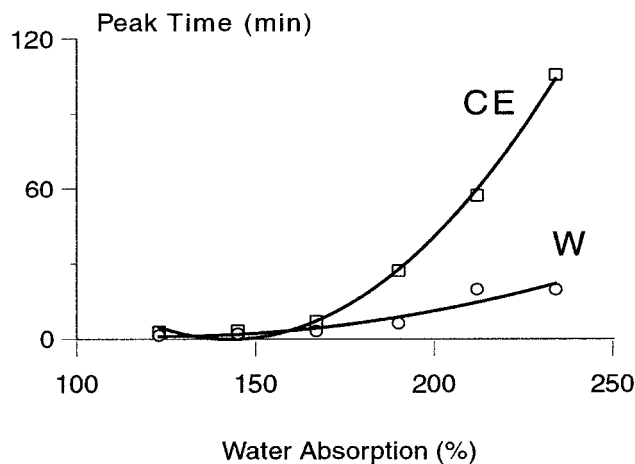


Fig. 3. Mixing time to peak as a function of water absorption or water content based on mixograms (Fig. 1) for water processed gluten (W-gluten, circles) and cold-ethanol processed gluten (CE-gluten, squares). Second-order polynomial regression coefficients for W-gluten were $a = 0.002$, $b = -0.414$, $c = 26.4$, and $r = 0.945$. Second-order polynomial regression coefficients for CE-gluten were $a = 0.013$, $b = -3.57$, $c = 255$, and $r = 0.999$.

RESULTS AND DISCUSSION

The research reported here sought to quantify similarities and differences between gluten concentrates produced by different methods. We sought to focus attention on the changes attributable to the process separation of starch from gluten by using a single flour as source for both W- and CE-gluten separation methods. We further sought to minimize heat effects due to removal of displacing liquid (water or ethanol) from the gluten by using freeze drying to dry the concentrated gluten samples.

The mixograph was used to measure the response of a gluten concentrate to a particular type of mixing. For testing straight gluten, the response primarily measures protein-protein interactions when all the protein is from processed gluten. For testing fortified flour, the response measures protein-protein interactions of three types: 1) fortifying or processed gluten protein with native or unprocessed flour protein, 2) native-flour-protein with native-flour-protein, and 3) fortifying, processed gluten protein with fortifying, processed gluten protein. The overall response depends on the native functional quality of both the protein in the fortifying gluten and the protein in the target flour. Therefore, the evaluation is specific only to the conditions of these experiments and flour-specific effects may be anticipated.

Mixograms for rehydrated W-gluten and CE-gluten containing 70.0% protein are shown in Fig. 1. Up to a water absorption of 167%, the most notable qualitative difference is that the CE-gluten shows a greater bandwidth for the duration of the experiments and this is reflected in part by the integral-at-peak values. In this range of absorption, the mixograms for both types also show a linear decrease in the peak-height value (Fig. 2) and a slight increase in the time-to-peak value (Fig. 3). This water absorption dependence is similar to that expected for flour (Baig and Hosney 1977; Kunerth and D'Appolonia 1985).

However, at >167% water absorption for CE-gluten and >190% water absorption for W-gluten, there is a distinct qualitative change to the mixograms. For both CE-gluten and W-gluten, there are progressively increasing peak time and integral-to-peak values (Figs. 3 and 4); but for a given water absorption, the values for W-gluten are always less than those for CE-gluten. In contrast, there was no break in the pattern of linear decline in peak height values throughout the range of measurement.

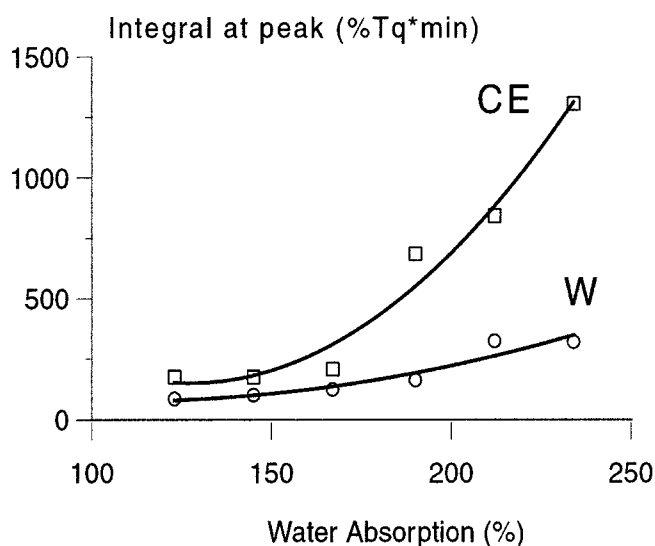


Fig. 4. Integral to peak as a function of water absorption or water content based on mixograms (Fig. 1) for water processed gluten (W-gluten, circles) and cold-ethanol processed gluten (CE-gluten, squares). Second-order polynomial regression coefficients for W-gluten were $a = 0.017$, $b = -3.55$, $c = 264$, and $r = 0.951$. Second-order polynomial regression coefficients for CE-gluten were $a = 0.102$, $b = -26.1$, $c = 1820$, and $r = 0.985$.

Interpretative guidelines developed for flour where protein is $\approx 25\%$ that of the straight gluten suggest that the CE-gluten samples were stronger and more tolerant to mixing. This is based on CE-gluten mixograms that show greater integral-to-peak values, and visual inspection of mixograms that show not only greater bandwidths but also a slower loss of bandwidth with increasing time. Slow development of resistance to mixing by CE-gluten also contributes to this conclusion (i.e., longer time-to-peak) (Kunerth and D'Appolonia 1985).

The use of high water absorptions in the testing of straight gluten, even though beyond the range of formulation for baking applications, could be of use in the evaluation of gluten concentrates to assess their value or to recommend concentrations in formulations. This possibility is suggested by the exaggeration in the differences in the mixograms over what might be considered to be subtle differences within the normal range of water absorptions used in testing. Other application uses of gluten, such as in chewing gum formulations (Shaw et al 1998), where performance at high absorptions would be desired, might also be evaluated using the mixogram at high absorption as a mechanical "chewing" model.

Moro flour with 9% protein and fortified with either W- or CE-gluten up to a total of 16% protein was mixed and developed on the mixograph (Fig. 5) using absorptions based on the amount of protein present in the fortified flour as prescribed in the Approved Method (AACC 2000). These mixograms reveal qualitatively relatively large changes by fortification with CE-gluten and lesser changes by fortification with W-gluten. However, quantitative parameters generated by the instrument software were compared and yielded only one measure, peak height (Fig. 6), that showed a consistent and differential pattern.

Height at peak values increased along with increases in the total protein content of the flour. All CE-gluten peak values were

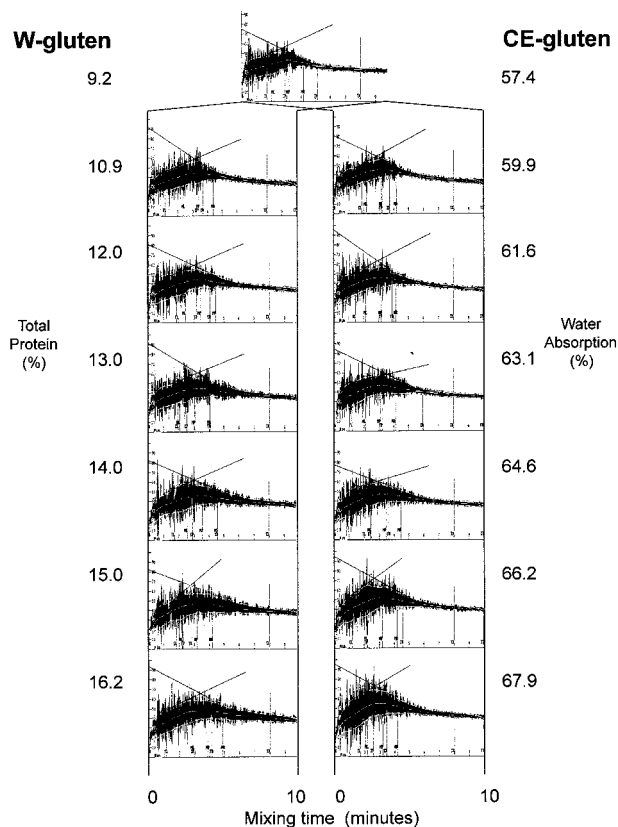


Fig. 5. Mixograms for water processed gluten (W-gluten, on the left) and cold-ethanol processed gluten (CE-gluten, on the right) in Moro flour (9.2% P, at top) demonstrating fortification and mixing or development characteristics influenced by protein content. Ordinate units in each frame are 0–100% torque.

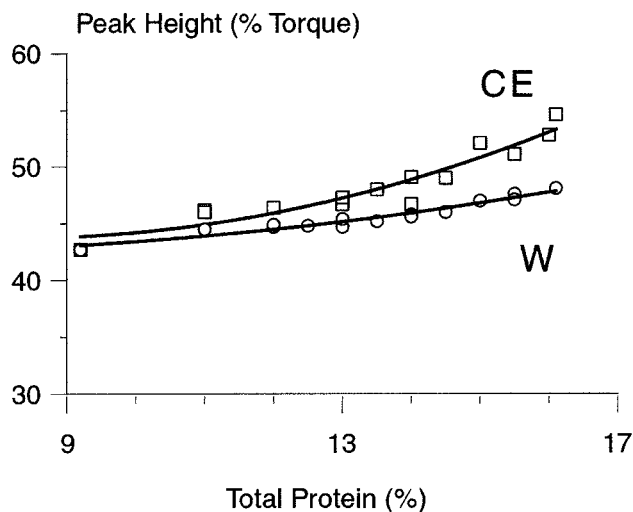


Fig. 6. Height at peak as a function of protein based on mixograms (Fig. 5) for water processed gluten (W-gluten, circles) and cold-ethanol processed gluten (CE-gluten, squares). Base Moro flour with no added protein is shown at left at 9.2%. Second-order polynomial regression coefficients for W-gluten were $a = 0.047$, $b = -0.501$, $c = 43.7$, and $r = 0.970$. Second-order polynomial regression coefficients for CE-gluten were $a = 0.154$, $b = -2.52$, $c = 54.0$, and $r = 0.945$.

greater than those for W-gluten, and the ratio of the increase in torque relative to the base flour for CE- to W-gluten at each fortification was calculated from the data as 1.9 ± 0.2 SD. The ratio of the marginal increase in torque per unit of increased protein (ratio of CE-slope to W-slope in Fig. 6) as determined from the regression equations in Fig. 6 increased uniformly from 1.7 at 11% protein to 2.4 at 16% protein (linear regression with $r = 0.984$). These ratios represent >100% increase for CE-gluten relative to W-gluten and suggest that about half as much of CE-gluten would accomplish the same mixing result as achieved by a quantity of W-gluten. These differences in development properties compare to a 57% higher (CE vs. W) marginal change in the farinograph stability time/unit of protein and a 24% decrease in the farinograph mixing tolerance index/unit of protein (Robertson and Cao 2001). Integral and time-to-peak also increased slightly with protein (not shown), slightly increasing values with increasing protein content, but protein-type-specific differential effects were not observed.

These results support the observation made previously that functional mixing properties of W-gluten and CE-gluten are not identical and that there may be distinct advantages for CE-gluten. It is possible that changes have been imposed on the W-gluten during freezing and drying that are not imposed on CE-gluten because of the absence of structure modifying ice crystals in the latter (Davies 1968). In addition, because of the reduction of water by dissolution in the ethanol, there is increased opportunity within the CE-gluten for more intimate contact leading to stronger protein-protein associations. The difficulty in reversing these associations upon rehydration and mixing is especially revealed by mixograms at high moisture.

These data form a baseline against which other processing-related changes may be compared. In commercial manufacturing, processed gluten likely will not be lyophilized, and W-gluten would be dried in hot air to remove water and CE-gluten desolvated to remove and recover ethanol. The conditions required for these drying processes may superimpose additional changes to functional properties. Property changes due to high-temperature drying will be the subject of a future report.

CONCLUSIONS

For development of hydrated fortified flour, gluten produced by the cold-ethanol displacement method has a greater ability on a per protein basis to increase peak height than gluten produced by aqueous displacement. For development of rehydrated gluten concentrates, mixograms reveal greater strength and stability for CE-gluten than for W-gluten. Mixograms of rehydrated gluten concentrates at high water absorptions exaggerate mixing properties and exaggerate and reveal differences that are less obvious at low water absorption. Greater definition of the interactions between compositional and functional properties of gluten concentrates is needed.

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