

Wheat Gluten Swelling and Partial Solubility with Potential Impact on Starch-from-Gluten Separation by Ethanol Washing

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ABSTRACT

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Swelling of wheat gluten may be a contributing factor in washing or displacement separation of gluten and starch using cold ethanol. To test this hypothesis, dissolution and swelling (settled volume or mass absorption) of a commercial gluten are reported here for the first time as a function of both temperature and ethanol solution concentration. In this test system, instant and substantial volumetric swelling was observed over most of the range of ethanol concentrations but not at 100%, v/v, ethanol. Settled volume reached a maximum of 50–70%, v/v, ethanol, and this

was up to 3.5× the volume in absolute ethanol at 22°C and 2× the volume at –15°C. This maximum closely corresponds to the maximum dissolution of whole gluten and prior literature reports of full dissolution of gliadin. The reduction of settled volume at low temperature reflects the possible role of undissolved, gliadin-class proteins in reinforcing the gluten structure and limiting the ultimate swelling. The data suggest gluten-swelling properties as a contributing factor to the success of the cold ethanol, gluten-from-starch separation process.

The washing of wheat flour dough to produce wheat gluten and wheat starch concentrates is conducted commercially in the United States by the Martin process and other water-based processes (Grace 1989). The Martin process uses water to mechanically remove starch from a water-developed protein matrix. We have developed a fundamentally analogous technology that substitutes aqueous or absolute ethanol for water (Robertson and Cao 1998a,b). In both aqueous and ethyl alcohol technologies, previously hydrated, mixed, and conditioned dough or batter is manipulated in excess fluid over a porous supporting screen. In this way, starch is displaced from the protein.

Both water- and ethanol-based technologies produce enriched starch and protein fractions. The Martin process succeeds because of low aqueous solubility of the starch and proteins; mechanical development of a cohesive protein matrix; generation of new surfaces during washing, poor adhesion between the starch, and protein matrix; and suspension of the starch by the wash fluid. Ultimately, these factors contribute to the formation of a protein matrix that is larger than the size of the openings in the supporting screen, while the size of the suspended starch particles is smaller. When wheat flour dough is washed with water, water-soluble proteins, enzymes, and gums may be lost.

The ethanol process developed in our laboratory is operationally similar to the commercial Martin process (Grace 1989), but the rate of separation was 5× greater than that achieved in a Martin-like, laboratory process (Robertson and Cao 1998a,b) or as reported for pilot-scale processes (Godon et al 1986). Furthermore, because ethanol is a more aggressive solvent for the wheat grain components, functional similarity between water and ethanol methods was achieved only by managing both the temperature and the water content of the wash fluid.

In the initial experimental program, progressively and uniformly improved separation efficiencies were obtained when we lowered the temperature of the ethanol to –13°C (at a constant water content) and increased the water content (at constant temperature) to 40%, v/v (60%, v/v, ethanol). The effectiveness of lowered tem-

perature was attributed to reduced solubility of gliadin proteins and lipoprotein complexes. However, the effectiveness of high water concentration contradicted anticipation by inference from the literature. Published data had suggested that the most successful protein-starch separation for this technology would be limited to the range of 0–15%, v/v, water (Meredith 1965), where percent of relative dissolution of gliadin is very low.

The contradiction between observation and inference created a need to identify concentration- and temperature-dependent properties that may have contributed to the separation efficiency for this size-dependent process. As reported here, these may have included solubility and swelling factors not previously reported.

MATERIALS AND METHODS

Chemicals

Vital wheat gluten at 86% protein ($5.7 \times N$) (moisture-free basis) obtained from a commercial supplier (Giusto, San Francisco, CA) was employed in all the experiments. Reagent-grade, anhydrous ethyl alcohol was diluted with distilled water to the desired concentration.

Solvent Dissolution and Swelling of Gluten

A gluten suspension containing 4.00 g of gluten (11.4% moisture) was added to 32 mL of the desired ethyl alcohol concentration at the selected temperature, mixed for 15 sec on a magnetic stirrer (Dataplate Stirrer, 730 series), and allowed to settle for 30 min at the same selected temperature. The volume was measured in a graduated tube for the $1 \times g$ settled volume. The sample was then centrifuged (Sorvall GLC-1 for 3 min at $1,800 \times g$) to separate the excess solvent from the retained solvent. Volume of gluten was measured and excess solvent was decanted. The solvated gluten was weighed, dried in an oven at 150°C for 3 hr, and reweighed to determine solvent uptake (absorption) by gluten. The solvent-soluble portion was determined by difference between the weight of the dried solvated gluten and the dry mass of the initial gluten (mass-loss method).

Alternatively, the solvent soluble portion was determined by weighing the hexane-extracted mass recovered following evaporation of the solvent used in the extraction of gluten (mass-recovery method). Specifically, 2.5 g of gluten in 50 mL of ethanol solution was vigorously stirred for 60 min. The suspension was decanted, centrifuged at $10,000 \times g$ for 10 min at temperature. The solvent was decanted, filtered at temperature through a Buchner funnel supporting cellulose filter media rated to recover particles $>2.5 \mu m$ and evaporated to constant weight (2 hr at 135°C). These solids were extracted with hexane, and residue evaporated to dryness (2 hr at 135°C) and weighed.

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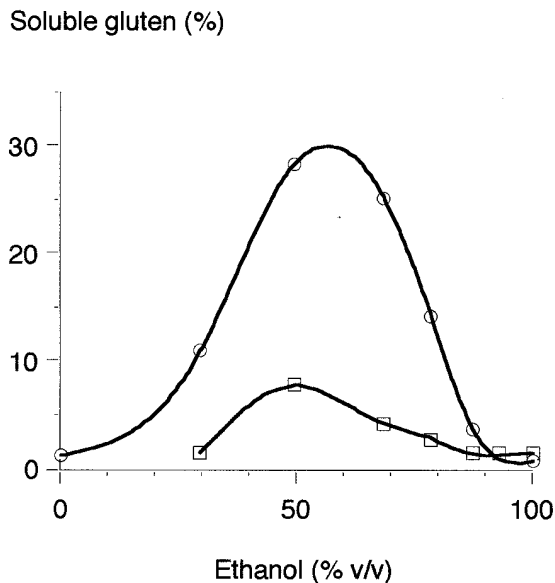


Fig. 1. Gluten sample soluble portion by mass-loss method at $22^{\circ}\text{C} \pm 2$ (\circ) and $-15^{\circ}\text{C} \pm 2$ (\square) using an 8:1 solvent-to-sample dilution.

Solvent Selectivity

Suspensions were prepared using 6.0 g of gluten added to 40 mL of ethyl alcohol solution at the selected temperature in a 25- × 150-mm test tube and stirred for 15 sec on a magnetic stirrer. The samples were allowed to rest at the desired temperature for 4 hr. Gas chromatography (HP 6890 Series) was employed to analyze solvent concentration before gluten addition, after gluten addition, and after 4 hr of settling.

RESULTS AND DISCUSSION

The yield of solvent-soluble whole gluten was strongly concentration- and temperature-dependent. When determined by the mass-loss method at a 8:1 solvent-to-solids dilution, the soluble portion was <5% of the sample at both low (0%, v/v) and high (>90–100%, v/v) ethanol concentrations, but increased to $\approx 30\%$ of the original sample when extracted with ethanol in the 50–65%, v/v, ethanol range (Fig. 1). Published data for a lipid-free gliadin preparation (Meredith 1968) indicated maximum relative solubility in the 50–70%, v/v, ethanol range. The solvent-soluble portion of whole gluten was substantially reduced at -15°C over the whole range of ethyl alcohol concentrations, and peak solubility at $\approx 50\%$, v/v, ethanol was only 25% of that at 22°C . Freezing of the suspension occurred at concentrations <30%, v/v, ethanol at -15°C .

The strong temperature and concentration sensitivity of gross gluten solubility was also seen in the extraction at a 20:1 solvent-to-gluten dilution and a 60-min extraction time. (Fig. 2). This data clearly indicates minimal dissolution (<5%) of gluten at $\leq 90\%$, v/v, ethanol concentration for temperatures as high as 35°C . At dilute ethanol concentrations in the 60–70% range, the gluten dissolved was $\leq 40\%$ of the original sample mass at temperatures $>15^{\circ}\text{C}$.

Wheat flour swelling, measured as volume after sedimentation, is used to estimate breadbaking and gluten quality. Sedimentation methods employing SDS and lactic acid have been reported (Zeleny 1947, Axford et al 1979, McDermott 1985). When isolated gliadin and glutenin were tested by the SDS or Zeleny methods, the glutenin proteins swelled and the gliadins dissolved (Eckert et al 1993). However, as described here for the first time, wheat gluten also swells (settled volume or absorption measurement) in aqueous ethanol solutions and may swell up to 50% more than in pure water (Figs. 3 and 4). The swelling is very rapid (seconds) and



Fig. 2. Gluten sample soluble portion by mass-recovered method 60 (\circ), 70 (\diamond), 80 (\square), and 90 (Δ) (v/v) ethanol solutions using a 20:1 solvent-to-sample dilution.

appears to be instantaneous when the sample is added to the test fluid. Swelling volume can reach $3.5\times$ the volume in 100%, v/v, ethanol. Similar swelling behavior was obtained when the samples were first suspended in anhydrous alcohol followed by titration of water into the stirred suspension. We observed no swelling of starch at similar experimental conditions. Swelling was considerably lower at -10°C (Fig. 3). This is a previously unreported phenomenon.

It is probable that the concentration dependence and the temperature dependence of swelling are related to the selective solvation of the gliadin protein. The gliadin proteins make up 30–40% of the mass of wheat gluten protein and are believed to form weak noncovalent bridges with other proteins through protein-protein and protein-lipid-protein interactions (Weegels and Hamer 1992). The gliadin protein reinforces the structure established by the much longer glutenin proteins that cross-link through end-to-end relatively strong, and relatively permanent, disulfide bonds. If the number of the noncovalent interactions are decreased by removal of the gliadin protein, the remaining protein matrix should be less rigid and may gain absorption capacity. This hypothesis is supported by the correlation noted between increased dissolution of the gluten (and gliadin) (Meridith 1965) and swelling.

Most importantly, however, swelling is still substantial at the lower test temperatures, reaching values more than double the volume in absolute ethanol, even in the presence of undissolved gliadin. There is close correspondence between improvement of separation at progressively lower temperatures (fixed concentration) and at progressively higher water concentrations (fixed temperature) (Figs. 6 and 7 of Robertson and Cao 1998)

Bulk properties such as surface tension should also play a role in the extent of swelling. Surface tension rapidly increases at ethanol concentrations <40%, v/v (Washburn 1928) and in this range it should create an increasingly more dense structure with reduced capillary solvent-holding capacity and, consequently, reduced swelling volume.

Commercial wheat gluten was chosen to test the relative importance of swelling and solubility properties that might also occur during the fluid displacement of starch from hydrated and developed dough. The intent was to correlate relative properties with the separation phenomenon reported earlier (Robertson and Cao 1998a,b). The use of these gluten and the test methods suggest a correlation between swelling and separation.

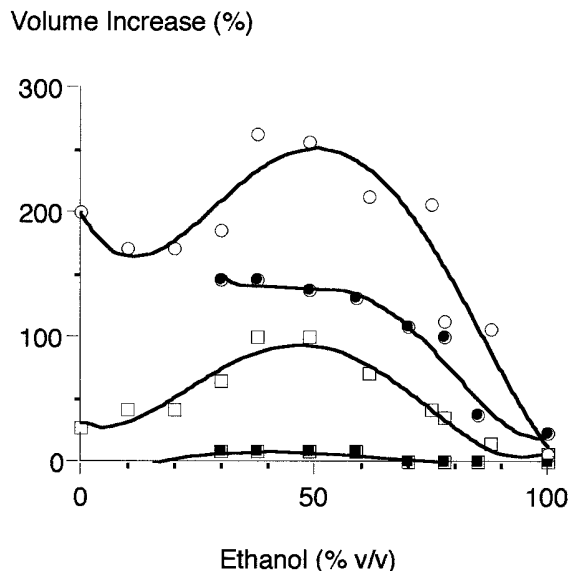


Fig. 3. Settled volume of wheat gluten in aqueous ethanol at $1 \times g$ and $23^\circ\text{C} \pm 2$ (\circ), $1,800 \times g$ and $23^\circ\text{C} \pm 2$ (\square), $1 \times g$ and $-10^\circ\text{C} \pm 2$ (\bullet), and $1,800 \times g$ and $-10^\circ\text{C} \pm 2$ (\blacksquare).

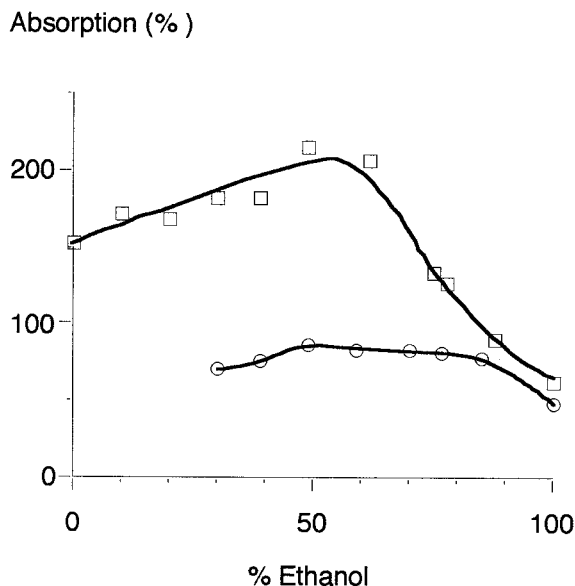


Fig. 4. Solvent absorption of wheat gluten in water and aqueous solutions of ethanol at $22^\circ\text{C} \pm 2$ (\square) and $-10^\circ\text{C} \pm 2$ (\circ).

However, there are very obvious differences between the present test method and the actual conditions of the separation experiment. For one, the present extraction times are 30–60 \times longer than those suggested for the wheat starch-gluten washing method (Robertson and Cao 1998a,b). Furthermore, the experimental ethanol-washing technology applies the ethanol wash to dough or batter that is fully hydrated and developed and is highly diluted with starch. Microscopy has revealed that the developed protein in the dough before displacement is in the form of bands with a diameter averaging perhaps 100 μm or more. Both the shorter extraction time and the large protein aggregate would retard dissolution of the gluten components, thereby leading to higher recovery at the process conditions.

We also considered the possibility that the swelling observed was due to selective uptake of water from the solution. To test this, ethanol concentration was monitored before and after exposure

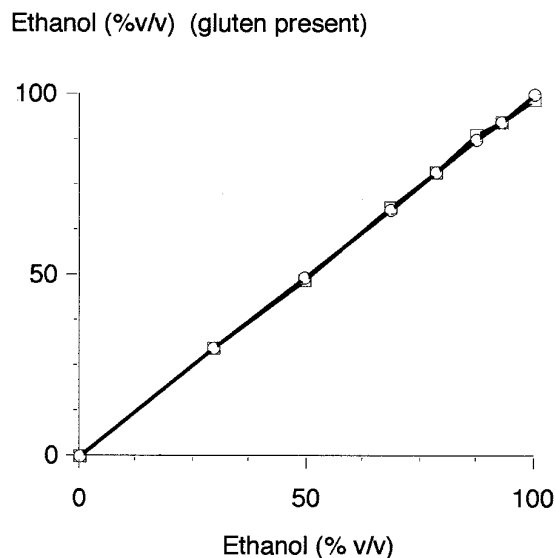


Fig. 5. Nonspecific absorption of aqueous ethanol by wheat gluten at 0 min (\circ) and 240 min (\square).

of the gluten to the solution. If water had been selectively absorbed, the concentration would have changed and would have been detected, but no changes were found (Fig. 5).

CONCLUSIONS

The data herein demonstrate previously unreported and substantial swelling extent in aqueous ethanol. Furthermore, the swelling extent increases as the water content increases up to $\approx 50\%$, v/v. Previously we reported that separation by a process employing cold-ethanol washing of wheat dough increased in the same range (Robertson and Cao 1998a,b). The data suggest that swelling contributes to the success of the separation. Interactions between ethanol solutions and wheat proteins and other constituents need additional investigation.

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