## COMMUNICATION TO THE EDITOR

# **Unexpected Solubility Changes in Wheat Proteins During Fermentation** and Oven Stages of the Breadmaking Process<sup>1</sup>

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### To the Editor:

It is well known that flour proteins play a central role in the ability of wheat flour to produce a satisfactory loaf of bread. To fully define the unique role of the proteins, more information is required on the compositional changes that occur during different stages of breadmaking process. In our studies, we focused on the changes of solubility. Early experiments produced "unexpected" results that may be of interest to other researchers working in this area.

Three flours of diverse quality were milled from pure cultivars or lines of hard red spring (HRS) wheat, sample A (221), B (219) and C (Butte 86). Protein contents were: A, 12.2%; B, 12.8%; and C, 12.6%, respectively. All three had the same high molecular weight (HMW) glutenin subunit composition, 2\*, 7+9, and 5+10, according to Payne et al (1987). Sample A had a medium farinograph dough mixing tolerance (MT) of 12 min and a loaf volume of 764 cm<sup>3</sup>, flour B had a very long MT of 33 min and a loaf volume of 835 cm<sup>3</sup>, and flour C had a short MT of 6 min and a loaf volume of 978 cm<sup>3</sup>. Baking was done according to the AACC approved method 10-09 (AACC 1995).

Analogous doughs were used for the fractionation study. Dough samples were taken immediately after mixing to optimum, just prior to first punch, after proofing, and after 3.5 min in the oven at 218°C. The samples were frozen and lyophilized. The crust was cut from around the lyophilized oven dough to leave about two thirds of the dough which was then ground with mortar and pestle. The ground material was used for protein fractionation.

Fractionation was as follows. Sample (1 g) was dispersed in 10 ml of 0.5M NaCl and extracted for 60 min with 30 sec of vortexing every 15 min. The dispersion was centrifuged (15 min at  $15,000 \times g$ ). The supernatant, containing the salt-soluble proteins, was decanted. The wet residue (containing salt from the solvent) was dispersed in 10 ml of solution comprising 2% sodium dodecyl sulfate (SDS) in 0.05M Na phosphate buffer (pH 6.8), and extracted and centrifuged as before. The supernatant, containing the SDS-soluble protein, was decanted. The remaining residue contained the SDS-insoluble proteins. A mixture of yeast-protease

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inhibitors (5 mM ethylenediaminetetraacetic acid, 1 mM  $\alpha$ -toluence sulfonyl fluoride, and 2 mM N-ethylmaleimide) (Pringle 1975) was included in the two solvents used for fractionation. The extraction procedure used is considered to be sufficiently gentle so as not to break any covalent linkages in gluten proteins.

Protein content (N  $\times$  5.7) of each fraction was determined by the Kjeldahl method. A blank was analyzed to determine the amount of nitrogen added in the form of protease inhibitor.

Composition of the SDS-soluble proteins was determined by SDS-polyacrylamide gel electrophoresis (PAGE) (Khan et al 1989). The percentage of total HMW glutenin subunits of the protein of the SDS-soluble fractions that entered the gel was determined by high resolution imaging densitometry of the stained gels (model GS-670, Bio-Rad, Richmond, CA).

Of the three protein fractions, the percentage (of total) of saltsoluble protein decreased after proofing and decreased further after 3.5 min in the oven (Fig. 1). The percentage of SDS-soluble protein increased substantially after heating for 3.5 min. The percentage of residue protein decreased slightly after heating in the oven.

The percentages of the SDS-soluble protein obtained here are lower than the values reported by He and Hoseney (1991), which were obtained after exhaustive extraction. The results for the three diverse flours were similar, except that the increase in the percentage of the SDS-soluble protein for the strongest flour (B) after proofing and heating in the oven was the lowest of the three.

The fluctuation in the percentage of the HMW glutenin subunits in the protein (Fig. 1d) of the SDS-soluble fraction that entered the gel on SDS-PAGE paralleled the changes in the percentage of SDS-soluble protein.

The results reported here for doughs appear contrary to the results reported by Schofield et al (1983) for gluten. Those workers reported a marked decrease of the solubility of the gluten proteins after heating for 100 min at 55°C. The results from the two studies are not directly comparable. In the present study, doughs (not gluten) were heated for 3.5 min, during which the temperature of the dough probably did not rise above 45°C (He and Hoseney 1991).

The increase in the percentage of SDS-soluble protein in dough during the initial 3.5 min of baking has not been reported previously. We present preliminary evidence that this increase may be related to the rapid changes of dough rheological properties at the stages of ovenspring. It is highly possible that gluten proteins in oven dough may become depolymerized or disaggregated into smaller aggregates such that the dough becomes more extensible. Details of the study will be reported in an article to follow.

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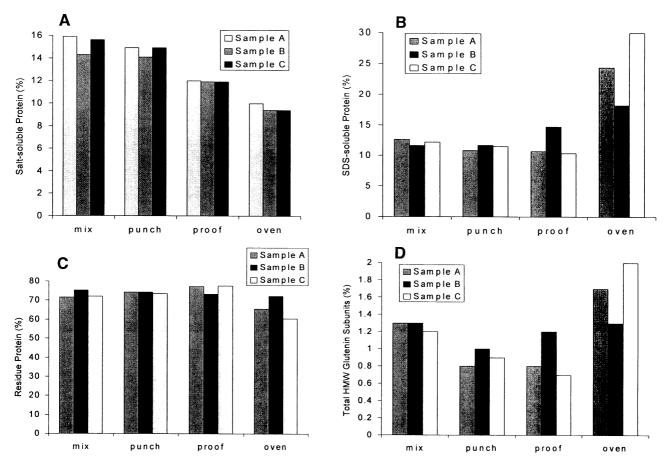


Fig. 1. Solubility changes in wheat proteins during the breadmaking process. Samples were taken at various baking stages: mix (at optimum mixing); punch (immediately before first punch); proof (after proof before baking); oven (after baking 3.5 min in the oven). A, Salt-soluble proteins; B, SDS-soluble proteins; C, Residue protein content; D, Total HMW glutenin subunits in SDS-soluble proteins. A, B and C represent sample A, B and C, respectively, as described in text.

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