Differences Between Flours in the Rate of Wheat Protein Solubility[1]

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ABSTRACT

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Extraction of flour with 1% sodium dodecyl sulfate (SDS), pH 7.0, showed that the proteins in poor-quality flour were solubilized at a faster rate than were those in good-quality flour, and the protein solubility of both flours increased with extraction time. When a sufficient extraction time was used, essentially all the flour proteins were solubilized. The relative viscosity of protein per milliliter of the protein solution from a strong flour was slightly higher (6%) than that of a similar extract from a weak flour. This indicates that the average molecular weight of the proteins in the strong flour was slightly larger. The relative viscosity per milligram of protein per milliliter of the protein solutions decreased with additional extraction time. A study of SDS-polyacrylamide gel electrophoresis and relative viscosity of unheated and heated protein samples showed that the decrease in viscosity was caused mainly by further dissociation of solubilized but still aggregated protein.

Since Finney and Barmore (1948) established that wheat gluten is the predominant factor affecting the quality of flour baking, the difference in gluten proteins between flours has received extensive study. A major problem in working with gluten has been the difficulty of solubilizing the total protein (Singh and MacRitchie 1989). Gluten has a relatively high average molecular weight and a strong tendency toward aggregation, which is generally assumed to result from the hydrogen-bonding potential of the unusually large numbers of glutamine side chains, the potential for apolar bonding of the many nonpolar side chains, and the low ionic character of gluten (Wrigley and Bietz 1988).

To completely solubilize the gluten proteins, various methods have been used to break up the glutenin polymers into smaller molecules. The techniques used involve reducing agents, dough mixing (Mecham et al 1962), or sonication (Singh and MacRitchie 1989). However, any conclusions based on the partially degraded proteins would not directly relate to their properties and/or functionality.

Arakawa and Yonezawa (1975) studied the aggregation behaviors of glutens from four flours of various baking qualities by recording the time required for turbidity formation of the gluten suspension. They found that the gluten from the flours of better baking quality aggregated more than did those from flours of poorer baking quality, and the aggregation behavior was mainly determined by the nature of its glutenin. Huebner (1970) showed similar effects. More recently, He and Hoseney (1991) showed that gluten from poor-quality flour had higher solubility than did gluten from good-quality flour. These differences in protein solubilization suggested that the proteins in poor-quality flour might have smaller molecular weights or less tendency to interact with themselves. Therefore, the purpose of this investigation was to solubilize wheat flour protein without significant degradation and to determine the factors affecting protein solubility in flours of different baking quality.

MATERIALS AND METHODS

Flour

Two hard red winter (HRW) wheat flours, CI 12995 and KS 501097, were used to represent flours of good and poor baking quality, respectively. Three additional flours—Shawnee (CI 14157), KS 644, and KS 619042—were used in limited studies. The flours were all HRW wheats; the chemical and baking properties are given in Table I.

Protein Extraction and Determination

Protein was determined by the AACC Kjeldahl procedure (AACC 1983). For extraction, 2 g of flour was first stirred gently

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by hand for 1 min in 5 ml of 1% sodium dodecyl sulfate (SDS) (w/v, pH 7.0) solvent to make a uniform slurry. The slurry was then transferred to a glass volumetric centrifuge tube, and 35 ml of 1% SDS solvent was added. After the tube was covered and shaken for 30 sec to obtain a uniform suspension, the tube was allowed to stand at room temperature (23°C) without additional stirring for 2, 10, 16, 24, 36, 48, and 72 hr. After the desired time, the sample was centrifuged at 10,000 × *g* for 15 min. This is a modification of the procedure of Danno and Hoseney (1982). Protein content, relative viscosity, and SDS-polyacrylamide gel electrophoresis (PAGE) of the supernatant were then determined. With extraction times of 24 hr or longer, the residue was found to contain insignificant protein.

Viscosity Measurement

The relative viscosity of extracted protein solution was determined using a capillary viscometer (Cannon-Fenske, size 50) in a water bath at a constant temperature (30 ± 0.1°C), as described by Moore and Hoseney (1989). The extracted protein was measured directly, with no adjustment of protein per cubic centimeter.

Electrophoresis

SDS-PAGE was carried out according to the method of Fling and Gregerson (1986). Polyacrylamide concentration was 9%.

RESULTS AND DISCUSSION

Protein extraction

In 1% SDS (pH 7.0), about 6% more protein was extracted from poor-quality KS 501097 flour than from good-quality CI 12995 flour during the first 2-hr extraction (Fig. 1). With longer extraction time, the protein solubilities of both flours increased. It took 10 hr to extract 98.5% of the total protein from KS 501097 flour and 24 hr to extract a similar percentage of the protein from CI 12995 flour. This suggests that if sufficient extraction time were used, essentially all the proteins from both flours could be solubilized. To confirm this, three additional flours were extracted. A strong flour, CI 14157, followed the same extraction curve as that of CI 12995, while two weaker flours, KS 501099 and KS 619042, gave extraction profiles similar to that of KS 501097. Therefore, the difference in protein solubility between flours is the rate of protein solubilization. Possible explanations for the difference in solubilization rate may be differences in the average molecular weight of proteins and/or differences in the aggregation tendency of proteins.

TABLE I
Properties of Different Hard Red Winter Wheat Flours

Variety	Protein[a] (%)	Ash[a] (%)	Water Absorption (%)	Mixing Time (min)	Loaf Volume (cm^3)
CI 12995	12.74	0.48	65	3.7	795
CI 14157	11.73	0.48	64	3.2	790
KS 644	10.93	0.47	63	2.0	610
KS 501097	13.95	0.48	65	1.2	480
KS 501099	13.85	0.48	65	1.2	445
KS 619042	12.39	0.47	58	1.1	455

[a] 14% mb.

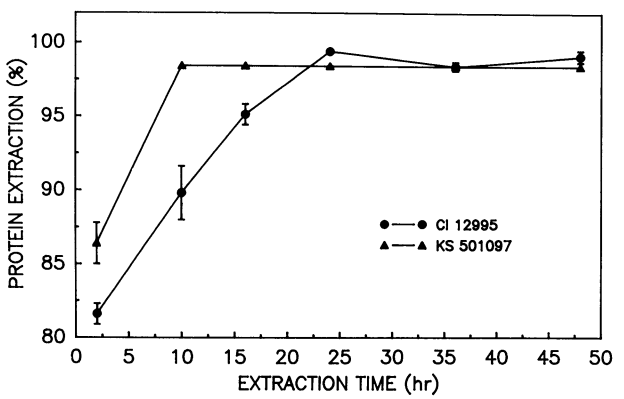

Fig. 1. Long-term protein extraction from CI 12995 and KS 501097 flours in 1% sodium dodecyl sulfate, pH 7.0 solution (average of at least three replicates; bars represent one standard deviation).

Relative Viscosity

To determine whether the average molecular weight of the proteins was responsible for the difference, the relative viscosity of the protein solution was measured (Fig. 2). The relative viscosity of protein solution after 2 hr of extraction from KS 501097 flour was 1.58, which is higher than that from CI 12995 flour, which was about 1.44. However, the results are difficult to interpret, as the protein content of the extracts also varies. Therefore, the results were calculated as relative viscosity per milligram of protein per milliliter (Table II). The values for CI 12995 (strong flour) were slightly higher (about 6%) than for KS 501097 (weak flour). This may suggest that the average molecular weight of the strong flour is slightly larger.

With increased extraction time, the relative viscosities per milligram of protein per milliliter of both protein solutions decreased. Two possible explanations for the decrease in viscosity are protein degradation, perhaps by proteolytic enzymes that are still active in the SDS, or simply by further dissociation of noncovalent bonds between aggregated proteins in the solution.

SDS-PAGE

The presence of proteolytic enzymes in 1% SDS flour extract has been reported (P. Payne, *personal communication,* 1989). Payne found that when the protein solution in 1% SDS was allowed to stand for several days, the high molecular weight (HMW) subunit bands of glutenin became lighter or disappeared.

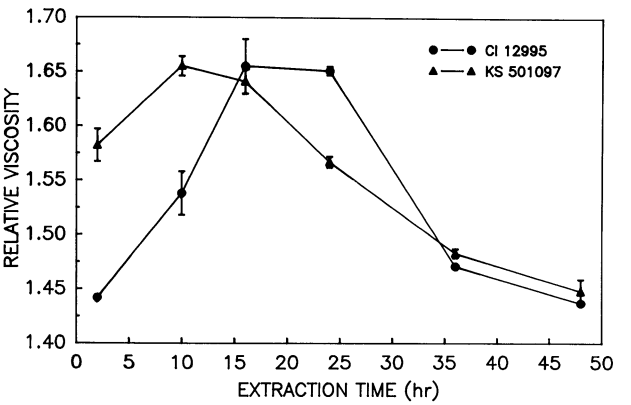

Fig. 2. Relative viscosity of protein extracted from CI 12995 and KS 501097 flours in sodium dodecyl sulfate, pH 7.0 solution (average of at least three replicates; bars represent one standard deviation).

TABLE II
Relative Viscosity per Milligram of Protein for Extracts From Flours of Good and Poor Quality

Extraction Time	Relative Viscosity per Milligram of Protein per Milliliter[a]	
	CI 12995	K 501097
2	0.278 ± 0.002	0.262 ± 0.001
10	0.269 ± 0.003	0.243 ± 0.001
16	0.274 ± 0.006	0.241 ± 0.002
24	0.261 ± 0.001	0.230 ± 0.001
36	0.235 ± 0.001	0.218 ± 0.002
48	0.228 ± 0.001	0.213 ± 0.002

[a] Calculated as: $\frac{\text{relative viscosity} \times 40 \text{ ml}}{\text{flour protein \%} \times \text{protein extractability} \times 2{,}000 \text{ mg}}$

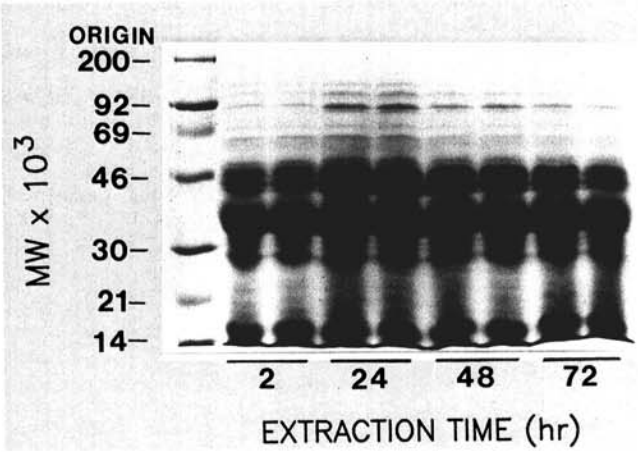

Fig. 3. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of protein extracted from CI 12995 flour for 2, 24, 48, and 72 hr with 1% SDS, pH 7.0 solution. The high molecular weight glutenins are about 92 kDa and higher.

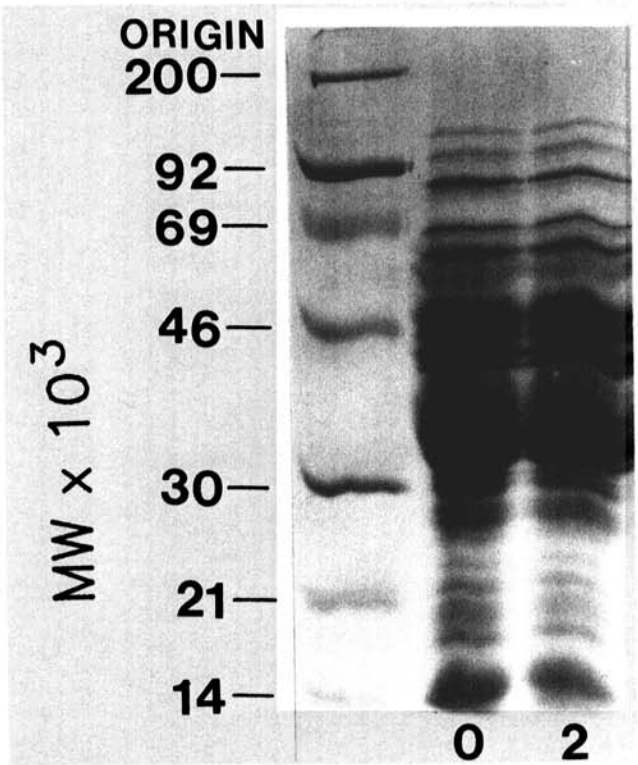

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of protein extracted from CI 12995 for 24 hr with 1% SDS, pH 7.0 solution. The protein solution was heated to 100°C for 6 min and tested immediately or allowed to stand for two days.

To clarify whether the decrease in viscosity was caused by enzymatic activity, the extracted proteins from CI 12995 flour were examined on SDS-PAGE after 2, 24, 48, and 72 hr of extraction with two replicates (Fig. 3).

Compared with 2-hr-extracted proteins, the amount of HMW subunits of 24-hr-extracted proteins increased substantially, mainly because more proteins were solubilized. With longer extraction time, the amount of HMW subunits appeared to decrease. After 72 hr of extraction, the HMW subunit bands became considerably lighter. This indicated that certain enzymes were still active in 1% SDS at pH 7.0. The protein degradation suggests that it may be at least partially responsible for the decrease in viscosity.

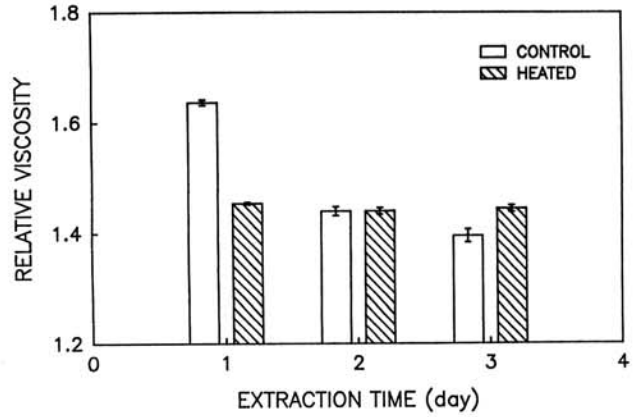

Fig. 5. Relative viscosity of protein extracted from CI 12995 flour with 1% sodium dodecyl sulfate, pH 7.0 solution before and after heating to 100°C for 6 min.

Heat Treatment

To further investigate how much the enzymatic protein degradation contributes to the decrease in viscosity, the 24-hr-extracted protein solution was heated in boiling water for 6 min to inactivate possible enzymes. During the heating, the protein solution became more transparent and less cloudy. Centrifugation at 10,000 × *g* for 15 min produced no centrifugate. Protein analysis of the supernatant before and after heating gave insignificant differences. The relative viscosity and the electrophoresis of the heated samples were examined both immediately and after standing for an additional two days at room temperature. SDS-PAGE (Fig. 4) showed that standing for an additional two days after heating did not change the amount of HMW subunits. This indicated that certain enzymes did exist in 1% SDS pH 7.0 and could be inactivated by heat treatment.

However, the relative viscosity immediately dropped from 1.65 to 1.44 as a result of heating (Fig. 5) and did not change during standing for an additional two days. The dramatic decrease in viscosity caused by heating cannot be explained by enzymatic degradation. Apparently, it is the result of the further dissociation of the aggregated proteins in the extract. Therefore, the data suggest that the decrease in viscosity of the extracts from day 1 to day 2 was mainly the result of the further dissociation of solubilized but still aggregated proteins. That dissociation was accelerated by heat treatment.

However, the enzymatic activity in the unheated protein solution also had some effect on the viscosity. This can be seen from the day 3 sample, of which the viscosity continued to decrease after two days of extraction, while the viscosity of the heated sample remained constant. However, compared with the effect of further dissociation of noncovalent bonds between solubilized proteins, the effect of the enzymatic degradation was much smaller and probably insignificant to the decrease in viscosity over the three-day period of extraction. This suggests that the enzymatic activity does not significantly affect the protein solubility, particularly during the first 24 hr of extraction.

ACKNOWLEDGMENT

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