Effects of Dough Mixing and Rheologically Active Compounds on Relative Viscosity of Wheat Proteins[1]

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ABSTRACT

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Changes in wheat proteins during dough mixing were studied by viscometry. Protein was extracted from flour and doughs with 1% sodium dodecyl sulfate, pH 7.0, and the relative viscosities of the extracts (3 mg of protein per milliliter in 1% sodium dodecyl sulfate) were determined. Only 72% of the total nitrogen was extracted from flour, but extractability increased to 95% from mixed doughs. The relative viscosities of proteins extracted from doughs were higher than those of proteins from flour. Overmixing decreased the viscosity of the extracted proteins. The relative viscosities of the proteins extracted from dough treated with fumaric acid, ferulic acid, or *N*-ethylmaleimide were lower than those of the extracts of untreated (control) dough. The results support the theory that disulfide bonds of wheat proteins were cleaved during dough mixing, thus causing depolymerization of those proteins. When the extracts were treated with 2-mercaptoethanol, the viscosities decreased markedly. With mercaptoethanol, the relative viscosities of protein extracted from optimally mixed, overmixed, and treated doughs were all equal, indicating that disulfide bonds break during dough mixing.

When wheat flour is overmixed, the dough's resistance to extension decreases. The effect is much greater if dough is mixed with activated double-bond compounds or fast-acting oxidants. A series of recent papers (Hoseney et al 1980, Schroeder and Hoseney 1978, Sidhu et al 1980, Weak et al 1976) dealt with the phenomenon. The general conclusion has been that disulfide bonds are cleaved during mixing to create thiyl radicals, which react with the activated double-bond compounds. MacRitchie (1975) has shown that disulfide bonds may rupture during mixing.

If disulfide bonds are ruptured during dough mixing, glutenin proteins will be partially depolymerized and should have lower relative viscosities. This study was to determine whether the viscosity of protein extracted from doughs decreased as a result of mixing and treatment with rheologically active compounds. Sodium dodecyl sulfate (SDS) was chosen as a solvent because of its ability to disrupt secondary forces.

MATERIALS AND METHODS

Flour

We used the following flours: KSU—a flour milled from a composite of hard winter wheat on the KSU experimental mill (10.0% protein, 0.37% ash); SRA flour (19.5% protein, 0.66% ash); and three experimental lines, 80172 (15.1% protein, 0.46% ash), 80179 (13.3% protein, 0.43% ash), and 80180 (15.8% protein, 0.46% ash), furnished by Seed Research Associates, Scott City, KS. Gluten plus starch (G + S) was prepared according to the method of Schroeder and Hoseney (1978).

Chemicals

SDS (99% pure) was obtained from Polysciences, Inc., Warrington, PA. All other chemicals were reagent grade.

Doughs

A 10-g mixograph was used to mix dough (Finney and Shogren 1972). The doughs were immediately frozen, lyophilized, and finely ground. Additives were dispersed in water and neutralized to pH 7.0 with 0.5*N* sodium hydroxide.

Protein Extraction

Flour and doughs (500 mg) were weighed directly into a 50-ml centrifuge tube, and 15 ml of 1% (w/v) SDS (pH 7.0) was added. The slurry was gently stirred by hand with a glass rod for 2 hr at room temperature and was then centrifuged for 10 min at 10,000 × *g* at 25° C. The supernatant was decanted to another tube and recentrifuged (20 min, 17,000 × *g*, 25° C). Protein was determined by micro-Kjeldahl (N × 5.7). The clear supernatant was diluted with 1% SDS (pH 7.0) to the desired protein concentration—generally 3 mg/ml.

Viscosity Measurement

Viscosities of extracts were determined with a Cannon-Fenske viscometer (size 50) at 30° C. Flow time of 1% SDS was about 300 sec. Five-ml sample solutions were used. Reported readings are the average of at least three determinations. The reproducibility was similar to that reported in Tables I and II.

RESULTS AND DISCUSSION

In an effort to identify the factors affecting protein viscosity, the effects of several factors on the relative viscosities of proteins extracted from overmixed dough (SRA flour, 15 min of mixing) were studied. A nearly linear relationship between relative viscosity and the protein concentration was obtained (Fig. 1). When the sample solution was treated with 2-mercaptoethanol, the relative viscosity decreased, presumably as a result of breaking disulfide bonds in the high-molecular-weight glutenin protein. Nielsen et al (1968) showed that the viscosity of gliadin protein was not changed

TABLE I
Relative Viscosities of Extracts from SRA[a] Flour and Its Gluten + Starch

Flour and Dough	Nitrogen Extracted (% of Total)	Relative Viscosity[b]	
		Without 2-ME[c]	With 2-ME[c]
Flour	72	1.223 + 0.013[d]	1.160 ± 0.010
Dough (3)[e]	94	1.434 ± 0.24	1.145 ± 0.020
Dough (15)	95	1.371 ± 0.024	1.148 ± 0.022
Gluten + Starch			
Powder	69	1.239 ± 0.009	1.139 ± 0.011
Dough (5)	86	1.395 ± 0.029	1.132 ± 0.004
Dough (15)	95	1.369 ± 0.023	1.141 ± 0.021
Solvent (1% sodium dodecyl sulfate)		(1.000)	(1.000)

[a] Seed Research Associates.
[b] Viscosity was measured at 0.3% protein concentration.
[c] 2-Mercaptoethanol added to be 2% of the extract, then stored overnight at 30° C.
[d] Standard deviation.
[e] Mixing times (in minutes) indicated in parentheses.

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by breaking their intramolecular disulfide bonds. The relative viscosity of the protein extract decreased slightly during incubation at 30° C (Fig. 2); after 5 hr the original relative viscosity (1.358) had decreased to only 1.341, showing that the effect of proteases on viscosity may be negligible. Heating the extract at 100° C decreased the relative viscosity (Fig. 2), but freezing at −20° C for two days had no effect (data not shown). The effect of pH on viscosity was studied by adjusting the extract pH with 0.5*N* sodium hydroxide or hydrochloric acid. No effect was observed in the range of neutral pH. Relative viscosity decreased slightly but not significantly as the concentration of SDS was increased.

Relative Viscosity of Extracts from Flour and Doughs

The relative viscosities of the extracts from doughs were higher than those from flour (Table I). About 72% of the total flour nitrogen was extracted from flour, but 94–95% of the total nitrogen was extracted from mixed doughs. Similar increases in protein solubility have been noted by previous workers as a result of dough mixing (Mecham et al 1962, 1963, 1965; Tsen 1967).

For all viscosity measurements, extracts were diluted with 1% SDS to 3 mg of protein per milliliter (N × 5.7). The high relative viscosity for the extracts from mixed doughs compared with extracts from flour shows that protein made soluble as a result of mixing has a high relative viscosity. Adding 2-mercaptoethanol to the extracts caused viscosity of the extract to decrease (Table I). After extracts were treated with 2-mercaptoethanol, no significant difference occurred in viscosities of proteins extracted from flour and those from doughs. Those results indicate that the additional protein components extracted as a result of dough mixing had interpolypeptide disulfide bonds and, thus, are glutenin. In this work, protein was gently extracted from flour with 1% SDS, because violent mixing during extraction can solubilize glutenin (Danno 1981).

Mixograms of G + S with the water-soluble fraction removed differed markedly from that of the original flour; total flour breaks down rapidly to give a narrow mixogram tail after mixing beyond the peak, but the G + S has a wide tolerance to overmixing. Even so, no difference existed in the relative viscosities of proteins extracted from flour and G + S (Table I).

The apparent lower viscosity of the G + S dough at optimum mixing time (5 min) compared to flour dough viscosities at optimum mixing time (3 min) probably resulted from the lower solubility of protein from the G + S.

Overmixed total dough (15 min) gave a much lower viscosity (1.371) than did optimum-mixed (3 min) dough (1.434), indicating that overmixing causes the protein to depolymerize. The G + S doughs gave the same trend, but the results were not so dramatic, primarily because the G + S at optimum mixing had both lower solubility and a lower relative viscosity than did the total flour dough. After 15 min of mixing, protein extracted from both the total flour and the G + S doughs had the same relative viscosity.

TABLE II
Relative Viscosities of Extract from Flour and Doughs

Flour and Dough	Nitrogen Extracted (% of Total)	Relative Viscosity[a]	
		Without 2-ME[b]	With 2-ME[b]
80172			
Flour	72.9	1.249 ± 0.004[c]	1.167 ± 0.003
Dough (1¾)[d,e]	91.1	1.509 ± 0.003	1.180 ± 0.004
Dough (15)	93.6	1.379 ± 0.006	1.164 ± 0.002
80179			
Flour	67.8	1.244 ± 0.004	1.166 ± 0.002
Dough (4)[e]	83.1	1.452 ± 0.006	1.169 ± 0.003
Dough (15)	93.7	1.376 ± 0.003	1.167 ± 0.003
80180			
Flour	71.3	1.247 ± 0.005	1.152 ± 0.003
Dough (2¼)[e]	81.4	1.365 ± 0.007	1.152 ± 0.004
Dough (15)	90.8	1.358 ± 0.006	1.152 ± 0.002

[a] Viscosity was measured at 0.3% protein concentration.
[b] 2-Mercaptoethanol, added to be 2% of the extract, then stored overnight at 30° C.
[c] Standard deviation.
[d] Mixing time (in minutes) indicated in parentheses.
[e] Optimal mixing time.

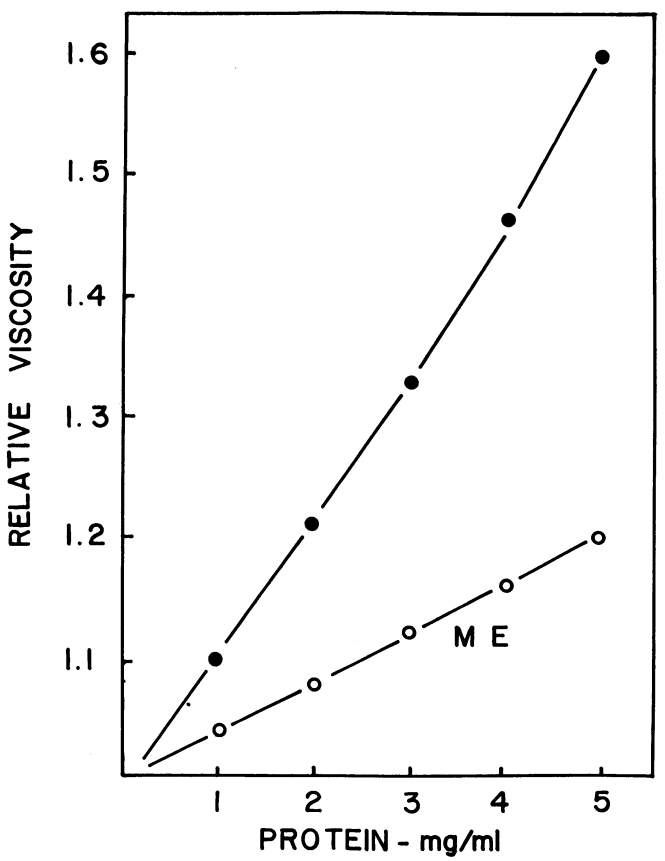

Fig. 1. Relationships between relative viscosity and protein concentrations. ME = 2-mercaptoethanol.

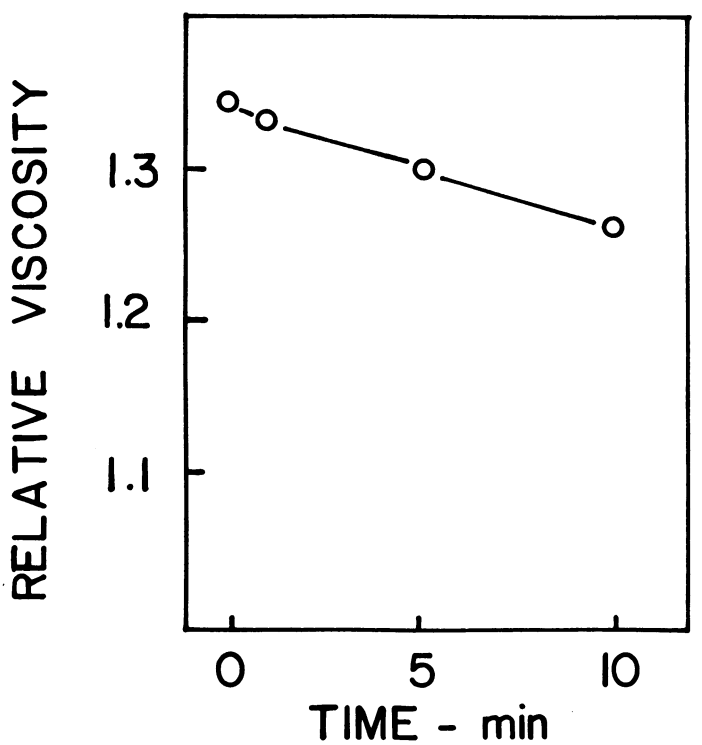

Fig. 2. Thermal stability at 100° C of the protein extracted with 1% sodium dodecyl sulfate.

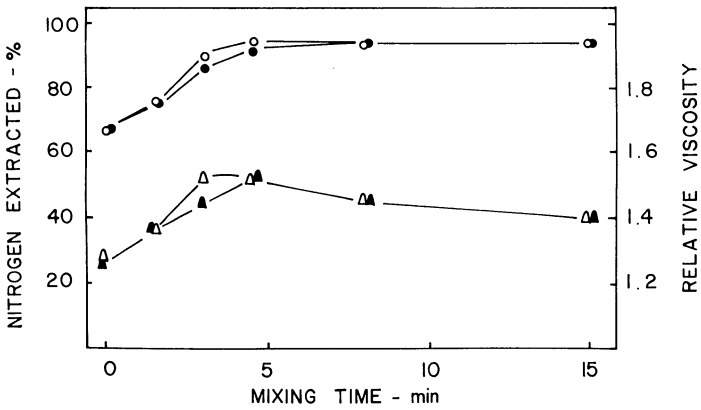

Fig. 3. Effects of mixing time on protein solubility and on the relative viscosity of extracted proteins. o, Δ = control flour; ●, ▲ = control flour plus 1% enzyme active soy flour; o, ● = nitrogen-extracted, Δ, ▲ = relative viscosity.

TABLE III
Relative Viscosities of Extracts from Doughs Treated with Additives[a]

Additives (ppm)	Relative Viscosity[b]	
	Without 2-ME[c]	With 2-ME[c]
Control (no additives)	1.445 ± 0.004[d]	1.178 ± 0.004
$KBrO_3$ (30)	1.466 ± 0.009	1.183 ± 0.005
KIO_3 (30)	1.398 ± 0.009	1.180 ± 0.005
K_2CO_3 (8,000)	1.473 ± 0.016	1.178 ± 0.006
Fumaric acid (2,000)[e]	1.378 ± 0.006	1.167 ± 0.005
Cysteine (50)	1.426 ± 0.007	1.175 ± 0.004
N-Ethylmaleimide (500)	1.362 ± 0.007	1.173 ± 0.004
Ferulic acid (250)[e]	1.375 ± 0.008	1.168 ± 0.004

[a] Kansas State University flour was used, and mixing time was 12 min.
[b] Viscosity was measured at 0.3% protein concentration.
[c] 2-Mercaptoethanol added to be 2% of the extract, then stored overnight at 30° C.
[d] Standard deviation.
[e] Na-salt (pH 7.0).

Thus, the large difference in mixing tolerance and width of the mixogram tail cannot be explained by depolymerization of the proteins. When the protein extracts were treated with mercaptoethanol, relative viscosities of all samples were essentially the same. This shows that depolymerization occurring as a result of overmixing is the result of breaking disulfide bonds.

The relative viscosities of three wheat flours with different mixograph properties are shown in Table II. Very weak flour 80172 had a short mixing time (1¾ min). Extracts from the dough mixed for 1¾ min had a high relative viscosity (1.509), which markedly decreased after 15 min of overmixing to 1.379. Those results suggest that glutenin of high molecular weight can be extracted after short mixing. This is similar to the findings of Khan and Bushuk (1978). Those results also suggest that glutenin depolymerizes during overmixing. Similar results were obtained with flours 80179 (strong) and 80180 (weak). The relative viscosities of proteins extracted from doughs mixed to optimum with those flours was lower than the protein extracted from 80172. However, the lower values can be explained by the lower solubilities obtained at optimum mix times.

Changes in protein solubility make it difficult to interpret the data. Thus, the KSU flour was mixed for various times between 0 and 15 min, and protein solubilities and relative viscosities of the extracted proteins were determined (Fig. 3). Relative viscosity of extracted proteins clearly increased as solubilized protein increased, and it decreased with overmixing. Enzyme-active soy flour (1%), which contains lipoxygenase, was added to the flour. Enzyme-active soy flour increases mixing tolerance and gives a wide mixogram tail (Hoseney et al 1980) but gave only minor differences in the rate of protein solubilization and no change in relative viscosity. Thus, again depolymerization was not related to mixing tolerance or width of the mixogram.

Relative Viscosity of Protein Extracts from Treated Doughs

The effects of certain rheologically active compounds on the relative viscosities of protein are shown in Table III. All doughs, made with Kansas State University flour, were overmixed (12 min) with the indicated compounds. Protein solubility was about 95% for all of the doughs.

Mixing dough with KIO_3 decreased the relative viscosity of the extract; mixing with $KBrO_3$, K_2CO_3, or cysteine did not. Fumaric acid, ferulic acid, and *N*-ethylmaleimide also decreased the relative viscosities of the extracts. The effects of fumaric acid, ferulic acid, and *N*-ethylmaleimide on the width of the mixogram tail have been explained as reactions with thiyl radicals created by cleavage of disulfide bonds in gluten proteins during dough mixing (Bloksma 1971, Hoseney et al 1980, Meredith and Bushuk 1962, Schroeder and Hoseney 1978, Sidhu et al 1980). The relative viscosity of extracted proteins treated with mercaptoethanol was similar for all doughs. The decrease in the relative viscosities of protein extracts from doughs mixed with those compounds supports the hypothesis that depolymerization of gluten proteins is accelerated by mixing in the presence of those compounds.

CONCLUSIONS

The relative viscosity of protein solutions can be affected by many factors. However, the use of SDS as a solvent eliminates most of those factors. The decrease in viscosity that is caused by overmixing, as well as the lack of change in viscosity of mercaptoethanol-treated protein extracts from overmixed doughs, are consistent with the hypothesis that disulfide bonds are broken as a result of dough mixing.

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