

Studies on Heavily Ground Flour Using Roller Mills. I. Alteration in Flour Characteristics Through Overgrinding

K. OKADA, Y. NEGISHI, and S. NAGAO¹

ABSTRACT

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Two commercial mill streams, extracted from the inner (stream A) and outer (stream X) part of the endosperm of no. 1 Canada western red spring wheat, were reground up to 10 times with a Miag Vario-Roller mill, and changes in flour characteristics were studied. As the number of passes through the rolls increased, the bromate requirement lessened, the SH content of the water-soluble fractions and the sulfhydryl/disulfide ratio decreased, and the valorimeter value of the farinogram increased. These changes may have resulted from the oxidation of flour components during overgrinding by roller milling. Activities of α - and β -amylase were not

altered, but protease activity decreased, and amylograph viscosity decreased slightly with progressive overgrinding. Proteins of streams A and X were fractionated into three fractions by gel filtration on Sephacryl S-300. Overgrinding of the flour streams caused some minor shifts in the proportions of the protein fractions. The bread-baking properties of stream A, except for an increase in baking absorption, were unchanged, whereas the bread-baking properties of stream X improved with severe overgrinding. When starch damage was over 11%, the peaks at 75°C in Do-Corder curves were higher than the peaks at 85 and 95°C.

Effects of starch damage on flour baking quality have been studied by many workers. Ponte et al (1961) found that the loaf volumes of bread made from a series of ball-milled flours decreases with increasing starch damage; general loaf quality also deteriorates, although little change was noted with the first moderate increase of starch damage. Schiller and Gillis (1964) studied the effect of increasing flour starch damage on continuous-process bread quality by repassing flour through steel rolls. They found that as starch damage increases, dough development time increases, and mixing tolerance decreases. When starch damage reaches 14.4%, bread quality is almost completely destroyed.

Farrand (1969) assumed that the characteristics of a bread dough depended on the ability of hydrated gluten to form a continuous matrix that completely covered the surface of the starch. He proposed that the optimum level of starch damage is a function of protein content. Tipples and Kilborn (1968) reported that pin-milling Canadian spring wheat flours to increase starch-damage level by 5 to 15 Farrand units permits the use of higher baking absorptions in short-time processes. This was translated into higher yields of bread, but bread characteristics were not greatly affected by pin-milling of the flours. Tipples (1969) reported that, in general, an increase in damaged starch will result in an increase in water absorption of flour, but for any flour there is an optimum level of starch damage (in terms of absorption and bread quality) depending on the flour protein content, level of α -amylase, and the type of baking process used (sponge-and-dough, continuous, Chorleywood, etc.)

Evers and Stevens (1984) reported the effects of the different types of starch damage on breadmaking quality. Baking trials were conducted on two blends of 10% starch and 90% high-protein flour. In both blends the flour component was identical, but the starches had been ground in different ways to produce high levels of different types of starch damage. Despite a substantial increase in starch damage, as measured by amylose extractability, differences in baking quality between samples were very small.

Alsberg and Griffing (1925) and Pulkki (1938), who studied the effect of fine grinding on flour, concluded that gluten quality is reduced with severe overgrinding. Atkinson and Fuehrer (1960) reported that loaf volume decreases with overgrinding, and that under particularly severe conditions the volume can drop off to a completely unacceptable level. Gluten quality does not seem to be adversely affected by minor degrees of overgrinding; however, after

additional overgrinding the gluten becomes shorter and tougher, which leads to changes in malting requirements, in bleaching and oxidation treatments, and in yeast levels needed in the bakery. Schlesinger (1964) concluded that the mechanical action of ball-milling is not sufficient to alter protein structure. In contrast, D'Appolonia and Gilles (1967) found that overgrinding flour causes a decrease in the total nitrogen contained in water solubles, an increase in nonprotein nitrogen, an increase in amino groups, an increase in lower-molecular-weight protein, and a slight variation in the shape of the peaks obtained by gel filtration.

Most of these reports concern the effects of milling conditions on the starch fraction, although a few report effects on the protein components of flour. The purpose of this study was to determine the effects of overgrinding on the properties of flour, dough, and baking quality. Two flour streams with markedly different baking quality were used: one flour (stream A) was an early reduction flour with good baking quality, whereas the other flour (stream X) was a late-break flour with poor baking quality.

MATERIALS AND METHODS

Wheat Flour

Two commercial mill streams were used: stream A (11.2% protein, 0.33% ash) was extracted from the inner endosperm and stream X (16.4% protein, 0.64% ash) from the outer part of the endosperm of no. 1 Canada western red spring wheat. All flour data are expressed on a 14.5% moisture basis. All chemicals used were reagent grade.

Preparation of Overground Flour

Overground flours were prepared with the Miag Vario-Roller mill under the following conditions: rolls, smooth (250 mm diameter, 150 mm length); speed of the fast roll, 450 rpm; roll differential, 1:1.5; roll load, 1.2 kW; and feed rate, 1.25 kg/min. Mill streams A and X were repassed through the mill one, three, and five times. An extremely overground sample was obtained by repassing stream X through the mill 10 times.

Determination of Starch Damage

Starch damage in flour was measured according to AACC method 76-30A (1982).

Determination of Diastatic Activity (Maltose Value)

Maltose values of flours were determined by AACC method 22-15 (1982).

Preparation of Dried Doughs

Doughs were prepared by operating the Do-Corder at 25, 75, 85, and 95°C for untreated stream X and stream A reground five times.

¹Nisshin Flour Milling Co., Ltd., Central Research Laboratory, 177 Tsurugaoka Ohi Iruma, Saitama 354 Japan.

The doughs were then immediately frozen by immersion in liquid nitrogen, lyophilized, and finely ground.

Scanning Electron Microscopy

Scanning electron micrographs of flours were obtained according to the method of Tanaka et al (1980).

Determination of Flour Particle Size

Distribution of flour particle size was determined in methyl alcohol with the Nikkiso Microtrac particle analyzer (Japan) using a laser system as described in the instruction manual.

Determination of Amylase Activity

α -Amylase activity was determined by the method of Mathewson and Pomeranz (1977). Extracts of α -amylase were prepared by the method of Hagberg (1961). Neo-Amylase-Test-Daiichi dyed amylose tablets (Daiichi Kagaku Yakuhin Co., Japan) were used as substrate; 10 ml of the α -amylase extract was warmed to 50°C, and the substrate was added just before the assay was started. After this mixture had been incubated at 50°C for 60 min, 0.5 ml of 1N NaOH was added to stop the reaction, and the solution was filtered through no. 2 Toyo Roshi filter paper. The activity was expressed as changes in absorbency values at 620 nm.

β -Amylase activity was determined by a modification of the method developed by Kneen and Sandstedt (1941).

Determination of Proteinase Activity

Proteolytic activities of flour on hemoglobin substrate in phosphate buffers were determined by the following method. Five grams of flour was dispersed in 30 ml of distilled water in a Waring Blender for 5 min and cooled in an ice bath. The mixture was centrifuged at 1,500 \times g for 10 min, and the supernatant was filtered through no. 2 Toyo Roshi filter paper. This filtrate was used as protease extract.

Hemoglobin substrate was prepared by the modified Anson method (Hagihara 1956). Hemoglobin (1.2 g) was dissolved in 70 ml of 0.1N NaOH containing 43.2 g of urea at 30°C for 60 min. The mixture was adjusted to pH 5.4 by 0.1M NaH₂PO₄ and was made up to 200 ml with distilled water.

One milliliter of protease extract was added to 5 ml of the substrate and incubated at 30°C for 6 hr. Five milliliters of 0.44M trichloroacetic acid was added to stop the reaction, and the mixture was kept at 30°C for 30 min before filtering through no. 2 Toyo Roshi filter paper. The colorimetric Folin method (Folin and Ciocalteu 1927) was used to determine the soluble protein content of the filtrates. Mixtures containing 2 ml of the filtrate, 5 ml of

0.55M Na₂CO₃, and 1 ml of the Folin phenol reagent diluted fivefold were kept at 30°C for 30 min before measuring absorbance at 660 nm on a Hitachi 220A spectrophotometer against a substrate blank. Proteolytic activities of flours were expressed as micromoles of tyrosine per minute per gram of flour.

Determination of Sulfhydryl and Disulfide Contents

Sulfhydryl (SH) and disulfide (SS) contents of flours were determined by amperometric titration, using the method developed by Sokol et al (1959) and modified by Tsen and Anderson (1963) and Okada and Yonezawa (1967).

Determination of SH Contents of Water-Soluble Proteins

SH contents of flour water-soluble fractions were measured by the method of Ellman (1959). Water extracts were prepared by stirring the mixture (5 g flour to 40 ml 0.02% ethylenediamine tetraacetic acid solution) for 5 min and centrifuging for 20 min at 20,000 \times g to remove insoluble material.

Operation of Dough Analysis Equipment

The Brabender amylograph was operated according to the instruction manual.

A Brabender farinograph was operated by AACC method 54-21 (1982). The thermostat was maintained at 30°C, and a large mixing bowl containing 300 g of flour was used.

A Brabender Do-Corder was operated as described by Tanaka et al (1980).

Birefringence Observation

Birefringence was measured with a polarizing light microscope as described by Watson (1964).

Gel Filtration on Sephacryl S-300

A Sephacryl S-300 superfine (Pharmacia) column (2.5 \times 57 cm) was equilibrated with 0.05M Tris-HCl buffer (pH 7.0) containing 0.5% sodium dodecyl sulfate. Flour proteins were extracted with 0.05M Tris-HCl buffer (pH 7.0) containing 0.5% sodium dodecyl sulfate and 1.6 \times 10⁻³ M *N*-ethyl maleimide. The suspension was stirred for 60 min at room temperature, and was then centrifuged at 28,000 \times g for 20 min at 25°C. The clear supernatant was collected.

Five milliliters of flour protein extract (8 mg protein/ml) was loaded onto the column, running at 23 ml/hr with upward flow at 25°C. The effluent was collected in 5-ml fractions. The protein concentration of the effluent was estimated by the difference in absorbance at 280 and 350 nm. Absorbance at 350 nm was used to correct for turbidity.

TABLE I
Analytic Data for Stream A and Stream X

Passes Through the Rolls (times)	Color Value ^{a,b}	Moisture ^a (%)	Starch Damage Values ^a (%)	Amylograph Peak			SH ^{e,f}	SS ^{e,f}	SH/SS	SH ^{e,g}
				Maltose Values ^{a,c}	Viscosity ^a (BU)	Protease Activity ^{a,d}				
Stream X										
0	5.7	15.0	5.3	166	530	0.41	1.9	3.9	0.49	2.5
1	5.5	15.0	5.8	174	525	0.31
3	5.9	14.9	7.0	184	490
5	6.5	14.9	8.7	238	490	0.26
10	6.8	14.7	10.7	292	470	0.24	1.9	4.1	0.46	2.3
Stream A										
0	-1.9	14.6	11.1	312	625	0.23	1.6	3.2	0.50	1.8
1	-1.8	14.5	11.9	341	600	0.19
3	-1.6	14.5	13.8	406	600	0.12
5	-1.2	14.5	15.4	449	575	0.13	1.6	3.6	0.44	1.8

^a Values are the average of duplicate determinations.

^b Color value was determined with the Kent-Jones and Martin flour color grader.

^c In milligrams of maltose per 10 g of flour.

^d Protease activity was expressed as micromoles of tyrosine per min g of flour.

^e Means of two independent experiments, analyzed in duplicate.

^f Sulfhydryl (SH) and disulfide (SS) contents were determined by amperometric titration (10⁻⁶ eq/g flour).

^g SH contents were measured by the method of Ellman (10⁻⁷ eq/g flour).

Baking Test

Breads were baked by the straight-dough method as described by Nagao et al (1981).

RESULTS AND DISCUSSION

Color and Moisture Content

As shown in Table I, color deterioration was observed with successive passes of the flour through the roller mill. In contrast, no significant change was seen in moisture content except when stream X was overground by passing through the rolls 10 times.

Starch Damage and Maltose Value

Table I shows the changes in starch damage and maltose value caused by overgrinding. Although the amount of starch damage for each stream increased with grinding, the starch damage after 10 passes of stream X was less than that of untreated stream A. This can be explained by differences in particle size and particle size distribution between streams A and X (Figs. 1 and 2).

The particle size distribution patterns in Figure 2 show a peak at 106 μm for untreated stream A and two peaks at 38 and 150 μm for untreated stream X. Overgrinding did not reduce the particle size of stream A, which seemed to be very hard, but it did increase starch damage. On the other hand, the main fraction of stream X was so small in particle size that most of it was unaltered in size by overgrinding, although larger particles (150 μm) were markedly reduced in size following 10 passes through the roller mill.

The correlation coefficient between starch damage and maltose value for both streams was very high (0.994). When α -amylase activity is low, such as in this study, the extent of starch damage can be estimated from maltose value, even though the flour samples are extracted from different parts of the endosperm.

Amylase Activity

Neither α - nor β -amylase activities were altered in either stream by overgrinding (results not shown).

Effect of Overgrinding on Amylograph Characteristics

Maximum amylograph viscosities are summarized in Table I. A slight decrease in the viscosities of both streams was seen with overgrinding. This agrees with the report of Tipples and Kilborn (1968), that amylograph viscosities decrease slightly with increasing damaged starch.

Protease Activity

As shown in Table I, protease activity decreased in both streams with progressive overgrinding. This suggests that wheat protease was more sensitive to overgrinding than wheat amylase.

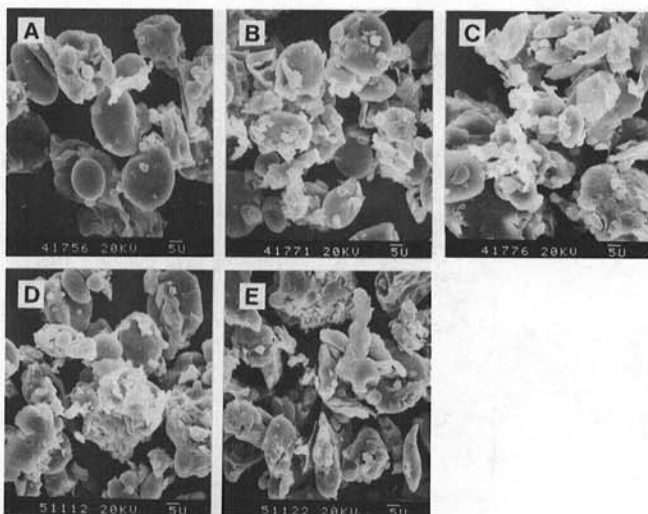


Fig. 1. Scanning electron micrographs of overground flours. Stream X: A, untreated; B, five passes; C, 10 passes. Stream A: D, untreated; E, five passes. Scale bar indicates 5 μm .

Sulfhydryl and Disulfide Contents

Table I shows SH and SS contents of both streams determined by amperometric titration. The SS contents in this study were lower than those previously reported in the literature (Tsen and Anderson 1963). Because stream X was extracted from the outer part of the endosperm, its SH content was higher than that of stream A. SS contents of both streams increased slightly with overgrinding, but SH levels did not show any significant change with overgrinding.

When SH contents of water-soluble proteins were determined by the DTNB method (Table I), the SH content of stream A did not change, but in stream X it decreased by 2.0×10^{-8} eq/g of flour with overgrinding. This value is very small but is equivalent to about 2.4 ppm of cysteine.

These results suggested that overgrinding may cause oxidation of SH groups in flour proteins.

Effects of Overgrinding on Farinograph Characteristics

As shown in Figure 3 and Table II, overgrinding of both streams increased farinograph absorption, valorimeter value, extended peak times, and development times. (The farinogram of stream X is now shown.) These results corroborated the report of Tipples and Kilborn (1968) that farinograph absorption increases almost linearly and dough development times become longer as damaged starch increases. Holas and Tipples (1978) reported that the increase in development time induced by overgrinding is caused by moderate heat damage. The possible decrease in flour protein SH groups observed following overgrinding in this study suggested that the increases in extended peak times, development times, and valorimeter values might be caused by the oxidation of SH groups in overground flours. To clarify the changes in the shape of the

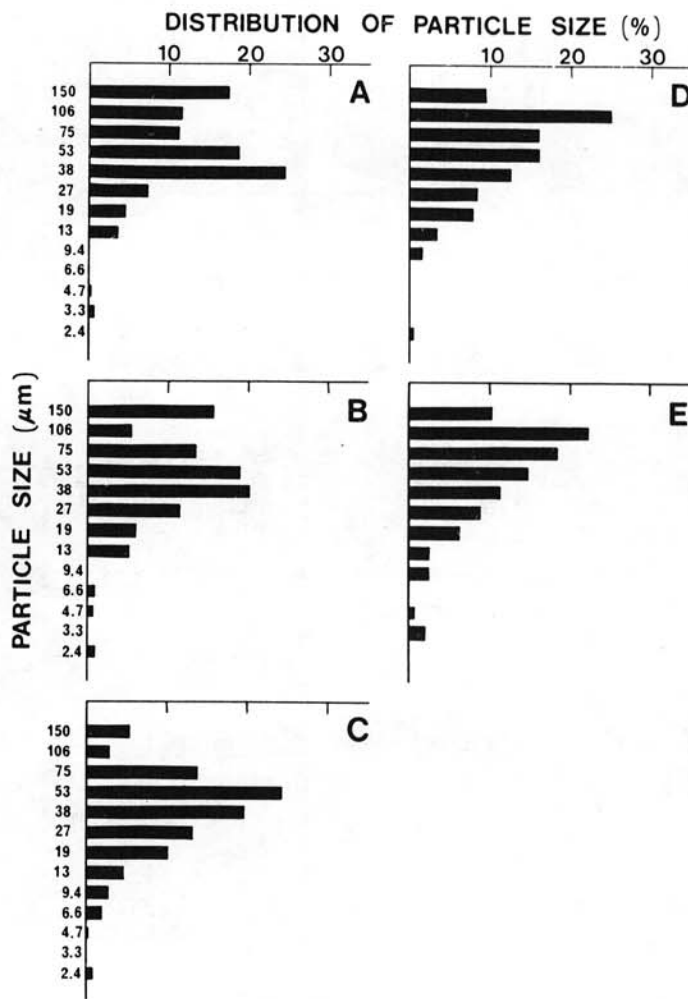


Fig. 2. Particle size distribution for various flours. Stream X: A, untreated; B, five passes; C, 10 passes. Stream A: D, untreated; E, five passes.

farinograph curves with respect to the oxidation of SH groups, farinograph curves were obtained in the presence of cysteine (10 ppm) for stream A after five passes through the rolls. The results were similar to the farinograph characteristics of untreated stream A except for its absorption (Fig. 3 and Table II).

TABLE II
Farinogram Data for Stream A and Stream X^a

Passes Through the Rolls (times)	Absorption (%)	Peak Time (min)	Development Time (min)	Mechanical Tolerance (BU)	Valorimeter Value
Stream A					
0	65.4	5.1	6.1	30	61
5	70.3	6.0	7.1	30	66
With cysteine (10 ppm)					
5	70.5	4.7	5.7	40	60
Stream X					
0	65.8	7.9	8.9	25	77
10	72.5	12.2	13.2	10	84

^a Means of two operations.

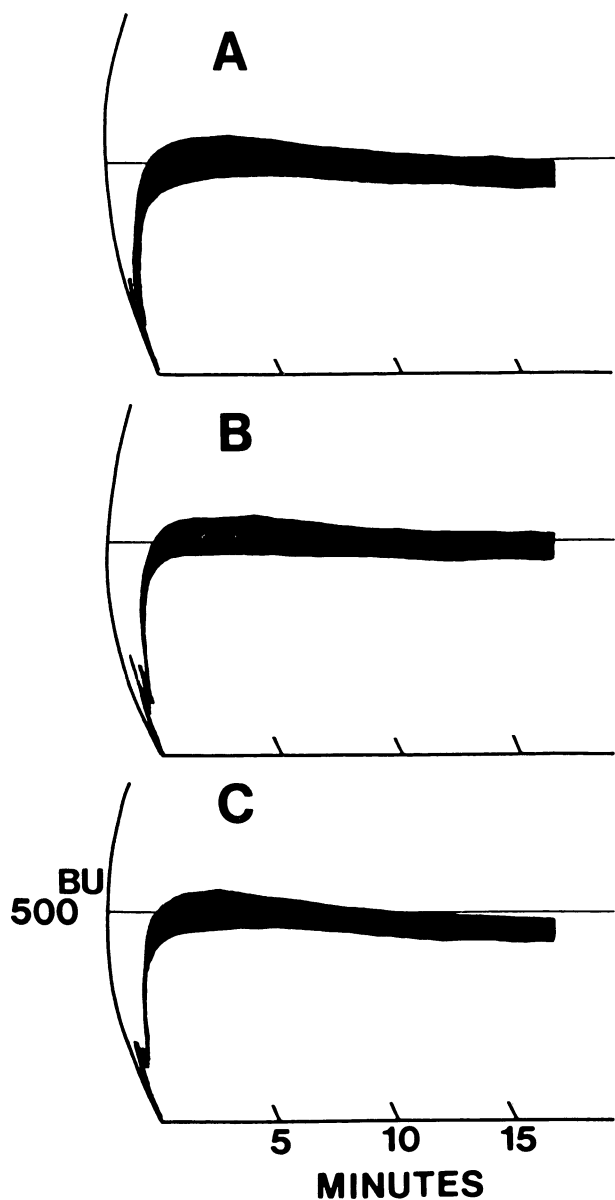


Fig. 3. Farinograms of stream A: **A**, untreated; **B**, five passes; **C**, five passes in the presence of 10 ppm cysteine.

In view of the above results, it appears likely that, except for absorption, the changes in farinograph properties induced by overgrinding were related to changes in SH/SS ratio caused by oxidation of SH groups in the flour during heavy roller milling.

Effects of Overgrinding on Do-Corder Characteristics

Endo et al (1981) reported that the major peak in Do-Corder curves shifts from 85 to 75°C as the period of vibrating ball-mill treatment increases. Figure 4 shows Do-Corder curves for flours with different levels of starch in the presence and absence of bromate.

The peaks in the Do-Corder curves in the presence of bromate were more obvious than those in its absence (Fig. 4). This agrees with the report of Tanaka et al (1980) that bromate promotes starch gelatinization. Comparing Table I with the results in Figure 4, the peak at 75°C was found to be higher than those at 85 and 95°C, when starch damage exceeded 11%.

To clarify changes in the shape of the Do-Corder curves with respect to the oxidation of SH groups, a Do-Corder was operated in the presence of cysteine (10 ppm) for streams A and X overground by five and 10 passes through the rolls, respectively. The results (not shown) were very similar to the curves on the left side of Figure 4. We concluded from these results that the changes produced in the Do-Corder curves by overgrinding were mainly

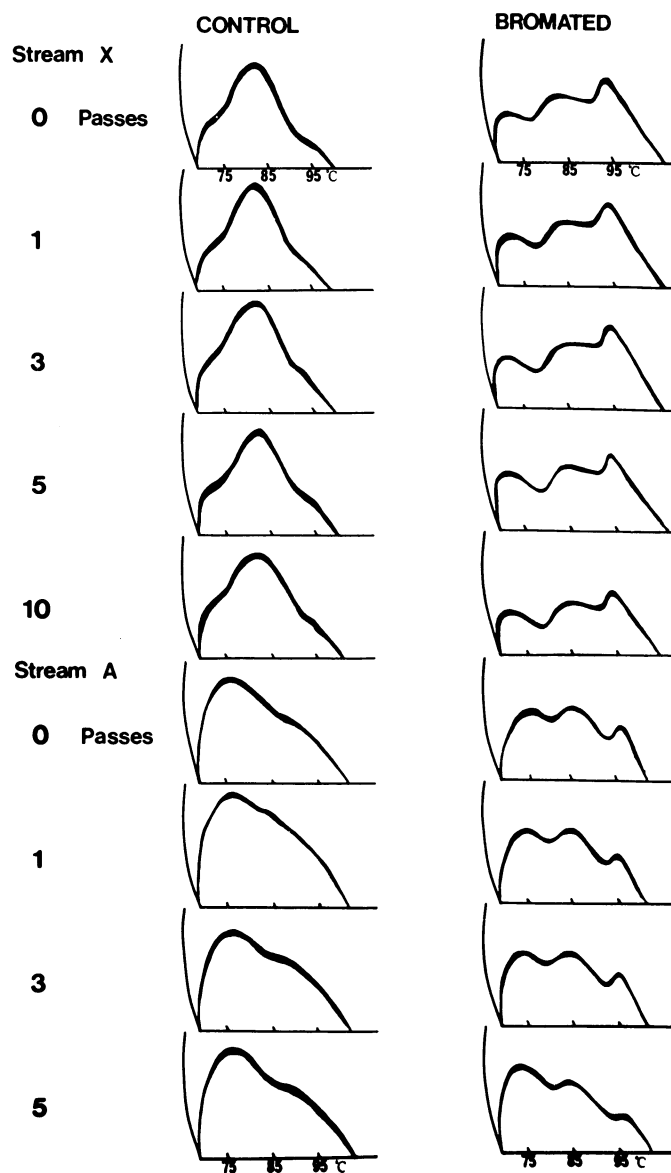


Fig. 4. Do-Corder curves for flours with different levels of starch damage in the absence (left) and presence (right) of bromate.

caused by starch damage rather than the oxidation of SH groups. Scanning electron micrographs of these doughs (Fig. 5) showed that starch granules in the untreated stream X still retained their shapes at 95°C (although they were slightly transformed into a flat shape), but those of the overground stream A collapsed even at 75°C. Maximum amylograph viscosity temperature of the overground stream A was lower (3.3°C) than that of untreated stream X (not shown). Starch granules of the overground stream A gelatinized faster than those of untreated X according to the difference of maximum amylograph viscosity temperature, birefringence observations (Table III), and scanning electron micrographs (Fig. 5).

From the above results, we concluded that the shift of peaks from 85 and 95 to 75°C in the Do-Corder curves was mainly attributable to a change in the rate of swelling of starch granules caused by damage to the starch.

Gel Filtration

Figure 6 shows the gel filtration profiles of proteins, extracted from streams A and X fractionated on Sephacryl S-300. Fractions I, II, and III were identified as glutenin, gliadin, and albumin, respectively, using the modified Osborne protein solubility classification of Chen and Bushuk (1970). Globulin was eluted between the glutenin and gliadin fractions.

TABLE III
Birefringence Value of Doughs Prepared by Do-Corder Operation at Different Temperatures

Do-Corder Temperature (°C)	Untreated Stream X (%)	Five Passes of Stream A (%)
25	100	100
75	9	0
85	0	0
95	0	0

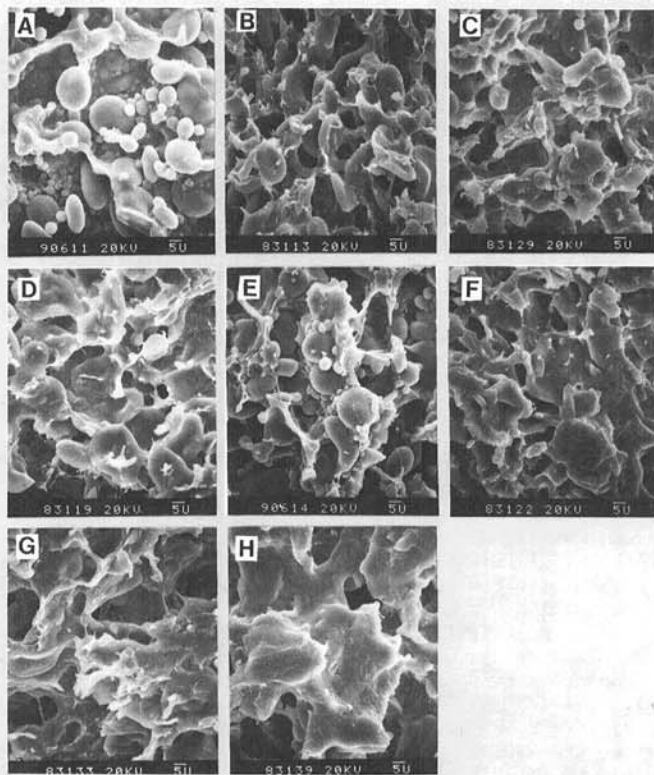


Fig. 5. Scanning electron micrographs of the dough prepared by Do-Corder operation at 25, 75, 85, and 95°C for the untreated stream X (A-D) and five passes of stream A through the rolls (E-H). A, dough of the untreated stream X at 25°C; B, at 75°C; C, at 85°C; D, at 95°C. E, dough of five passes of stream A at 25°C; F, at 75°C; G, at 85°C; H, at 95°C. Scale bar indicates 5 μm.

Similar profiles were obtained from streams A and X but some differences in the proportion of each fraction were observed, as shown in Table IV. Initially, stream A had a greater proportion of fraction I than stream X. Overgrinding of stream A slightly decreased the proportion of fraction I and slightly increased that of fraction II. In contrast, there was a slight increase in the proportion of fraction I and a slight decrease in the proportion of fraction II for overground stream X. Although highly repeatable, the magnitude of these shifts was not great enough to indicate a major change in protein size by overgrinding.

Baking Test

The effect of overgrinding on bread-baking properties was tested by the straight-dough method as described by Nagao et al (1981). As shown in Table V, the bromate requirements for untreated streams A and X were 2 and 15 ppm, respectively. The lower

TABLE IV
Changes in the Proportion of Protein Fractions Eluted From Sephacryl S-300

	Stream A		Stream X	
	Untreated (%)	Five Passes (%)	Untreated (%)	10 Passes (%)
Fraction I	55.4	51.5	45.9	50.7
Fraction II	27.8	31.2	38.0	32.6
Fraction III	16.8	17.3	16.1	16.7

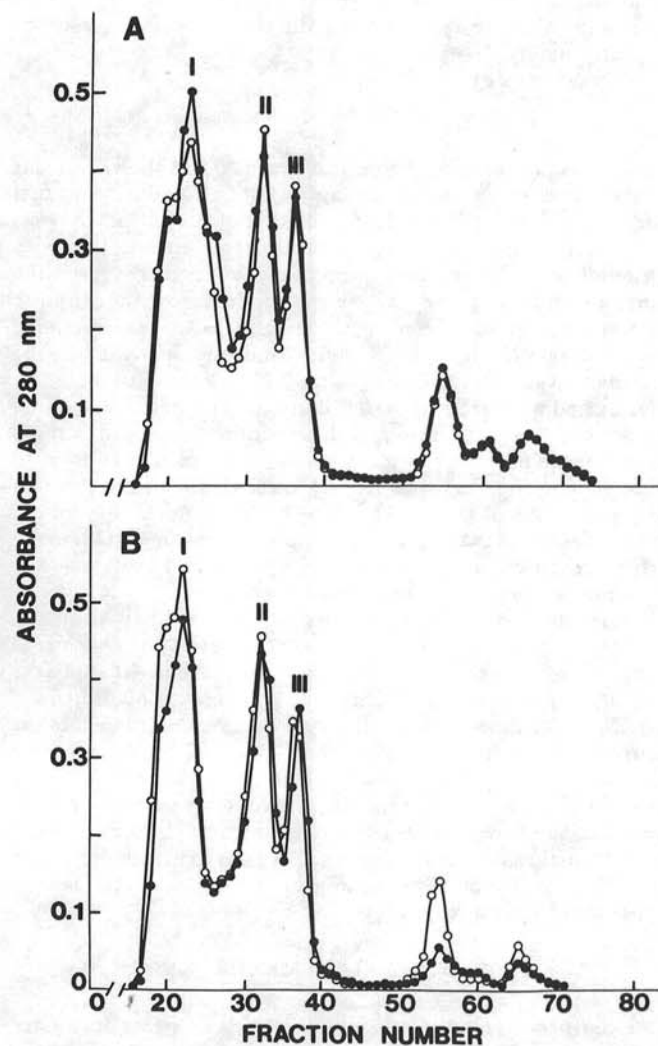


Fig. 6. Elution profiles from gel filtration chromatography on Sephacryl S-300 of various flour proteins. A, stream A: ●—● untreated, ○—○ five passes; B, stream X: ●—● untreated, ○—○ 10 passes.

TABLE V
Effects of Overgrinding on Breadmaking Properties Using the Straight-Dough Method^a

	Stream A								Stream X							
	0	0	0	0	1	3	5		0	0	0	0	10	10	10	10
Passes through the rolls (times)	0	0	4	0	1	3	5		0	10	15	20	0	5	10	15
Potassium bromate (ppm)	0	2	4	6	0	0	0		0	10	15	20	0	5	10	15
Water absorption (%)	75.0	75.5	76.7	77.0	77.0	79.5	81.5		71.5	72.5	74.0	74.2	80.5	80.5	82.0	82.5
Loaf volume (cm ³)	1,770	1,860	1,790	1,680	1,760	1,720	1,700		1,640	2,190	2,300	2,250	1,740	2,030	2,300	2,280
Ratings ^b																
Loaf volume (1-15)	10.7	11.6	10.9	9.8	10.6	10.2	10.0		9.4	14.9	15.0	15.0	10.4	13.3	15.5	15.0
Crust color (1-10)	7.1	7.2	7.2	7.1	7.3	7.3	7.5		7.0	7.2	7.2	7.2	7.5	7.5	7.5	7.5
Crust characteristic (1-15)	11.3	11.1	11.1	11.1	11.1	11.1	11.1		9.0	11.3	11.8	11.3	9.3	11.3	11.8	11.4
Crumb color (1-10)	7.3	7.3	7.3	7.3	7.3	7.3	7.3		6.0	6.3	6.3	6.3	6.2	6.4	6.4	6.4
Grain (1-20)	15.0	14.6	14.8	14.8	15.0	14.4	14.6		14.0	15.0	15.4	15.1	14.2	15.0	15.4	15.2
Texture (1-20)	14.6	14.6	14.6	14.4	14.6	14.4	14.4		14.0	15.0	15.6	15.4	14.2	15.0	15.6	15.5
Flavor (1-10)	7.0	7.0	7.0	7.0	7.0	7.0	7.0		6.5	7.0	7.0	7.0	6.7	7.0	7.0	7.0
Total ^c	73.0	73.4	72.9	71.5	72.7	71.7	71.9		65.9	76.7	78.3	77.3	68.5	75.5	78.7	78.0

^a Mean of two separate bakes.

^b Values in parentheses give possible ranges. In all rating systems, 1 = least desirable.

^c 100 possible.

bromate requirement for stream A compared to stream X was probably attributable to the lower content of SH groups in stream A (Table I). The bread-baking properties of untreated stream A reground one time were similar to those of stream A before regrinding in the presence of bromate (4 ppm); but even after regrinding 10 times, stream X still required 10 ppm of bromate. The baking properties of stream X improved after regrinding; dough stickiness was reduced and baking absorption, loaf volume, and bread characteristics were improved. In contrast, except for an increase in baking absorption, regrinding had little effect on stream A baking quality.

CONCLUSION

The inner part of the endosperm (stream A) was shown to incur more starch damage than the outer part (stream X) during normal roller milling. After being reground, stream A (but not X) was oxidized, resulting in a lower bromate requirement. Excessive regrinding by roller milling appeared to oxidize SH groups in the flour, as indicated by the observed decrease in bromate requirement, the reduction in SH content of the water-soluble fractions, the lower SH/SS ratio, and the increase in the valorimeter value of the farinogram.

Overgrinding increased starch damage. The degree of starch damage was related to farinograph absorption but not to changes in the shape of the farinograph curve. Starch damage did appear to affect dough properties with respect to starch swelling at elevated temperatures, as observed in the Do-Corder. Competition for the uptake of water between gluten and starch is very important in the early stages of baking; changes in both starch and protein due to overgrinding must therefore be considered.

Previously, it was shown that the activities of lipase (Yamada and Machida 1962 a,b) and lipoxidase (Surrey 1964, Walsh et al 1970) do not change with overgrinding. In the current study the level of amylase did not change, but the activity of proteinase decreased. The decrease in proteinase may have contributed to the improving effect of overgrinding on the poor baking flour, stream X.

Investigation of protein structure changes caused by overgrinding showed that the proportion of the high-molecular-weight protein in stream X increased slightly. This change might also contribute to the observed improvement in the breadmaking properties of stream X.

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