Studies on Dough Development. III. Mixing Characteristics of Flour Streams and Their Changes During Dough Mixing in the Presence of Chemicals

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ABSTRACT

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Changes in analytical and physical properties of dough during extended mixing were studied using a 60% extraction flour and flour steams obtained from an experimental Buhler mill. The effects of mixing on the rheological properties of doughs were measured by the Do-Corder at temperatures comparable to dough temperatures during the early stages of baking. The break flours were characterized by three peaks (75, 85, and 95° C) on the Do-Corder curve for bromated doughs, whereas the reduction flours gave only one peak (85°C) and a shoulder (75°C). Unlike the reduction flours, the break flours also showed a significant change in the Do-Corder curve during dough mixing (after longer mixing). Quantitative distribution of acetic acid-soluble protein (ASP) varied among the flour streams. Adding 1% (v/v) 2-mercaptoethanol to 0.05*N* acetic acid increased the amount of ASP in the extracts (mercaptoethanol-soluble protein, MSP). Dough mixing gradually increased both the amounts of ASP and MSP. The direct addition of bromate, 2-mercaptoethanol, or *N*-ethylmaleimide to the dough, however, specifically decreased the amount of MSP.

The variations of dough properties among wheat flour streams are well recognized (Pomeranz 1971). Baking quality of an individual flour mill stream depends on many factors, including wheat quality and milling conditions.

The chemical and rheological dough properties of individual flour streams might be affected by the distribution of wheat flour components during milling. Many previous workers have observed the different baking qualities of flour streams.

Pomeranz and McMasters (1968) reviewed the structural and compositional differences of the wheat kernel in detail. Holas and Tipples (1978) attempted to clarify the factors that affect water absorption of a dough, using flour mill streams. Working with flour streams from a commercial mill, Noguchi et al (1976) observed the correlation between sulfhydryl (SH) content and dough stickiness. More recently, Davis and Eustace (1984) illustrated the kernel disruption patterns of wheat during the milling process.

Although the importance of wheat proteins in breadmaking has been documented in detail (Finney and Barmore 1948, Bushuk et al 1969, Tanaka and Bushuk 1972, Orth and Bushuk 1972), limited information is available on the factors affecting baking qualities of individual flour streams.

We reported the contribution of the unmasked SH groups to the rheological properties of a dough during dough mixing (Endo et al 1984, 1985). The primary purpose of this study was to compare the rheological properties of flour mill streams using a Brabender Do-Corder. The secondary purpose was to clarify the flour components that contribute to dough properties. In addition, the effects of some chemicals on the protein solubility and consistency of dough were studied.

MATERIALS AND METHODS

Wheat

A sample of No. 1 Canada Western red spring wheat used in this study had a protein content (%N × 5.7) of 13.9%, an ash content of 1.60%, and a moisture content of 12.8%.

Milling

The cleaned wheat was tempered to a moisture content of 15.5% for 24 hr and milled on an experimental Buhler mill. The flour stream yields are listed in Table I. All yields were calculated on the basis of total recovered products (as is moisture basis).

Preparation of Flour Samples

Three samples were prepared for this study. A flour sample of 60% extraction, commonly used for quality assessment in Japan, was prepared according to the method described by Nagao et al (1976) by compositing the first and second break flours and the first and second reduction flours with a portion of the mixture of the third break and reduction flours. Break flour was prepared by compositing the three break streams, and reduction flour was prepared by compositing the reduction streams proportional to their flour yields.

Preparation of Premixed Dough Samples

Premixed dough samples were prepared in triplicate according to AACC method 54-40 (AACC 1983), except that sufficient water was added to 30 g of flour to give an absorption level of 70%. Doughs were mixed in a mixograph with a 35-g bowl (National Mfg. Co., Lincoln, NE) until they reached maximum consistency (peak time) and mixing was continued for another 5 min (peak time + 5).

Doughs containing chemicals were prepared by dissolving either potassium bromate (200 ppm), *N*-ethylmaleimide (NEMI; 200 ppm), or 2-mercaptoethanol (285 μeq) in an aliquot of the added water. All chemicals used were of reagent grade.

At the end of mixing, the dough was immediately frozen by immersion in liquid nitrogen. The frozen dough was freeze-dried, pulverized in a mortar, and ground in a coffee grinder to pass through a 100 mesh-sieve.

Do-Corder Operation

A Brabender Do-Corder was operated as described by Tanaka et al (1980) at a constant water absorption level of 70%. Potassium bromate (1,200 ppm) was dissolved in an aliquot of the added water. The x-axis of the Do-Corder curves shows the range of temperatures. The Do-Corder curves show the change in dough consistency with temperature increasing from 70 to 100° C.

Testing of Dried Dough Samples in the Do-Corder

The premixed dough samples (freeze-dried and ground) were again mixed with water to 70% absorption and heated in the Do-Corder to 100° C. For comparison, a flour sample was mixed in the same way.

Analytical Methods

Moisture and ash contents of wheat and flours were determined by AACC methods 44-15A and 08-01, respectively (AACC 1983). Protein (%N × 5.7) was determined by the Kjeldahl procedure,

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AACC method 46-11. Determinations were made in duplicate. All analytical values are reported on a 14% moisture basis.

Extraction of Proteins

Proteins were extracted with 0.05*N* acetic acid; 1 g (db) of sample was suspended in 20 ml of 0.05*N* acetic acid, and the mixture was stirred with a magnetic stirrer for 1 hr in a cold room (5° C). After centrifugation (5,000 × *g*, 20 min), the supernatant was freeze-dried. Protein contents of the dried solids were determined by the micro-Kjeldahl method. The values for acetic acid soluble-protein (ASP) are reported as percentage of total flour protein. The study was conducted in duplicate with replicate extractions.

Mercaptoethanol-soluble protein (MSP) was determined by the same procedure except that 0.05*N* acetic acid containing 1% (v/v) 2-mercaptoethanol was used.

Determination of Sulfhydryl (SH) and Disulfide (SS) Contents

SH and SS contents of the flour and dough samples were determined by an amperometric titration method developed by Sokol et al (1959) and modified by Tsen and Anderson (1963). The results are expressed in μmole per gram of flour protein. The changes in SH and SS contents of premixed doughs were calculated by the following formulas: change in SH content of premixed doughs (%) = (SH content per 1 g of protein in premixed dough at appropriate mixing time) × 100/(SH content per 1 g of protein in flour). When SS contents of premixed doughs were calculated, SS replaced SH in the equation. Determinations were made at least in duplicate.

RESULTS AND DISCUSSION

Analytical Properties

As summarized in Table I, the flour streams showed variable analytical values. Protein contents varied from 10.9% for 3M flour to 19.1% for 3B. Break flours and reduction flours exhibited different protein solubility trends. ASP of break flours progressively decreased from 1B to 3B. On the other hand, reduction flours showed more constant values. Thus, protein solubility of a wheat flour depends on the qualities (protein solubilities) and quantities (proportion of break flour streams to flour yield) of break flours. Although further studies are needed, it is interesting to note that protein solubility appears to be related to the extraction rate of flour. In addition to inherent differences among wheat cultivars and classes, both wheat milling quality and milling conditions might affect protein solubility.

Extractions were also performed with 0.05*N* acetic acid containing 1% (v/v) 2-mercaptoethanol to minimize the possible effects of SS bonds on the protein solubility. The addition of 2-mercaptoethanol increased the protein content (MSP) in the extracts with the exception of 3B flour, bran, and shorts.

SH and SS contents showed values somewhat lower than those reported by other researchers (Sullivan et al 1963, Tsen and Anderson 1963, Tanaka and Bushuk 1973). The 3B flour showed the highest SH and SS contents. This agreed well with the fact that SH contents are higher in the aleurone layer and germ than any other portions of wheat kernel (Pomeranz and Shellenberger 1961). Third break flour is derived from the peripheral regions of the endosperm rather than the center of the endosperm.

Rheological Properties

The Do-Corder curves for 60% extraction, break, and reduction flours were compared with and without the addition of potassium bromate (Fig. 1). The addition of bromate accentuated the difference in rheological properties among these flours. The bromated break flour was characterized by the presence of three peaks at 75, 85, and 95° C. The reduction flour with bromate gave a

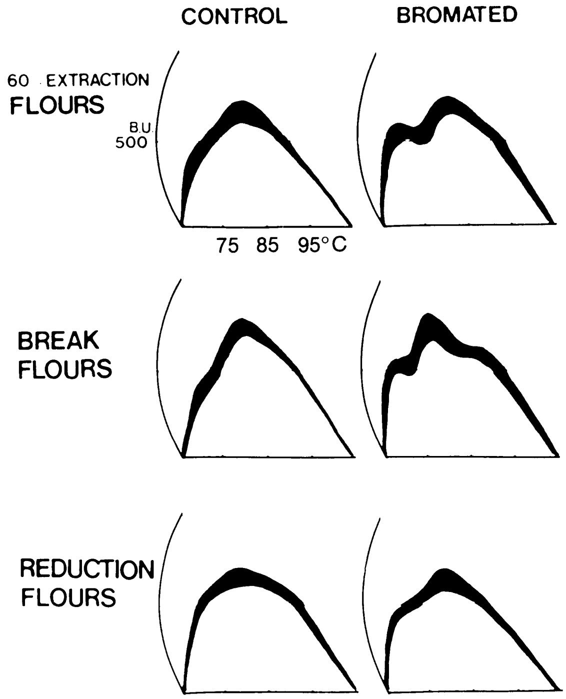

Fig. 1. Do-Corder curves for 60% extraction, total break, and reduction flours in the absence (control) or presence (bromated) of potassium bromate (1,200 ppm).

TABLE I
Flour Yields and Analytical Properties of Flour Streams

Flour Stream	Yield[a] (%)	Ash[b] (%)	Crude Protein[c] (%)	Solubility (%) ASP[d]	MSP[e]	SH[f] (μmol/g protein)	SS[g] (μmol/g protein)
1B	10.1	0.43	13.1	70.0	76.5	12.6	40.0
2B	8.8	0.38	16.0	46.3	55.7	15.9	40.9
3B	1.9	0.52	19.1	13.8	16.5	20.2	52.1
1M	18.8	0.38	12.7	41.9	48.3	16.5	32.3
2M	14.4	0.40	11.2	65.4	70.3	16.1	41.1
3M	11.3	0.50	10.9	56.7	68.0	17.1	41.3
Bran	23.1	4.67	14.2[h]	20.4	21.0	36.1	16.4
Shorts	11.6	1.95	14.7[h]	20.6	20.8	47.8	15.4
Flour of 60% extraction	60.0	0.40	12.6	64.5	71.2	15.0	41.4

[a] All yields were calculated on basis of total recovered products (as is moisture basis).
[b] 14% moisture basis.
[c] Crude protein (N × 5.7) reported on 14% moisture basis.
[d] ASP = Acetic acid-soluble protein (% of total flour protein).
[e] MSP = Mercaptoethanol-soluble protein (% of total flour protein).
[f] SH = Sulfhydryl content.
[g] SS = Disulfide content.
[h] N × 6.25.

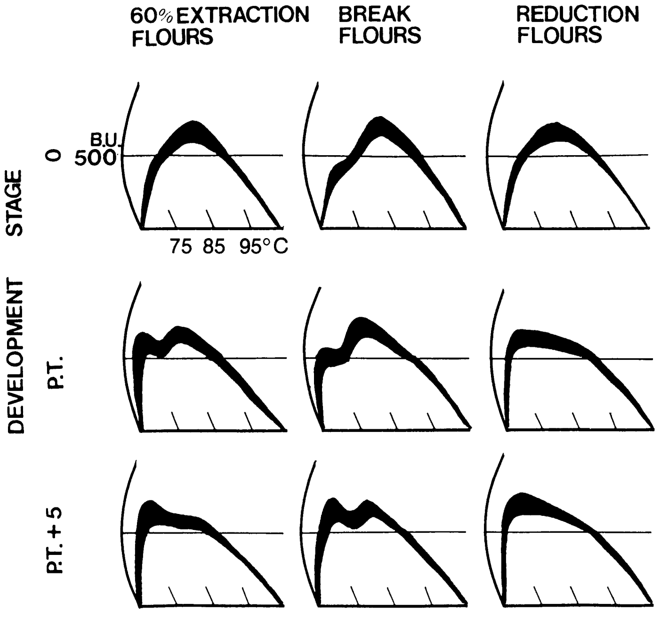

Fig. 2. Do-Corder curves for 60% extraction, total break, and reduction flours (**top**) and for powdered freeze-dried doughs premixed in a mixograph at 70% absorption level. Doughs were premixed until they reached maximum consistency (peak time, **P.T.**) and then for another 5 min (**P.T.**+5).

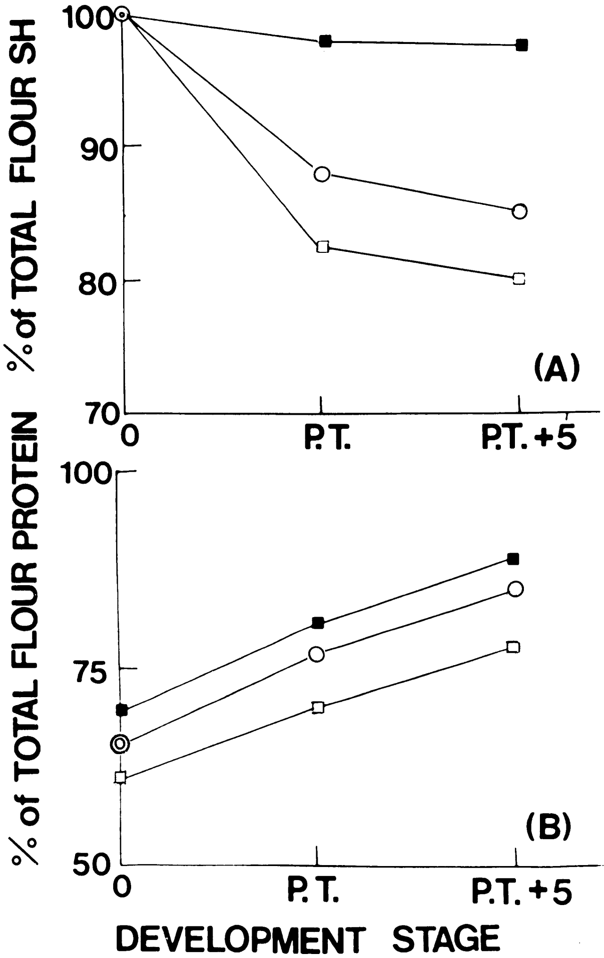

Fig. 3. Effects of dough-mixing on **A**, the sulfhydryl (**SH**) content and **B**, the amount of acetic acid-soluble protein. Doughs were premixed in a mixograph until they reached maximum consistency (**P.T.**), and then for another 5 min (**P.T.**+**5**) at a 70% absorption level. The results of SH content are shown as a percentage of the SH content of a powdered, freeze-dried, premixed dough compared to that of a flour: ◎ = original level, o—o = flour of 60% extraction, □—□ = total break flour, and ■—■ = total reduction flour.

Do-Corder curve similar to the 60% extraction flour without bromate, exhibiting a predominant peak at 85° C and a slight rise or a plateau at 75° C. The 60% extraction flour gave two clear peaks at 75 and 85° C in the presence of bromate. Thus, the break flour was the only one that responded to the addition of bromate by exhibiting a 95° C peak. In the absence of bromate, this peak did not appear on any of the Do-Corder curves.

In a previous study (Tanaka et al 1980), we suggested that protein and starch play an important role in producing the peaks at 75 and 85° C, respectively. The mechanism for the formation of a characteristic peak at 95° C for the bromated break flour, however, remains obscure.

Changes in the Properties of Flour Streams During Dough Mixing

Effect of mixing on the properties of doughs prepared from flour streams were examined after they had been premixed in a mixograph to peak time and peak time + 5 (Fig. 2).

The break flour underwent a dramatic change in the Do-Corder curve during dough mixing; the major peak showing higher consistency shifted from 85 to 75° C. On the other hand, the curves of the reduction flour after dough mixing differed only slightly from the flour without dough mixing. These results showed that the break flours responded greatly to both chemical (bromate) and mechanical (dough mixing) dough development. However, the peak at 95° C observed for unmixed, bromated break flour was not observed for premixed doughs.

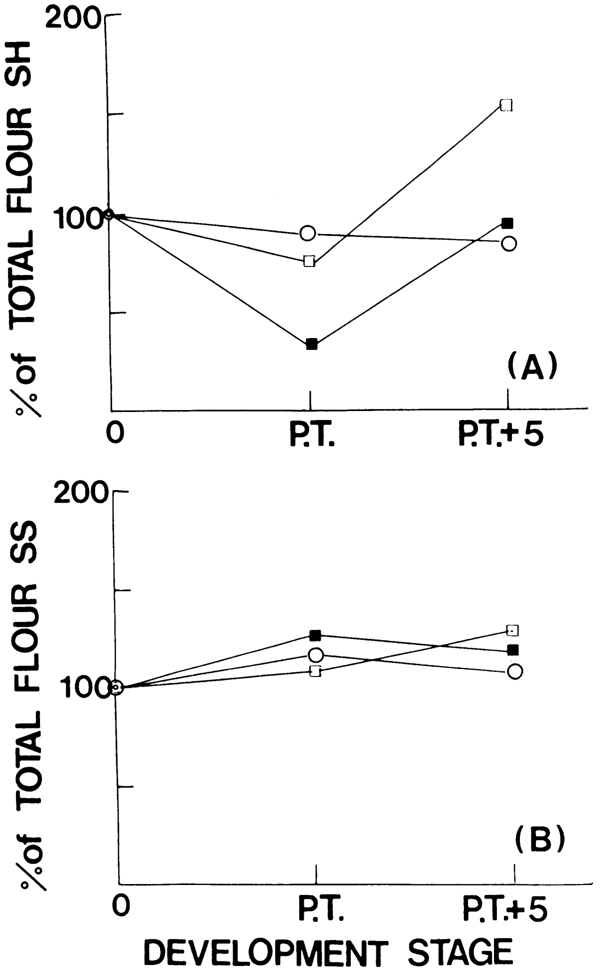

Fig. 4. Effects of chemicals on the contents of **A**, sulfhydryl (**SH**) and **B**, disulfide (**SS**) of a flour of 60% extraction. Doughs were premixed in a mixograph in the absence or presence of bromate (200 ppm) and *N*-ethylmaleimide (200 ppm) until they reached maximum consistency (**P.T.**), and then for another 5 min (**P.T.**+**5**) at a 70% absorption level. No chemical was added at the original level. The results are shown as a percentage of the SH (or SS) content of a powdered, freeze-dried, premixed dough compared to that of a flour: ◎= original level, o—o = control dough, □—□ = dough containing bromate and ■—■ = dough containing *N*-ethylmaleimide.

Previously, we observed that the changes of Do-Corder curves during dough mixing were affected by the water-absorption levels of doughs (Endo et al 1984, 1985). To examine the possible effects of water-absorption levels, doughs premixed at both lower and higher absorption levels (65 and 75%) were also tested. The rapid changes of Do-Corder curves for the break flour during dough mixing were confirmed for these doughs. The reduction flours showed minimal changes (data not shown). Therefore, the differences in the rheological properties of break and reduction flours could be attributed to intrinsic differences in their components.

These results suggest that there may be some complex of oxygen (e.g., linoleic acid hydroperoxide) formed during the premixing which reacts with SH. Meredith and Bushuk (1962) showed the effect of oxygen during dough mixing. In the current study, the break flour showed a more rapid decrease in SH content than the reduction flour (Fig. 3A). Thus, changes observed in Do-Corder curves during dough mixing appeared to be related to the removal of SH. A greater proportion of SH was removed from the break flour.

In contrast to these results, the proportionate increase of ASP during dough mixing was comparable in break and reduction flours (Fig. 3B).

Effect of Chemicals on SH and SS Contents

Levels of SH and SS during dough mixing were compared in the presence and absence of bromate (200 ppm), 2-mercaptoethanol (285 μeq), or NEMI (200 ppm), using a flour of 60% extraction.

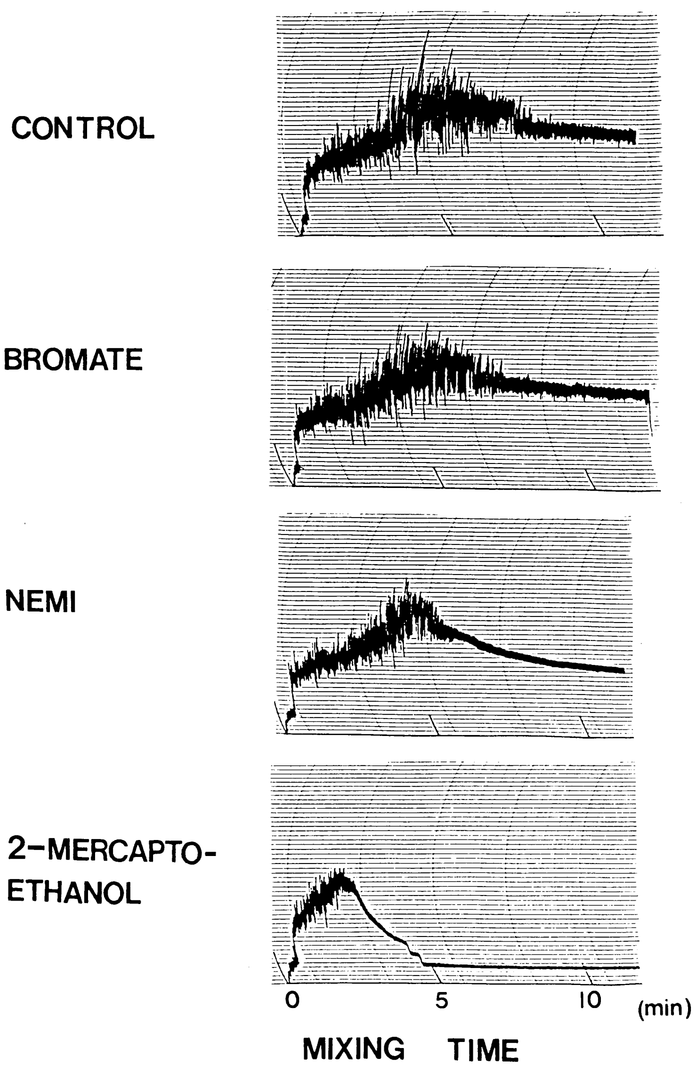

Fig. 5. Comparison of the mixograms for doughs containing bromate (200 ppm), *N*-ethylmaleimide (**NEMI**) (200 ppm), and 2-mercaptoethanol (285 μeq).

The direct addition of bromate and NEMI to the flour increased the decrease in SH content during the early stage of mixing (peak time; Fig. 4A). These results are consistent with the findings of Tanaka and Bushuk (1973). When the doughs containing bromate or NEMI were mixed for an additional 5 min, a large amount of SH was produced. In contrast, the control dough (without chemicals) did not exhibit an increase in SH with additional mixing. When a bromated dough was mixed beyond maximum consistency (peak time + 5), it contained almost 50% more SH content than the original level of the 60% extraction flour. These results are inconsistent with the findings of others (Sullivan et al 1963, Tsen and Anderson 1963, Tanaka and Bushuk 1973). However, it should be noted that in the current study excess amounts of NEMI and bromate were added to doughs to enhance their effects.

Compared to the control dough, the doughs that contained excess amounts of NEMI and bromate exhibited a decreased mixogram band width beyond maximum consistency (peak time). These effects were somewhat similar but less pronounced than the effect of adding of 2-mercaptoethanol (Fig. 5).

The critical increase of SH content during prolonged dough mixing could be partly attributed to the cleavage of SS bonds in flour proteins. The doughs used in the present study, however, did not show any definite changes in SS content (Fig. 4B). Both the control dough and the dough containing NEMI exhibited a slight

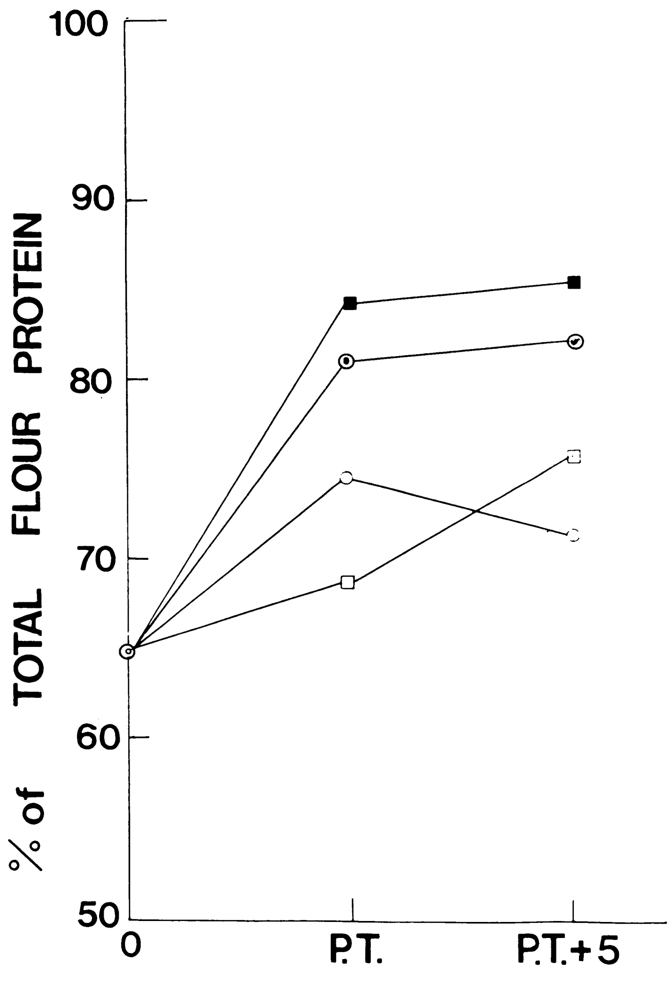

Fig. 6. Changes of the contents of acetic acid-soluble protein during dough-mixing for doughs containing bromate (200 ppm), *N*-ethylmaleimide (200 ppm), and 2-mercaptoethanol (285 μeq). Doughs were premixed in a mixograph in the absence or presence of chemicals until they reached maximum consistency (**P.T.**), and then for another 5 min (**P.T.+5**) at a 70% absorption level. No chemical was added at the original level. ◎= Original level, o—o = control dough, □—□ = dough containing bromate, ■—■ = dough containing *N*-ethylmaleimide, and ◉—◉ = dough containing 2-mercaptoethanol.

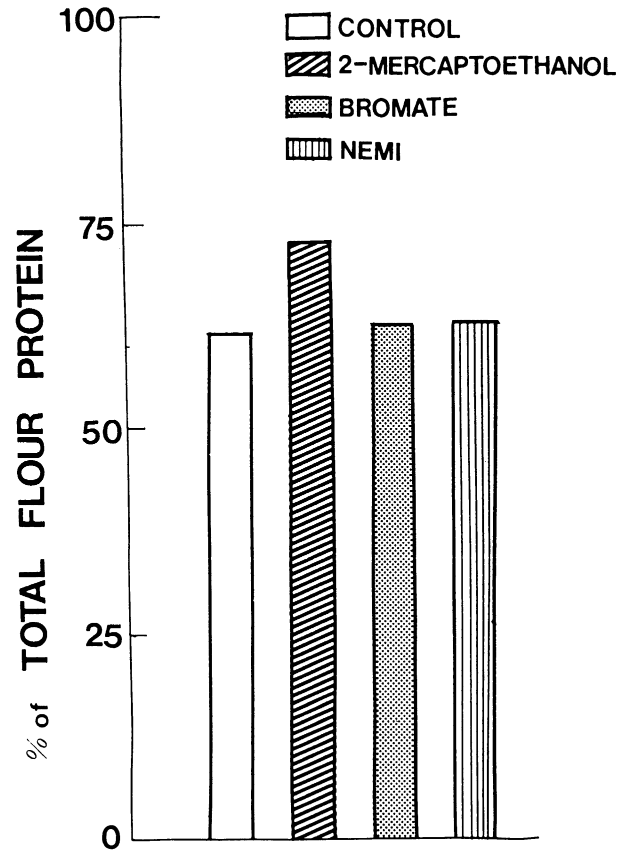

Fig. 7. Comparison of the contents of acetic acid-soluble protein for the flour of 60% extraction extracted with $0.05N$ acetic acid contained bromate (200 ppm), *N*-ethylmaleimide (**NEMI**) (200 ppm), and 2-mercaptoethanol (285 μeq).

maximum in SS bond content at peak time during dough mixing. On the other hand, the bromated dough showed a slight progressive increase in SS bond content with increased mixing.

In conclusion, highly precise analytical measurements are required to study the changes of SS content under the conditions of dough mixing investigated. Again, it should be noted that both bromate and NEMI, if present in excess amounts, can enhance the unmasking of SH groups or breakdown of SS bonds that occur during dough mixing. The production of unmasked SH groups might be similar to the cleavage of SS bonds by reducing agents such as 2-mercaptoethanol.

ASP and MSP

The direct addition of NEMI and 2-mercaptoethanol to doughs caused a marked increase in the amount of ASP during dough mixing (Fig. 6). Bromate had no effect.

Figure 7 shows the effects of adding these chemicals to the extraction medium on the amounts of ASP extracted from the 60% extraction flour. As observed in Table I, 2-mercaptoethanol was the only chemical that increased protein solubility.

On the basis of these results, the increase in ASP of doughs containing 2-mercaptoethanol can be attributed to the combined effects of dough mixing and the addition of additive. NEMI had no effect on the amount of ASP in the absence of dough mixing.

Changes in protein solubility during dough mixing using $0.05N$ acetic acid containing 1% (v/v) 2-mercaptoethanol were used to study MSP. Figure 8 shows the changes in the amounts of MSP for doughs containing bromate, NEMI, or 2-mercaptoethanol. The increase in the amounts of MSP was, in general, similar to that observed for ASP for a control dough (no additives), although MSP showed higher values. In contrast, the direct addition of bromate, NEMI, or 2-mercaptoethanol to doughs drastically decreased the amounts of MSP during dough mixing.

On the basis of these results, some relation was suggested between the trends of SH contents and the amount of MSP. MSP might become insoluble with the production of unmasked SH groups during dough mixing.

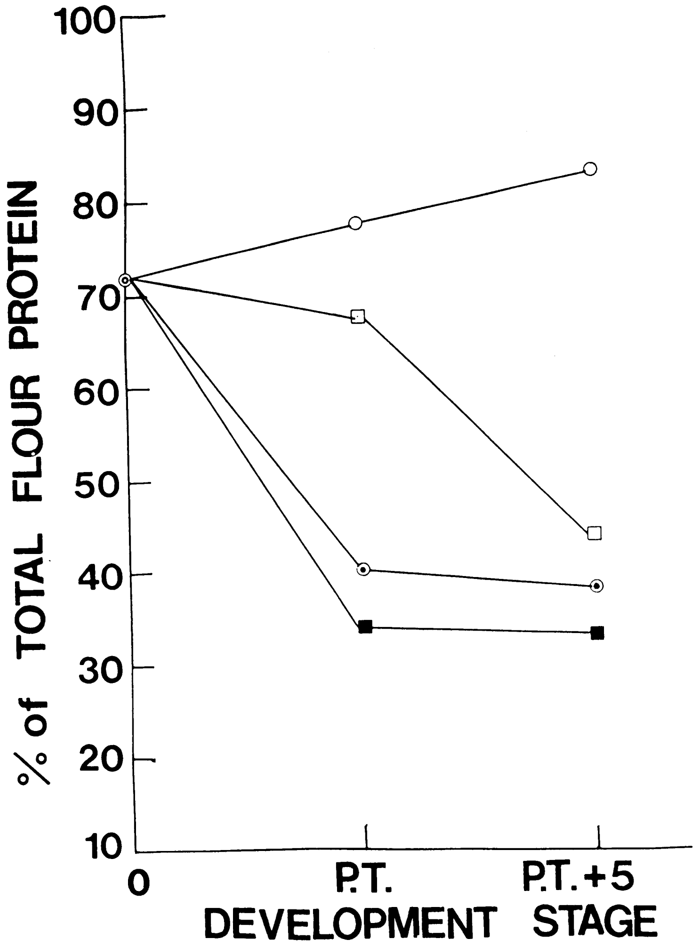

Fig. 8. Effect of chemicals on the contents of mercaptoethanol-soluble protein during dough-mixing. Doughs were premixed in a mixograph in the absence or presence of bromate (200 ppm), *N*-ethylmaleimide (200 ppm), and 2-mercaptoethanol (285 μeq) until they reached maximum consistency (**P.T.**), and then for another 5 min (**P.T.+5**) at a 70% absorption level. No chemical was added at the original level. ◎= Original level, o—o = control dough, □—□ = dough containing bromate, ■—■ = dough containing *N*-ethylmaleimide, and ◉—◉ = dough containing 2-mercaptoethanol.

CONCLUSION

An uneven distribution of flour components that play critical functional roles in dough mixing occurred during milling. Excess amounts of chemicals and their combined effects with dough mixing promoted the production of unmasked SH groups.

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