Effect of Potassium Bromate, Potassium Iodate, and L-Ascorbic Acid on the Consistency of Heated Dough

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ABSTRACT

The Do-Corder was used to investigate the effect of potassium bromate, potassium iodate, and L-ascorbic acid on dough mixed at high temperatures. The reagents were tested at a level of 1,200 ppm, based on flour. The Do-Corder curve generally showed two peaks at 75 and 85°C, which were assigned to the consistency effects of gluten and starch, respectively. The peak at 75°C was largely eliminated by addition of 1,200 ppm *N*-ethylmaleimide. Potassium bromate at 1,200 ppm also promoted starch consistency, as shown by the Do-Corder. Scanning electron microscopy and light microscopy of dough that had been heated and dried confirmed that bromate promoted gelatinization and swelling of starch.

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In most breadmaking processes, after mechanical development of the gluten network, the structure must be stabilized by oxidants such as acetone peroxides (Tsen 1964), azodicarbonamide (Tsen 1963), potassium bromate (Bushuk and Hlynka 1961), potassium iodate (Tsen and Bushuk 1963), and dehydroascorbic acid (Tsen 1965).

The extremely rapid reaction of most of these oxidants with sulfhydryl (SH) groups of flour has been demonstrated using dough-testing devices at room temperature (Tsen 1964, 1965). Bushuk et al (1960) reported, however, that the bromate reaction is temperature dependent. They recovered most of the bromate added initially to a fermented dough but essentially none from a dough that had progressed to an early stage of baking.

Bromate is the most widely used oxidant in flour; it gives dough with better machining properties and produces an improved loaf of bread. However, despite much work on the action of the improver, the mechanism remains obscure (Sullivan et al 1961).

This research was designed to study the improving mechanism of oxidants, using the Brabender Do-Corder fitted with a closed mixing bowl and operated at a temperature as high as that in the early stages of baking. A high level of oxidant (1,200 ppm) was used to accentuate the effects on flour components.

MATERIALS AND METHODS

Materials

The flour was an unbleached commercial blend milled by the Nisshin Flour Milling Co. from hard red spring wheat imported from North America. Protein content was 12.4%; moisture was 14.6%, and ash 0.41%. Oxidants were potassium bromate, potassium iodate, and L-ascorbic acid.

All chemicals were reagent grade.

Analysis of Wheat Flour

The flour data on protein, moisture, and ash content were obtained using approved methods of the AACC (1962).

Operation of Brabender Do-Corder

A Brabender Do-Corder, with an almost completely enclosed mixing bowl (roller mixer type 50 with a pressure ram), was operated under the following conditions: mixing speed, 60 rpm; setting of the scale head, XI; position of buckle connector, 1:1; and chart paper speed, 1 cm/min. The measuring ranges of the torque were shifted by adjusting a lever from position 0 to position 5. The temperature of the mixer was controlled at 35–110°C by a Brabender oil circulation system. The temperature of the test material was measured with a thermocouple attached to the mixer and a continuous recording chart.

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Various amounts of water were added to the mixer at 35°C to give desired absorption levels of 60, 70, 85, and 100%. For oxidant addition (1,200 ppm), the reagent was dissolved in an aliquot of the added water. The correct amount of sample (70 g for flour and starch and 50 g for vital gluten) was put into the rotating mixer, and mixing was continued. After 12 min of mixing, the temperature was raised gradually to almost 100°C.

Preparation of Dried Doughs

Doughs containing 0 or 1,200 ppm of oxidant were mixed in the Do-Corder and heated at a rate of about 3°C per min from ~35°C until the appropriate temperature was reached. During this time, the dough consistency was recorded to produce the tracings shown in the figures. When a dough sample was to be taken, mixing and heating were stopped at the appropriate stage and the sample was quickly frozen by immersion in liquid nitrogen. The frozen dough was freeze-dried, pulverized by hand, and ground in a coffee grinder to pass through an 80-mesh sieve.

Degree of Starch Gelatinization

The degree of starch gelatinization of the samples was determined by measuring diastase accessibility as described by Migumo et al (1954).

Birefringence Observation

The birefringence was measured with a polarizing light microscope as described by Watson (1964). Sufficient water was added to a small portion of the sample to make it suitable for microscopy.

Scanning Electron Microscopy (SEM)

The dried samples were sprinkled onto double-sided adhesive tape attached to specimen stubs. The mounted specimens were coated with a layer of platinum about 150 Å thick, in an Eiko ioncoater (Type IB-3) and viewed with a Hitachi S450 at 20 kv.

Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis (PAGE)

The SDS-PAGE technique used in this study to determine the molecular weights of the components of Osborne fractionation (Chen and Bushuk 1970) was described by Orth and Bushuk (1973). Where a comparison between reduced and unreduced protein was required, an additional electrophoresis experiment was carried out by blocking the SH groups of the unreduced protein with *N*-ethylmaleimide (NEMI) as reported by Tanaka and Bushuk (1973). For electrophoresis of the insoluble residual proteins, 2 g of the starch residue was extracted with 20 ml of AUC (0.1 *M* acetic acid, 3 *M* urea, and 0.01 *M* cetyltrimetylammonium bromide) by stirring overnight in a cold room (4°C). This suspension was centrifuged for 25 min at 20,000 × *g*, and the supernatant was freeze-dried. The freeze-dried protein was reduced with β-mercaptoethanol and complexed with SDS according to Orth and Bushuk (1973).

Preparation of Starch and Vital Gluten

The flour sample (200 g) and 300 ml of water were homogenized in a Waring Blendor. The homogenate was then centrifuged at 2,000 $\times g$ for 10 min. The supernatant solution was discarded. Gluten was obtained in the upper layer and starch in the lower layer of the precipitate. Both were freeze-dried and ground in a coffee grinder to pass an 80-mesh sieve. The preparations were subjected to Do-Corder mixing analysis to investigate the oxidizing reaction on starch and protein. In the analysis, 70 g of starch and 50 g of gluten were examined at 70% absorption. Mixing and heating were stopped at 75°C, the point at which consistency reached a maximum. The starch was also subjected to SEM after preparation as described for dried doughs.

RESULTS

L-Ascorbic acid is a reducing agent. Kuninori and Matsumoto (1963, 1964) have shown, however, that during dough mixing L-ascorbic acid is oxidized rapidly to dehydroascorbic acid, which can act as an oxidant. In this study this reagent is considered an oxidant.

Effects of Bromate on Do-Corder Curves at Various Absorptions

The changes in curve characteristics caused by the various water absorption levels in doughs with and without potassium bromate are illustrated in Fig. 1. All curves tended to show either a double rise or two peaks that closely coincided with the temperatures of 75 and 85°C.

As the absorption level was decreased, the major peak (higher consistency) shifted from 75 to 85°C. With bromate (1,200 ppm), however, the first peak at 75°C was clearly seen at any absorption. Accordingly, the effect of bromate was to produce characteristic curves with two clear peaks. Because this was especially true at 70% absorption, 70% was employed thereafter in this study. This level is also very close to that used in commercial baking.

The amount (1,200 ppm) of bromate is greater than the level (10–30 ppm) employed in the baking industry. However, the effects of bromate and of other oxidants could not be clearly detected by the Do-Corder unless oxidant levels were greater than 500 ppm. (Results are not shown.) The amount (1,200 ppm) was arbitrarily chosen to accentuate the effects of the oxidants.

Oxidizing Reaction on Protein

Effects of Oxidants With and Without NEMI. The Do-Corder curves on the left side of Fig. 2 show the effects of the various oxidants on doughs at 70% absorption. As shown in Fig. 1, the bromated dough gave two distinct peaks at 75 and 85°C. Both iodated and ascorbated dough gave one clear peak (at 85 and 75°C, respectively) and a small rise (at 75 and 85°C, respectively). In the presence of NEMI (1,200 ppm), however, all curves were similar (Fig. 2); they all showed a prominent peak at 85°C and a slight rise or a plateau at 75°C. These results imply that protein plays an important role in forming the peak at 75°C. Other workers (Mecham et al 1960, Schroeder and Hoseney 1978) have shown that wheat doughs lose their strength when mixed in the presence of NEMI at ~25°C. The peak at 85°C in the curves is attributed to the gelatinization and swelling of the starch. The consistency decreases as the temperature rises above 85°C and as mixing (shearing) is continued.

SDS-PAGE Result. PAGE was used to examine flour and dough protein after complexing with SDS directly or after reduction with β-mercaptoethanol. This procedure eliminates the effects of intrinsic charge differences between the proteins and separates the components according to molecular weight.

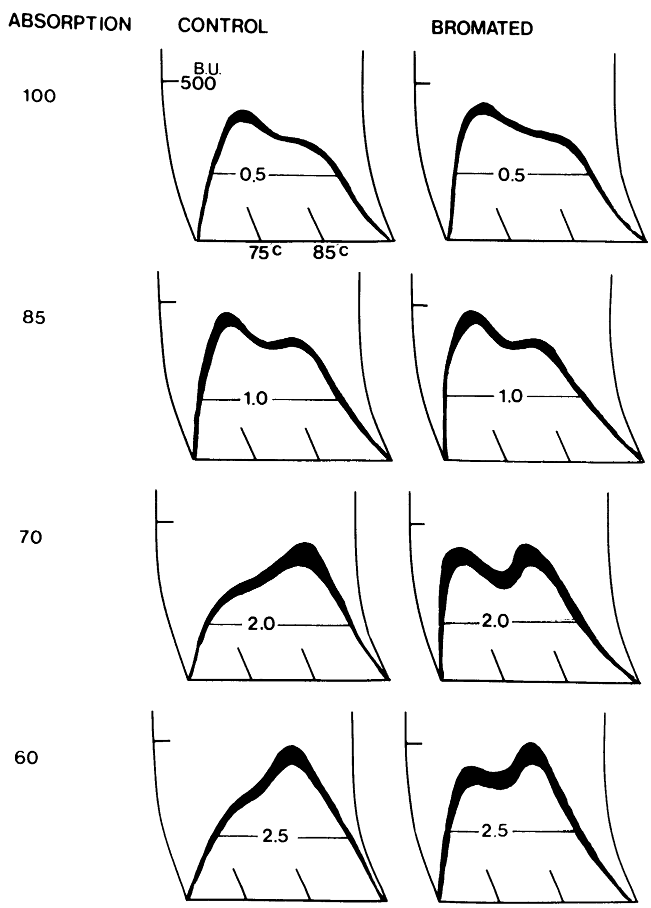

Fig. 1. Do-Corder curves for control and bromated doughs at various absorptions. The figure under each curve stands for the lever position of the instrument.

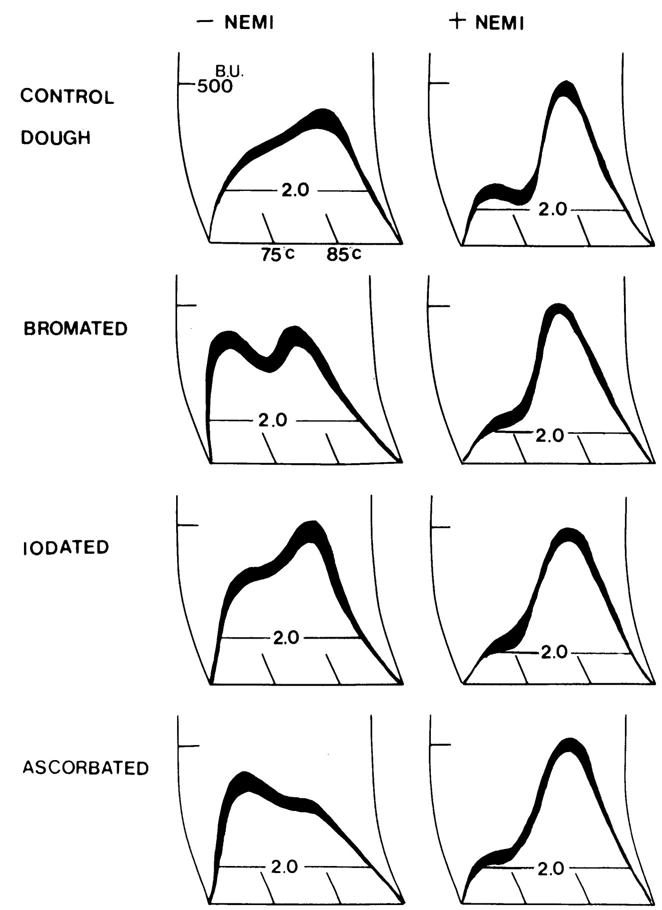

Fig. 2. The effect of *N*-ethylmaleimide on Do-Corder curves for control and oxidized doughs. The figure under each curve stands for the lever position of the instrument.

With Osborne fractions of flour and dough proteins, SDS-PAGE without prior reduction could only be used to examine the water-soluble, salt-soluble, and alcohol-soluble fractions. The intact protein of the acetic acid-soluble or the insoluble residue fraction is too large to enter the gel. Neither heat treatment nor oxidants affected the electrophorectic patterns of any protein fraction. Patterns of starch and flour protein fractions were identical.

Do-Corder Results for Vital Gluten Isolated from Flour. As shown by the Do-Corder curves in Fig. 3, the control and the ascorbated gluten increased in viscosity at 70°C and quickly decreased as temperature increased. The loss of consistency of the gluten is due to heat denaturation of the protein. With the addition of bromate and iodate, the peak at ~70°C became sharper, although the decrease in viscosity with continued heating was not affected.

In the presence of NEMI (1,200 ppm), all the curves became almost identical. The increase at 70°C was greater and the subsequent decrease in viscosity was smaller than those in the absence of NEMI. These results again indicate the importance of SH groups with regard to the reaction with oxidants. In the presence of NEMI, the consistency of gluten doughs does not decrease as much as does that of a wheat dough. This may possibly be explained by the lower proportion of NEMI to protein in the gluten dough or by the accelerated mechanical breakdown of the wheat dough from other constituents.

Oxidizing Reaction on Starch

The Extent of Starch Gelatinization. The data given in Table I show two variables to indicate the extent of starch gelatinization. One, birefringence, is a visual observation to monitor the degree of starch gelatinization. Starch in the dried ground sample of control dough heated to 75°C retained 67% of birefringence and seemed to be at the first stage of swelling (Miller et al 1973). The sample heated to 85°C, however, retained only 5% of birefringence and was nearly completely gelatinized, according to Watson's definition (1964).

Hill and Dronzek (1973) reported that wheat starch was gelatinized in the range of 56–64°C in a large excess of water. In this study, the doughs were prepared at 70% absorption, and starch would have to compete with other flour components for the limited amount of water. Therefore, even at 75°C, starch was still at the first stage of swelling (Miller et al 1973) and was not gelatinized completely until the temperature reached 85°C. Derby et al (1975) found that starch isolated from pie crust was more than 50% birefringent, which confirms that the amount of water is critical for starch gelatinization.

Another variable to indicate the extent of gelatinization was expressed as DG in Table I and is measured by an enzymatic technique (Migumo et al 1954). In this case, gelatinization is defined as the complete susceptibility to the action of diastase. According to this measurement, starch was nearly completely gelatinized even at 75°C for all samples.

Starch in the bromated dough heated at 75°C showed 26% birefringence, which is nearly one-third of that for the control. But iodate and ascorbic acid had no effect on the starch birefringence. In general, the extent of gelatinization was promoted by the presence of bromate (Table I), which is particularly interesting because bromate has generally been assumed to have little effect on starch in baking.

SEM of Starch Isolated from Dried Doughs. SEM was employed to further observe the effect of oxidants on the extent of starch gelatinization. A sample of the wheat starch control is shown in Fig. 4G. The large lenticular-shaped or A-type granules and the smaller spherical or B-type granules, as described by Kent (1966), are strikingly evident in this photograph. Flour protein is also observed on the surface of the granules.

The SEM pictures of starch isolated from control, iodated, and ascorbated doughs heated to 75°C (Figs. 4A–C, respectively) show that some of the large granules and many of the smaller granules have collapsed to form doughnut-shaped structures. For the same samples heated to 85°C, the process has developed further, but folding and deformation of granules are far from complete (Figs. 4D–F). Most granules are expanded with loss of their original shapes, especially those from ascorbated dough (Fig. 4F).

The gelatinizing effect of bromate on starch granules can be observed clearly under SEM by comparing starch from bromated dough at 75°C (Fig. 4H) and at 85°C (Fig. 4I) with starch from control doughs at these temperatures (Figs. 4A and C). At 85°C, individual starch granules from the bromated dough are barely visible (Fig. 4I).

Do-Corder Results on Starch Isolated from Flour. Do-Corder curves in Fig. 5 show that at 75°C bromate brought about a large increase and ascorbic acid a small increase in viscosity for starch isolated from the flour, whereas iodate slightly decreased the viscosity. The increase can be attributed to water being taken up by the starch as the granules swell. Bromate and ascorbic acid enhanced the swelling of the starch, as was shown by SEM, and thereby caused the increase in viscosity.

TABLE I
Effects of Oxidants (1,200 ppm) on Starch Gelatinization in Doughs Heated and Mixed in a Do-Corder

Dough	75°C		85°C	
	Birefringence (%)	DG[a] (%)	Birefringence (%)	DG (%)
Control—no oxidant[b]	67	93.2	5	96.2
Bromated	26	94.1	0	100
Iodated	62	86.8	5	91.5
Ascorbated	63	89.8	0	98.4

[a] Degree of gelatinization, ie, susceptibility to diastase (Migumo et al 1954).
[b] The starch in the flour showed 100% birefringence and 15.8% DG.

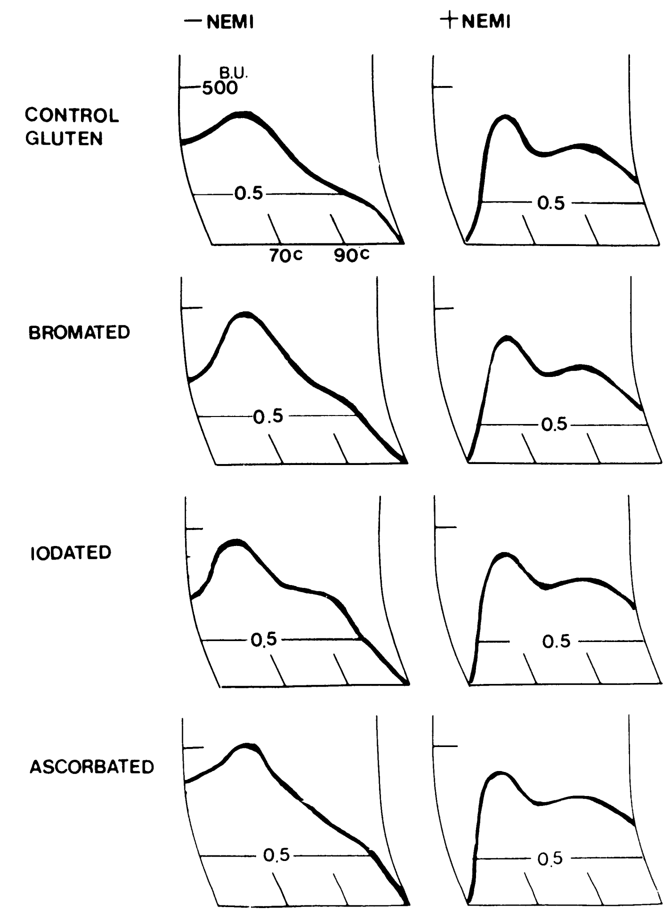

Fig. 3. Do-Corder curves for vital gluten isolated from flour with and without *N*-ethylmaleimide. The figure under each curve stands for the lever position of the instrument.

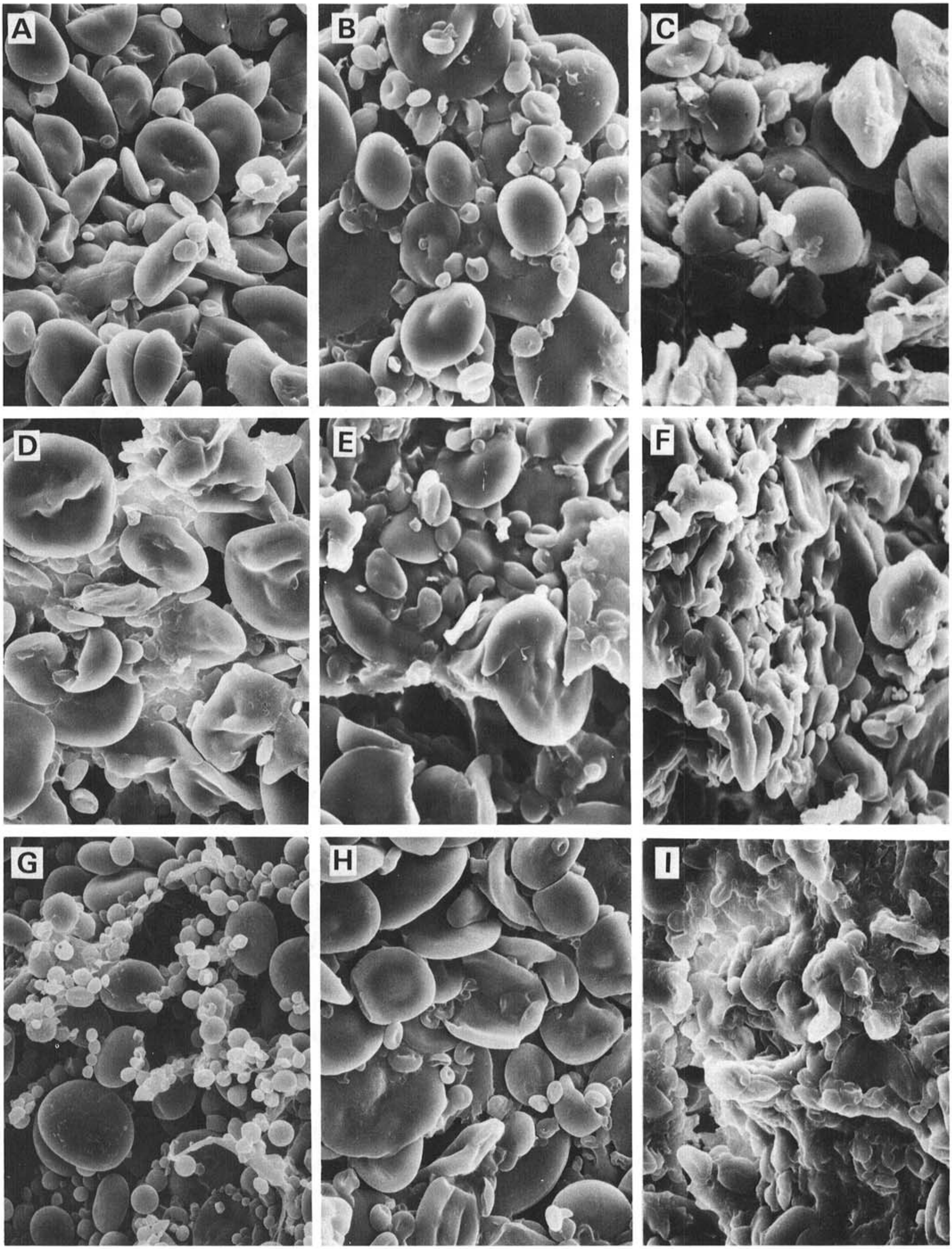

Fig. 4. Scanning electron micrographs of starch isolated from samples of: **A,** control dough at 75° C; **B,** iodated dough at 75° C; **C,** ascorbated dough at 75° C; **D,** control dough at 85° C; **E,** iodated dough at 85° C; **F,** ascorbated dough at 85° C; **G,** flour; **H,** bromated dough at 75° C; **I,** bromated dough at 85° C. (×1,000)

Effects of Oxidants on Gelatinization of Starch Isolated from Flour. SEM indicated that bromate promoted the gelatinization and swelling of starch in heated dough. It did not make clear, however, whether the effect was caused by bromate reacting directly on starch or by an intermediate such as a peroxide of a lipid generated by the oxidant. In this part of the study, starch isolated from flour was treated directly with oxidants in the Do-Corder to investigate their direct effect on the degree of the gelatinization. The SEM pictures of control, iodated, and ascorbated starch showed essentially no differences (Figs. 6A–C). Bromated starch (Fig. 6D) showed more pronounced gelatinization compared with the others. We concluded from these results that, of the oxidants used in the study, only bromate reacted with starch directly.

DISCUSSION

Water is one of the most important constituents of dough and plays a major role in dough characteristics (Bushuk 1966, Bushuk and Hlynka 1964). The amount of water in dough affected the Do-Corder curve, as shown in Fig. 1. With increasing absorption, the main distinct peak shifted from 85 to 75°C. This was true for bromated doughs as well as for those without bromate. Bushuk (1966) determined the water distribution in dough and estimated that 45.5% of the total water was associated with the starch, 31.2% with protein, and 23.4% with the extremely small amount of pentosan. A marked change in the distribution of water in dough occurs during baking. When wheat starch gelatinizes, its hydration capacity increases from 55 to 98% (Yamazaki 1955). On the other hand, thermal denaturation of gluten reduces its hydration capacity from 2.15 g of water per gram to essentially zero. The peaks of the Do-Corder curve may largely result from the combined effects of starch gelatinization and thermal denaturation of gluten because these two events govern the distribution of water in dough between starch and gluten.

All doughs showed distinctive curves at the 70% absorption level (Fig. 2). The results in Fig. 2 suggest that the first peak or shoulder of the curve, occurring at 75°C, was associated with the gluten fraction in the dough. Furthermore, because the peak at 75°C nearly disappeared in the presence of NEMI, this peak intensity may be due to oxidation of SH to disulfide groups.

The increase in viscosity at 85°C was mainly brought about by starch gelatinization, as indicated by the fact that, in the presence of NEMI, each dough showed a distinct peak in the Do-Corder curve at 85°C. On the other hand, starch isolated from the flour caused a peak at 75°C (10°C below that in flour) regardless of the presence or type of oxidant (Fig. 5). In the complete dough system, not enough water was available for starch gelatinization and swelling until the protein started to lose water by thermal denaturation above 70°C (Fig. 3). Thereafter the viscosity in the curve for the control dough (Fig. 2) gradually increased to the maximum at 85°C. Thermal treatment and oxidants had no effect on the intrinsic structure of the proteins or subunits, as judged by SDS-PAGE.

The promotion of starch gelatinization by bromate, as shown in the SEM results (Figs. 4 and 6) and with the Do-Corder (Fig. 5), is particularly significant because no attention has been paid to the

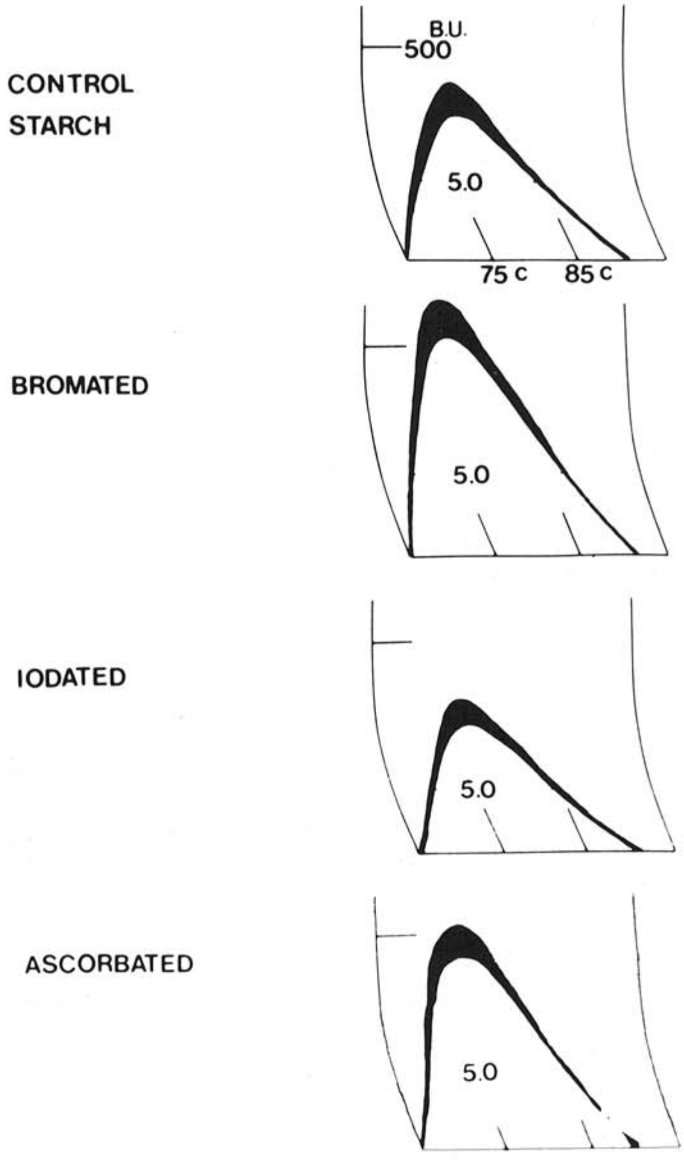

Fig. 5. Do-Corder curves for starch isolated from flour. The figure under each curve stands for the lever position of the instrument.

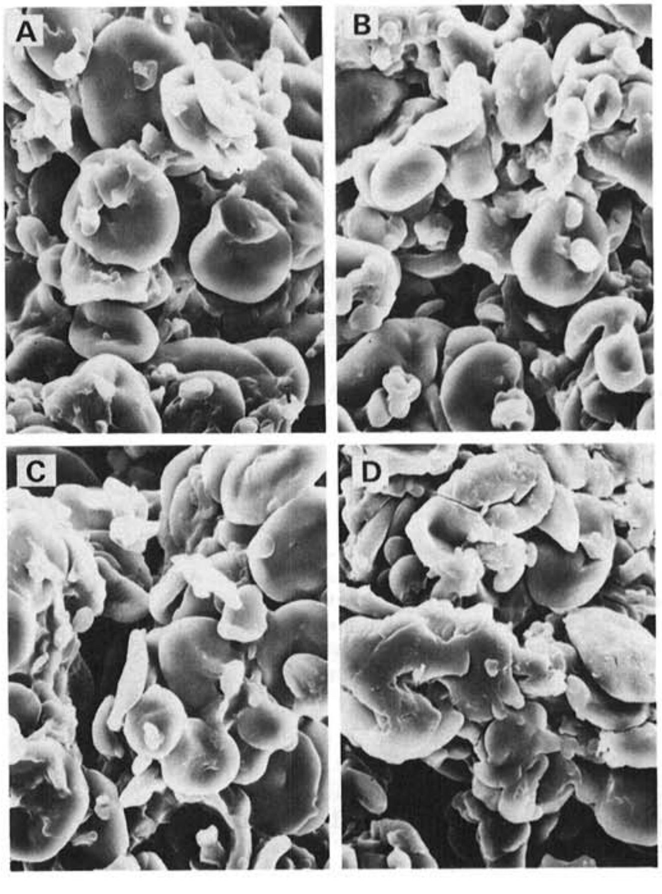

Fig. 6. The effects of oxidants on gelatinization of starch isolated from flour under scanning electron micrographs. **A,** Control starch; **B,** iodated starch; **C,** ascorbated starch; **D,** bromated starch.

starch fraction with regard to bromate reaction. The mechanism of the bromate reaction with starch remains to be clarified, but the production of keto or aldehyde groups might be expected.

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