EFFECTS OF SULFHYDRYL-BLOCKING AGENTS AND FLOUR IMPROVERS ON GASSING POWER, YEAST ACTIVITY, AND SOME FLOUR ENZYME SYSTEMS[1]

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ABSTRACT

The study was conducted to evaluate inhibitory effects of sulfhydryl (–SH)-blocking agents and flour improvers on the gas production (gassing power) of yeast with flour-water dough. The three –SH-blocking agents, N-ethylmaleimide, iodoacetic acid, and *p*-hydroxymercuribenzoate, all could inhibit the gas production markedly at the addition levels of 135, 405, and 1,215 p.p.m. (on flour basis). However, of the common flour improvers including bromate, iodate, acetone peroxides, ascorbic acid, and azodicarbonamide, tested at the levels of 5 to 1,215 p.p.m., only iodate clearly exhibited an inhibitory effect on the gas production. The gas production was reduced to a greater extent by iodate with glucose and ammonium phosphate than with dough, indicating that iodate could inhibit yeast activity directly and that flour –SH and other oxidizable components could dilute this inhibition. In addition, flour enzyme systems involving diastatic and proteolytic activities were also inhibited by iodate; the inhibition of these enzymes could also be directly and indirectly responsible for the decrease in gas production.

The activities of a great number of enzymes can be inhibited by sulfhydryl(–SH)-blocking agents. Flour improvers are known to oxidize –SH groups of proteins in dough (1–4). It is then reasonable to assume that these flour improvers could also oxidize –SH groups of enzymes in dough and in yeast and inactivate those with –SH groups essential for their activities. As a result of such inhibition, yeast fermentation rate in dough might be reduced and so would gas production and loaf volume. The level of improvers used in the continuous dough processes introduced in recent years is often four or five times higher than that formerly used in most conventional processes; it thus becomes increasingly important to examine any inhibitory effect that the large addition of improvers may exert on yeast fermentation in dough.

An interesting study of Lee and Reynolds (5) on effects of –SH-blocking agents on gassing power of yeast and on loaf volume demonstrated that –SH-blocking agents, including maleimide (MI), N-ethylmaleimide (NEMI), and N-phenylmaleimide (NPMI), exhibited an inhibitory effect on the gas production. The inhibition corresponded closely with a decrease in loaf volume. However, their gassing-power values were obtained from mixtures of 3.0 g. yeast, 10.0 g. sucrose,

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and 10.0 ml. of 25% ethanol containing one of the –SH-blocking agents. Results obtained with such mixtures may deviate from those with dough. Furthermore, the action of –SH-blocking agents may be different from that of flour improvers. Thus it seemed desirable from scientific and practical standpoints to steady (a) whether –SH-blocking agents and flour improvers exert any inhibitory action on the gassing power of yeast with dough as a substrate; and (b) if they do, whether the inhibition is due to the inactivation of yeast or dough enzyme systems. Results of such a study are reported and discussed in this paper.

Materials and Methods

Reagents. All chemicals used in this study were reagent grade. Difco Bacto-hemoglobin was used as substrate for measuring proteolytic activity. Azodicarbonamide and acetone peroxides used in this study were in the form of Maturox (a starch mixture containing 10% azodicarbonamide) and an acetone peroxide blend (a starch mixture containing 10% hydrogen peroxide equivalent of acetone peroxides), kindly supplied by Wallace & Tiernan, Inc., and the Research Laboratory, J. R. Short Milling Co., respectively.

Flour. An untreated straight-grade flour milled from a blend of Canadian hard red spring wheat was used throughout the study. The protein (N × 5.7) and ash contents of the flour were 14.0 and 0.48% (14% moisture basis), respectively. The –SH content was 1.03 μeq. per g. of flour.

Analytical Methods. Unless otherwise indicated, gassing power was measured on a dough made up of 10.0 g. flour, 7.0 ml. yeast suspension containing 0.3 g. yeast (Fleishmann cake, 71.4% moisture), and 0.33 ml. of a solution containing one of the agents by the AACC pressuremeter method using a modified pressuremeter (6). Azodicarbonamide and acetone peroxides, however, were added directly to flour. Pressure in mm. for total gas evolved was taken every hour during fermentation. The amount of agent used was calculated on a flour (14% moisture) basis and expressed in p.p.m.

Diastatic activity was determined on a 5-g. sample by the AACC method (7). Results were reported as mg. maltose per 10 g. flour. For the inhibition tests, iodate was added to flour suspension before and after the incubation at 30°C. for 1 hr. The titration value, obtained from the one with iodate added after the incubation, was used as the control.

Proteolytic activity was assayed according to the AACC method (a modified Ayre-Anderson hemoglobin method) (7). For the experiments

on the inhibitory effect of flour improvers, a mixture containing 5 g. flour and the required amount of azodicarbonamide or acetone peroxides was mixed well with 10 ml. buffer. Iodate, bromate, and ascorbic acid were dissolved in 10 ml. buffer and added to the sample. The mixture was allowed to stand for 30 min. at room temperature with swirling every 10 min. Then hemoglobin, pumice, and an additional 40 ml. buffer were added (previously warmed to 40°C. in a water bath), and the mixture was agitated to ensure uniform suspension before being placed in a 40°C. bath.

All analytical results reported are average figures obtained from duplicate tests. Most experiments were repeated at least once, in order to give more accurate and confirmative data.

Results and Discussion

Effect of –SH-Blocking Agents on Gassing Power. Figure 1 presents data on gassing power obtained from a mixture prepared according to Lee and Reynolds (5). It is evident that 25% ethanol can inhibit gas production substantially and mask the effect of NEMI when added at 5, 15, 45, or 135 p.p.m. (on sugar basis). However, at 405 and 1,215 p.p.m., NEMI does show an inhibitory effect on the gas production, as

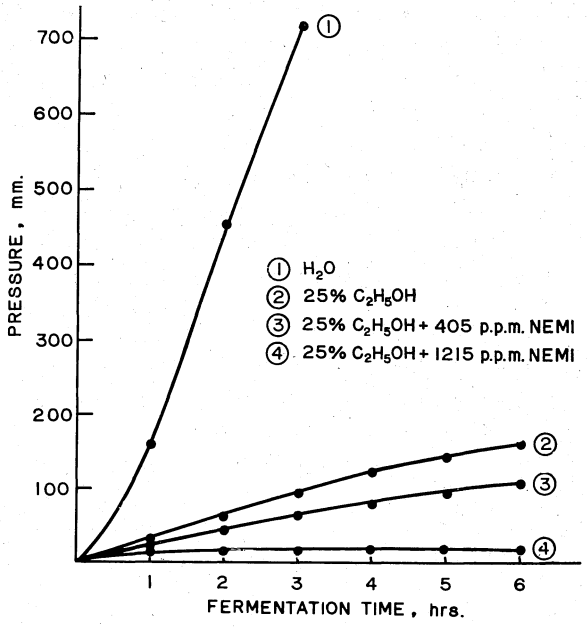

Fig. 1. Effect of N-ethylmaleimide (NEMI) and ethanol on gassing power of yeast with sucrose as substrate.

shown in the graph. The results concerning the effect of NEMI confirm the finding of Lee and Reynolds (5).

To obtain more convincing evidence to illustrate the inhibitory action of –SH-blocking agents, experiments were then extended to measure gas production of flour-water dough according to the AACC method. Since NEMI is only slightly soluble in water, the low levels (5, 15, and 45 p.p.m.) of NEMI are added as solution to flour; for the high levels (135, 405, and 1,215 p.p.m.) of NEMI, the calculated amounts of NEMI are mixed directly with flour and water. However, it should be pointed out that some solid NEMI, when added at the high levels, may remain in dough because of its low solubility.

Figure 2, A, shows that NEMI, when added to flour at 135, 405, and 1,215 p.p.m., definitely inhibits gas production. However, no inhibition was discernible when 5, 15, or 45 p.p.m. of NEMI was used. The results were confirmed by using two other –SH-blocking agents, iodoacetic acid (IA) and *p*-hydroxymercuribenzoate (sodium salt) (PHMB): like NEMI, they all exert an inhibitory action on the gas production at the addition levels of 135, 405, and 1,215 p.p.m., as shown in Fig. 2, B and C. It is evident from Fig. 2 that on a weight basis PHMB is less effective, as an inhibitor, than NEMI or IA. Although the concentrations of these agents are not expressed in terms of moles, it is conceivable, upon comparison of the inhibitory effects of 135 p.p.m. NEMI or IA and 405 p.p.m. PHMB, that NEMI or IA still inhibits the fermentation to a greater extent than does PHMB, even if the comparison is made on a molar basis. The comparatively low inhibitory effect of PHMB could be due to a number of factors. The obvious one, as shown in Fig. 2, is that the initial fermentation rate for PHMB-treated doughs is faster than that for NEMI- or IA-treated doughs. The rate difference indicates that –SH groups of yeast and/or dough may not be as readily accessible to PHMB as to NEMI or IA.

Effect of Flour Improvers. In view of the inhibitory action of the –SH-blocking agents, further experiments were then conducted to ascertain whether flour improvers exert any inhibitory effect on gassing power. The improvers used were bromate, iodate, ascorbic acid, azodicarbonamide, and acetone peroxides — all commonly used in milling and baking industries. The inhibition tests were carried out in the following ways: (a) An improver, either in solution or in powder form, was mixed directly with flour and yeast suspension for 2 min. before incubation. (b) An improver was mixed with yeast suspension first for 2 min., and allowed to stand for 30 min. at room temperature; the mixture was then added to flour and mixed together for incubation.

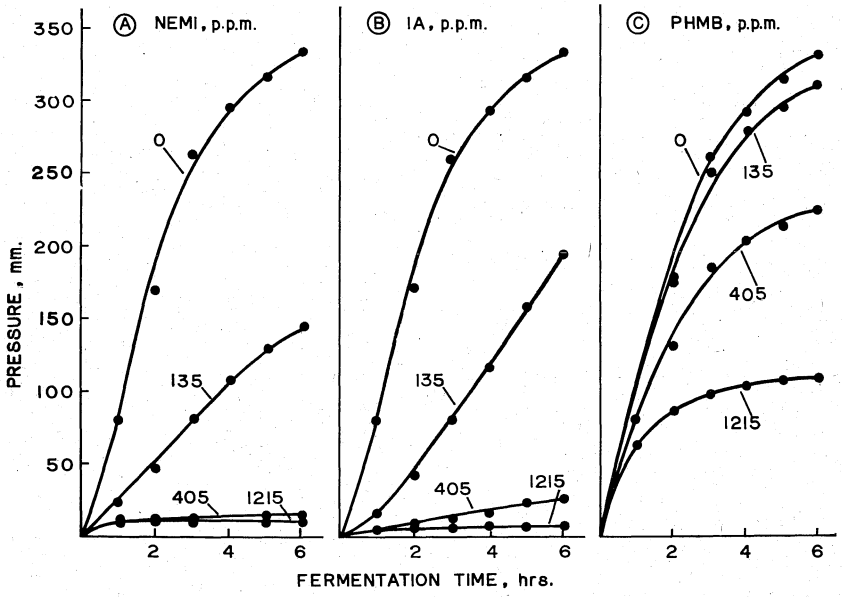

Fig. 2. Effect of N-ethylmaleimide (NEMI), iodoacetic acid (IA), and *p*-hydroxymercuribenzoate (PHMB) on gassing power of yeast in dough.

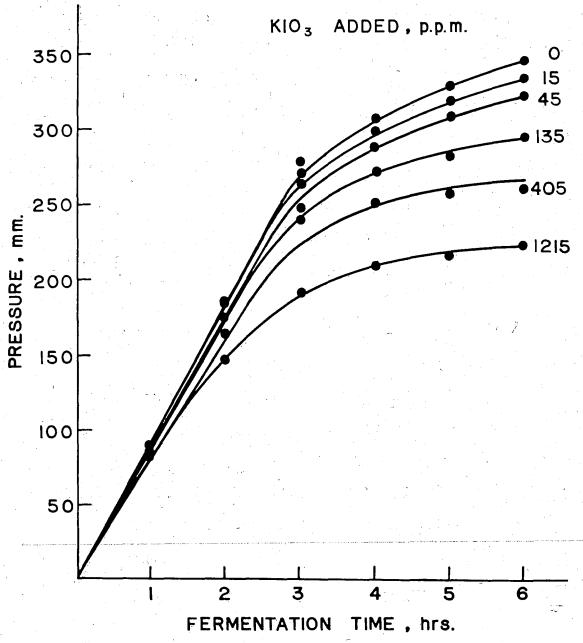

Fig. 3. Effect of potassium iodate on gassing power of yeast in dough.

Of all these improvers tested at a concentration range from 5 to 1,215 p.p.m. by both methods, only iodate clearly shows an inhibitory effect on gas production (see Fig. 3). No marked difference is observed for gas production whether iodate is mixed with yeast and flour directly or mixed with yeast first and then with flour. Further, no inhibitory effect is observed for potassium iodide on gassing power, when it is checked under the same experimental conditions.

On the basis of the results presented in Figs. 2 and 3, it is evident that the –SH-blocking agents are stronger inhibitors than iodate, probably because they are more specific for –SH groups than iodate; iodate is an oxidizing agent which could oxidize other readily oxidizable compounds besides –SH groups in dough and yeast.

Inhibitory Action of Iodate on Yeast Activity. Since iodate could inhibit gas production, further studies were pursued to establish whether iodate inhibits yeast or dough enzyme systems, or both. To eliminate the effect of flour, D-glucose and ammonium phosphate (monobasic) were used instead of flour as a substrate for determining gassing power. Figure 4 shows the gas production obtained from a mixture of 0.5 g. glucose, 0.1 g. ammonium phosphate, 7.0 ml. yeast suspension (0.3 g. yeast), and 0.33 ml. iodate. The levels of iodate used

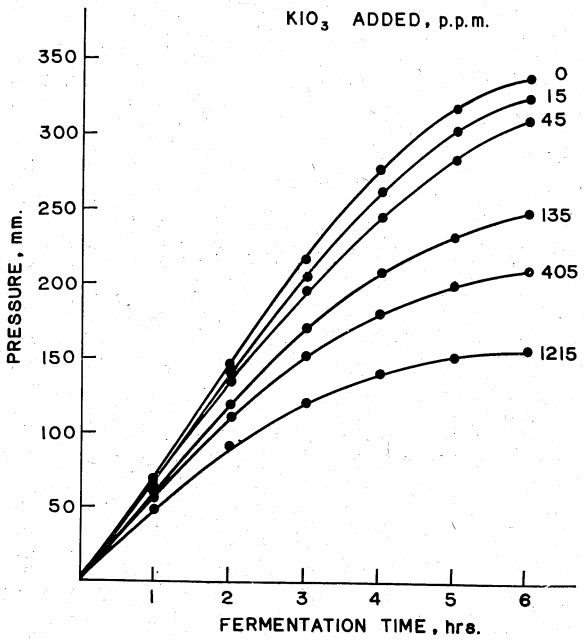

Fig. 4. Effect of potassium iodate on gassing power of yeast with glucose.

were the same as those used for flour. The graph shows that the increments of iodate cause a progressive decrease in gas production; this indicates that the iodate exerts an inhibitory action on yeast directly.

A comparison of the gassing power of yeast with glucose and with flour (see Figs. 3 and 4) reveals that iodate inhibits the gas production to a greater extent with sugar than with flour. The difference is presumably due to the fact that in flour there are various –SH and other oxidizable compounds which could react with iodate and dilute its inhibition.

Iodate Inhibition on Flour Enzyme Systems. In addition to yeast, the diastatic activity of flour of course can influence gas production. A study was made to examine the effect of iodate on the diastatic activity of flour.

Results, presented in Fig. 5, show that iodate does reduce maltose formation. The reduction indicates that iodate can inhibit activity of either alpha- or beta- or both amylases in flour.

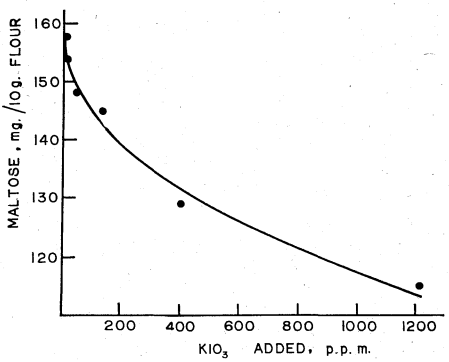

Fig. 5. Inhibition of flour diastatic activity by potassium iodate.

Following the inhibition test on diastatic activity, it was desired to know whether iodate could also inhibit proteolytic activity of flour. Proteolytic activity, though it is not closely associated with the gas production from fermentation, is related to dough development during mixing and fermentation. In the literature there are several papers dealing with the inhibition of flour proteases (8). Particularly, Jørgensen (9,10) and Balls and Hale (11,12) found that flour proteases could be inhibited by bromate, iodate, persulfate, and ascorbic acid with gelatin or autolysis tests. In fact, they proposed, on the basis of their findings, that the improving effect of flour improvers could be explained by their inhibitory actions on flour proteases. Therefore it also seemed desirable to re-examine the inhibitory effect of other improvers

in addition to iodate on proteolytic activity of flour with the AACC (hemoglobin) method. All presently used improvers such as bromate, iodate, ascorbic acid, acetone peroxides, and azodicarbonamide were included in this test.

Of all these improvers studied at addition levels from 5 to 405 p.p.m., only iodate has inhibitory action on the proteolytic activity. The inhibition increases with increasing concentrations of iodate added, as shown in Fig. 6. The finding that iodate inhibits proteolytic activity agrees well with those of others (13,14). However, our results on the effects of bromate and ascorbic acid do not accord with those of Jørgensen or Balls and Hale. The discrepancy, in part, is probably due to the differences in testing methods for measuring proteolytic activities.

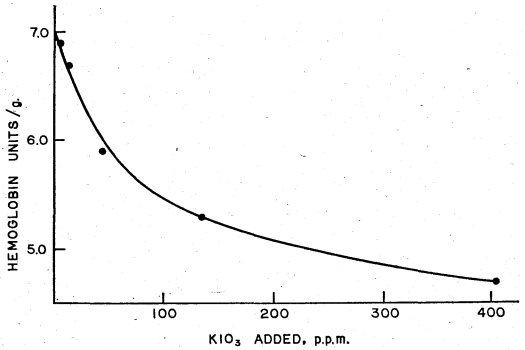

Fig. 6. Inhibition of flour proteolytic activity by potassium iodate.

General Discussion

Though it is reasonable to speculate that flour improvers, like –SH-blocking agents, could inactivate yeast and some enzyme systems in dough, it is interesting to note from this study that the common flour improvers, with the exception of iodate, do not have any inhibitory effect on the gas production even at an addition level as high as 1,215 p.p.m. In practice an improver is rarely used over 75 p.p.m. Since the amount of improver added to flour is minute, and since various –SH compounds in flour can react with the added improver and dilute its inhibitory action (see Figs. 3 and 5), it is apparent that these flour improvers used at the levels presently employed by the milling and baking industries will not exert any inhibitory effect on fermentation.

Iodate, on the other hand, exerts inhibition on the gas production discernibly even when the amount of iodate added is as low as 15 p.p.m. It is not clear why iodate is more powerful, as an inhibitor,

than the other improvers; but several contributing factors may deserve consideration. Iodate is a fast –SH-oxidizing agent; equivalently it can oxidize more –SH groups in dough than the others (1,2,4); and, like *o*-iodosobenzoate, iodate may also oxidize other groups essential for the enzyme activity (15).

The inhibitory action of iodate on the gas production is primarily due to the inactivation of yeast and of the diastatic activity of dough (see Figs. 4 and 5). There are a number of yeast enzymes such as alcohol dehydrogenase, aspartic dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase, which are known to be inhibited by –SH-blocking agents (16). The reduction of yeast activity is most likely the result of the inhibition of these and other enzymes in yeast by iodate. In flour, other enzymes besides those involving diastatic and proteolytic activities could also be inhibited by iodate.

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