EFFECT OF VARIOUS CONCENTRATIONS OF PAPAIN AND POTASSIUM IODATE ON THE LOAF VOLUME OF BREAD

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INTRODUCTION

The mechanism of the action of chemical bread improvers has recently been the subject of wide discussion. Jørgensen (9, 10, 11, 12, 13) and Balls and Hale (2, 3) have advanced the theory that the improvement of baking quality caused by oxidizing agents, such as potassium bromate and potassium iodate, is due to the fact that they inactivate the proteinases of flour, thereby inhibiting the action of these enzymes on the flour proteins. These writers also believe that the flour proteinases belong to the papain group.

This theory is supported by Flohil (6) and is opposed by Read and Haas (16, 17, 18), Carbonnelle (5), and Ritter (19). The arguments for and against the theory have been fully and ably summed up by the opposing sides in recent articles (12, 13, 14, 18).

In studies on the total and free amylase content of ungerminated seeds, baking tests were made on a sample of commercial wheat flour to which were added various concentrations of papain and potassium iodate. The results, reported here, may throw some added light on the disputed theories with regard to the mechanism of action of certain bread improvers.

PROCEDURE

The baking tests were conducted according to the standard procedure of the American Association of Cereal Chemists (1). The respective quantities of papain and potassium iodate specified in table 1 were added to the water (60 cc. per 100 gm. of flour) before it was mixed with the flour. The concentrations, percentage as well as molecular, are given on the basis of the water added. These tests were carried out in three series of bakings on different days. The respective concentrations of papain were the same in all three series. They ranged from 0.0005 to 0.01 percent, or from 0.3 to 6.0 mg. per 100 gm. of flour. Balls and Hale, who were the first to obtain a depression in the loaf volume of bread with papain, used 50 mg. per 315 gm. of flour. The concentrations of the potassium iodate solution, however, varied in the different series. They ranged from 0.00002 to 0.00008 molar or from 0.25 mg. to 1.0 mg. per 100 gm. of flour.

RESULTS

Table 1 and figures 1, 2, and 3 deal only with the loaf volume of the bread. The data in table 1 represent the products of the longitudinal and transverse circumferences of the loaves and serve only as a numerical expression of the relative volume of breads obtained with the vari-

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1 Received for publication August 16, 1941.
2 Italic numbers in parentheses refer to Literature Cited, p. 151.
ous concentrations of papain with and without potassium iodate. The measurement data and the photographs are in good agreement.

In the first series of bakes (fig. 1), the concentration of the potassium iodate solution was 0.00008 molar (1 mg. in 100 gm. of flour). Without potassium iodate the loaf volume consistently decreased with the increased concentration of papain. The greatest depressions, caused by the concentrations of 0.01 and 0.005 percent of papain (6 and 3 mg. per 100 gm. of flour, respectively) were overcome to a large extent by the potassium iodate. But with the concentrations of 0.002 and 0.001 percent of papain (1.2 and 0.6 mg. and 0.05 mg., respectively).
respectively per 100 gm. of flour), the loaf volume was less with potassium iodate than that of the bread made without potassium iodate. This would indicate that these low concentrations of papain were not sufficient to overcome the depressing effect of an 0.00008 molar potassium iodate solution. That some concentrations of potassium iodate added singly depress the loaf volume of bread is shown by the work of Jørgensen (9) and by unpublished results of this laboratory.

The concentrations of potassium iodate that evidently caused a depression in the loaf volume of bread in these experiments are much lower than those that caused a depression in Jørgensen's tests. The most likely explanation of the discrepancy is that different flours were used in the two sets of experiments.

In the second series the concentration of the potassium iodate solution was 0.00004 molar (0.5 mg. in 100 gm. of flour) or half that of the first series. Here, too, the results show that the depression in loaf volume was directly related to the concentration of papain. It is further shown that the concentration of 0.00004 molar of iodate was not so efficient in counteracting the depressing effect of 0.01 and 0.005 percent of papain (6 and 3 mg. per 100 gm. of flour) as the concentration of iodate used in the first series but it completely overcame the depressing effect of 0.002 percent of papain. These results indicate that the concentrations of 0.001 and 0.0005 percent of papain (0.6 and 0.3 mg. per 100 gm. of flour) were not strong enough to counteract the depressing effect of this concentration of potassium iodate.
Table 1.—Loaf volume of bread obtained with various concentrations of papain and potassium iodate

<table>
<thead>
<tr>
<th>Concentration of papain solution (percent)</th>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without potassium iodate</td>
<td>With a 0.00008 molar solution of potassium iodate</td>
<td>Without potassium iodate</td>
<td>With a 0.00004 molar solution of potassium iodate</td>
</tr>
<tr>
<td>None</td>
<td>199</td>
<td>209</td>
<td>216</td>
</tr>
<tr>
<td>.01</td>
<td>156</td>
<td>160</td>
<td>165</td>
</tr>
<tr>
<td>.005</td>
<td>162</td>
<td>162</td>
<td>177</td>
</tr>
<tr>
<td>.002</td>
<td>177</td>
<td>182</td>
<td>197</td>
</tr>
<tr>
<td>.001</td>
<td>192</td>
<td>196</td>
<td>201</td>
</tr>
<tr>
<td>.0005</td>
<td>205</td>
<td>205</td>
<td>211</td>
</tr>
</tbody>
</table>

1 Longitudinal circumference multiplied by transverse circumference. The figures are numerical expressions of the relative volume of the loaves.

2 On the basis of water added. To obtain percentages on the basis of flour, multiply by 0.6.

3 1.0 mg. in 100 gm. of flour.

4 0.5 mg. in 100 gm. of flour.

In the third series, in which a 0.00002 molar (0.25 mg. in 100 gm. of flour) potassium iodate solution was used, the results consistently follow the same trend as in the two previous series. This weakest concentration of potassium iodate used completely counteracted the depressing effect of only the weakest concentration of papain.

The results of these three series of baking tests indicate clearly that potassium iodate, which is classed among the bread improvers, counteracts the depressing effect of an added proteinase on the loaf volume of bread. Flohil (6) was able to overcome the depression in loaf volume of bread caused by papain by adding potassium bromate, another bread improver. Read and Haas (17) admit that potassium bromate inhibits the proteolytic action of papain but doubt whether the wheat proteinases belong to the papain group. The evidence in the literature indicates that at least some of the proteinases of wheat belong to the papain group (3, 4).

The results also show that under certain conditions the proteinases in flour may be beneficial, because they tend to overcome the depressing effects of oxidizing agents, such as potassium iodate. Possibly flour contains natural oxidizing substances, an excess of which would depress the loaf volume just as an excess of potassium iodate does. Under such conditions the addition of a proteinase might prove beneficial. Indeed, Read and Haas (16) found that the addition of proteinases had a beneficial effect on the baking qualities of flours that develop "bucky doughs."

Discussion

In the baking tests reported here the loaf volume was depressed by the added papain in direct relation to its concentration, the added potassium iodate counteracted this effect, and an excess of iodate in turn depressed the loaf volume. Since it is generally accepted that the loaf volume of bread is associated with the strength of the gluten, the logical interpretation of these findings would be that the papain attacked the proteins, destroying their strength, and that potassium...
iodate counteracted the effect of the papain. Consistent with this reasoning is the theory that potassium iodate, added in excess, depressed the loaf volume of the bread because it carried the inhibition of the proteinases too far, as a certain degree of proteolytic action is supposed to be necessary for the proper maturing of the gluten. This is essentially the theory advanced by Jørgensen (9, 13) and by Balls and Hale (2, 3).

Is a different interpretation of these facts possible?

It is known that papain liberates amylase in some ungerminated cereals (7). The tests reported here showed that papain added to the flour in the concentrations used in the baking tests also liberates amylase (table 2). Even the lowest concentrations of 0.0005 percent of papain had a pronounced effect in increasing the diastatic power of the flour. Potassium iodate in the concentration that counteracted the effect of papain and depressed the loaf volume of the bread in the baking tests (table 1) also depressed the diastatic activity of the flour (table 3). The extractions of the amylase in the last two experiments (tables 2 and 3) were carried out at about 70° F., according to the approximate procedure of the British Institute of Brewing (8). The effect of papain on the liberation of the flour amylase and the hydrolizing power of the latter, as well as the inhibiting effect of the potassium iodate, would be expected to be stronger at the temperature of dough ripening and of the first stages of baking (before the bread reaches the temperature of inactivation of the enzymes), than at 70° F. Therefore, while it is realized that the diastatic power of flour as determined by the Lintner method is not the only factor that conditions diastasis in dough, is it not still possible that the respective effects of the papain and potassium iodate on the loaf volume in these baking tests were due to their effect on the liberation or depression of the flour amylase, largely beta amylase? Indeed, Stamberg and Bailey (20) found that added beta amylase produced poorer loaves of bread than the controls. Kosmin (15) also found that excessive starch hydrolysis is injurious to the baking qualities of flour but attributed this effect to the alpha amylase. This is at variance with the results of Stamberg and Bailey, who found that the baking qualities of bread were improved by the addition of alpha amylase (20). Kosmin based her conclusions on results obtained with sprouted-wheat flour, while Stamberg and Bailey used separate alpha and beta amylase preparations.

### Table 2. Effect of various concentrations of papain on the development of diastatic power in flour

<table>
<thead>
<tr>
<th>Concentration of papain (percent)</th>
<th>3 hours</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
<th>144 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>26.5</td>
<td>25.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.5</td>
<td>120.5</td>
<td>120.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.01</td>
<td>111.1</td>
<td>117.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.005</td>
<td>89.3</td>
<td>117.7</td>
<td>119.0</td>
<td>115.0</td>
<td>120.5</td>
</tr>
<tr>
<td>.0025</td>
<td>66.7</td>
<td>85.5</td>
<td>109.0</td>
<td>104.2</td>
<td>110.2</td>
</tr>
<tr>
<td>.001</td>
<td>54.8</td>
<td>86.2</td>
<td>89.3</td>
<td>104.2</td>
<td>114.5</td>
</tr>
<tr>
<td>.0005</td>
<td>43.5</td>
<td>55.6</td>
<td>59.2</td>
<td>62.2</td>
<td>72.0</td>
</tr>
</tbody>
</table>

1 On the basis of water added.
The beneficial effect of natural aging of flour on the baking qualities of bread, which Balls and Hale attribute to its inhibitive effect on the flour proteinases, may similarly be explained by its effect on the diastatic power of the flour, for the results presented here (controls in tables 2, 3, and 4) indicate that the diastatic power of flour gradually declines with time. The changes involved are relatively small, but they have been consistently verified in many tests.

In the concentration commonly used in bread making, however, sodium chloride also has a strong stimulating effect on the diastatic activity of flour (table 4). Even one-twentieth of the salt concentration used in the baking test causes a distinct stimulation of the diastatic power of flour. The maximum stimulation caused by salt, however, does not reach that caused by papain, and there are other differences between the action of papain and that of salt, which will be discussed elsewhere. The 2 percent of sodium chloride used in these baking tests did not cause any depression in loaf volume, notwithstanding the fact that it stimulated the diastatic activity of the flour. This would seem to exclude the possibility of explaining the results of the baking tests reported here by the effects of papain and potassium iodate on the stimulation or inhibition of the diastatic power of flour.

**SUMMARY**

The results of baking tests show that there is a consistent interaction between various concentrations of papain and potassium iodate in their effect on the loaf volume of bread.

When added singly, some concentrations of potassium iodate and papain depress the loaf volume of bread, the extent of depression being directly related to the concentration. When added together, certain of these concentrations counteract each other.
While papain stimulates diastatic activity and potassium iodate depresses it, the action of these substances on the loaf volume of bread cannot be ascribed to their effect on the diastic power of the flour since sodium chloride, which is always used in bread baking, also stimulates diastatic activity, yet causes no depression in loaf volume.

The results seem to support the theory of the mechanism of action of bread improvers advanced by Jørgensen and Balls and Hale.

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