

USE OF LACTASE IN BREADMAKING¹

Y. POMERANZ, B. S. MILLER,² D. MILLER, AND J. A. JOHNSON

ABSTRACT

An acceptable bread can be obtained by using lactase and lactose without adding other sugars, provided a sufficiently high level of alpha-amylase is maintained. Lactases from bacterial and fungal sources exhibited similar properties, but the latter with their lower pH optimum were more suitable for use in panary fermentation. The action of lactase was not influenced by a variety of lactose-containing substrates. Possibilities of enhancing hydrolytic action of lactases by varying breadmaking formulations and schedules are outlined.

Milk is used in baking in many countries because it contributes to the nutritive value of wheat proteins. Nonfat milk powder, dry whey, and dry buttermilk are readily available and contain substantial amounts of lactose which is not fermentable by baker's yeast (4). Hydrolysis of lactose, however, by lactase produces glucose which is fermentable by yeast, and galactose which is not fermentable but which contributes to crust color through the Maillard reaction (7). Converting lactose to monosaccharides should improve substantially the quality of bread baked without adding fermentable sugars and might enhance toasting properties of bread baked by rich formulas employed in the U.S.

Lactase has been used to increase the sweetening properties of lactose through hydrolysis to hexoses, which are sweeter than the original substrate, and to counteract the undesirable effect of lactose crystallization in ice cream and cheese (11).

Browne (2), in connection with a study of the utilization of the lactose present in the milk products, followed, by means of gassing-power determinations, the effect of adding a lactose fermenter, *Torula cremoris*, in addition to baker's yeast. The authors know of no published study, however, regarding the use of lactase in breadmaking.

A number of lactases have been isolated from plants, molds, and bacteria (5) and also from the intestinal mucosa of mammals (12). Different β -galactosidases (lactases) vary in their activity, pH, and temperature optima. However, no information is available to indicate

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²Present address: General Mills, Inc., Central Research Laboratories, Minneapolis 27, Minnesota.

their action in baking. The objective of this investigation was to study the effect of bacterial and fungal lactase on gas production in doughs containing either lactose or milk products, and to determine the feasibility of using lactase preparations to supply sugars for panary fermentation.

Materials and Methods

A commercial unmalted hard red winter, straight-grade flour with a protein content of 11.9%, ash content 0.43%, and water absorption (as determined by the farinograph) of 64.9% was used for most of the experiments. A malted commercial hard red winter baker's patent flour was used in part of the preliminary experiments.

Six lactase preparations were used: a crude extract of β -galactosidase from *Escherichia coli* (3), a crude bacterial extract from *Paracolobactrum aerogenoides* containing 24 mg. of protein per ml. (1), a lyophilized extract of *E. coli* (8), a commercial, fungal crude extract of β -galactosidase from a lactase-producing yeast (*Saccharomyces fragilis*) (10), and two commercial dry products having optimal activity at pH 4.5 to 5.5 and 6.0 to 7.0, respectively, according to the suppliers. These latter preparations are designated as fungal lactase A and fungal lactase B. Fungal lactase A was a product of fungal origin from the *Aspergillus oryzae-niger* group; fungal lactase B was a product prepared from a number of lactose-fermenting yeasts of *Saccharomyces*, *Torulopsis*, and *Candida* groups. The lactases were tested by a procedure for evaluation of lactase preparations for use in breadmaking (6). Employing baker's yeast, a buffered nitrogen base medium, and the glucose which was split off from lactose by lactase as the only source of energy for yeast fermentation, the amount of carbon dioxide evolved was measured manometrically, using ethyl lactate-filled pressure meters. The relative activities of the tested preparations (expressed in arbitrary lactase units per mg. dry weight) were: 1.4 for the crude extract of *E. coli*, 2.7 for the crude bacterial preparation from *P. aerogenoides*, 14.0 for the lyophilized extract of *E. coli*, below 1 for the yeast lactase, 10.5 for the fungal lactase A, and 2.7 for the fungal lactase B.

The lactose-containing substrates used were: lactose, analytical grade; and the following, both spray process and roller process: dry whole milk, nonfat dry milk, dry buttermilk, and dry whey.

Methods. Gassing-power determinations were made with Sandstedt-Blish pressuremeters (9) using 10 g. of flour and 0.5 g. of lactose (or 1.0 of nonfat dry milk, buttermilk, or whey). At 1-minute intervals the

following were added: 4 ml. of a yeast suspension (7.5 g. of baker's yeast suspended in 95 ml. of water) and 6 ml. of a lactase suspension. The contents were mixed thoroughly with a spatula, the lids screwed on tightly, and the pressuremeters placed in a water bath at 30°C. The pressure was released after 5 minutes of temperature equilibration and recorded at 1-hour intervals for 5 hours.

The baking experiments were on a laboratory scale employing the straight-dough procedure. The basic formula was:

| <i>Ingredients</i> | <i>Parts</i> |
|--------------------------------------|--------------|
| Flour | 100 |
| Water | variable |
| Yeast | 2 |
| Sucrose (when used) | 5 |
| Milk solids | variable |
| Malted wheat flour (when used) | 0.5 |
| Shortening | 3 |
| Sodium chloride | 2 |
| Arkady | 0.5 |

After mixing to optimum consistency, the doughs were fermented at 30°C. After 110 minutes the doughs were punched, and 50 minutes later punched again, divided, rested for 20 minutes, and moulded. The doughs were proofed at 30°C. for 45 minutes and baked for 25 minutes at 210°C. Variations in the ingredients used, or in the baking schedule are indicated, where applicable, in the following section.

Results and Discussion

The effects of adding various amounts of fungal lactase A on the gas production of flour to which dry spray-processed buttermilk and dry spray-processed whole milk were added are shown in Figs. 1 and 2, respectively. A similar relationship between the amounts of enzyme used and response, measured as pressure, was obtained for all the other active lactase preparations and milk products tested.

The rate of carbon dioxide formation in flour doughs supplemented with lactose and lactase was low. There was practically no increase in gas production during the first 2 hours of fermentation, despite an eightfold increase in lactase levels. Use of sugars readily available for yeast fermentation (sucrose or glucose) resulted in highly increased gas production almost from the beginning of fermentation. The effect of adding lactase becomes evident only in the later stages of fermentation. These findings are confirmed and discussed more fully in the section covering the baking investigations. As a result of pressuremeter determinations, the major part of the baking experiments employed the more potent fungal lactase A.

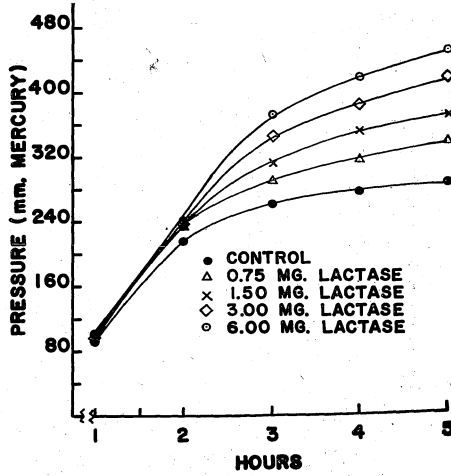


Fig. 1. Effect of adding various levels of fungal lactase A on gas production in a dough containing buttermilk.

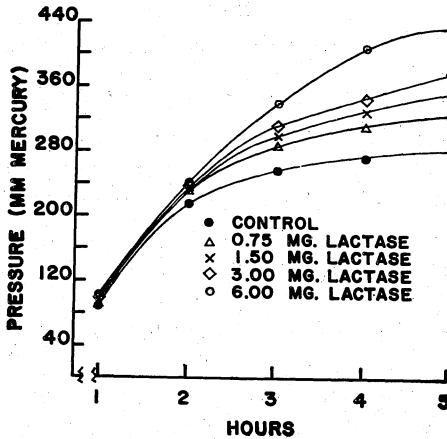


Fig. 2. Effect of adding various levels of fungal lactase A on gas production in a dough containing whole milk.

A comparison among the activities of four lactase preparations employing lactose as the substrate is given in Fig. 3. The lactase preparation from *S. fragilis* was inactive under conditions of the test. The third bacterial lactase (lyophilized extract of *E. coli*) was comparable in activity to the others, but the small amount of material available limited experimental data obtained for this preparation. It should be noted that both lactases from yeast were kept in storage for several years prior to their use in this study. It is likely that, in addition to the

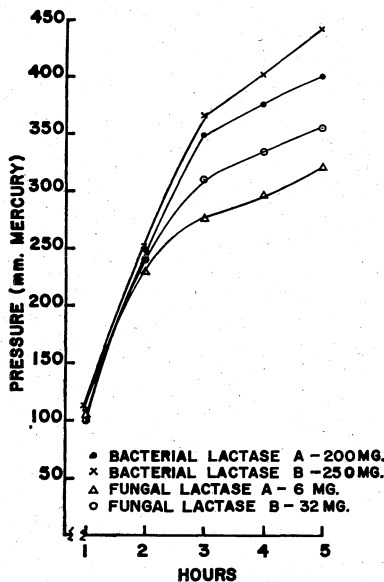


Fig. 3. Gas production in doughs containing lactose and various lactases.

fact that these enzymes have pH optima higher than those of fermented dough, the activity of these lactases, evaluated at their optimal pH values, decreased substantially as a result of long storage. On a comparative weight basis, the activity of the bacterial lactases was substantially lower than that of fungal lactase A. This may be due in part to fungal lactase A exhibiting a lower pH optimum than the bacterial lactases (about 5 for fungal lactase A and 7 for bacterial lactase). The lower pH optimum of fungal lactase A is more compatible with breadmaking conditions.

Preliminary results (Table I) showed that use of lactose (or whey or nonfat dry milk) plus lactase, with no sucrose in the formula, produced as good bread from commercial malted flour as that made with sucrose and nonfat dry milk. High levels of lactase (2% based on weight of lactose used) were beneficial. Essentially the same results were obtained by using unmalted flour. In this case, however, the addition of malt was necessary to produce an acceptable bread when sugar was used or when lactose and lactase were added. The absence of malt in a sucrose-containing mix resulted in a poorer, but still acceptable, loaf; however, the absence of malt in formulations containing lactose and lactase resulted in unacceptable bread (Table II and shown in Fig. 4).

The levels of lactase used were the lowest possible that still pro-

TABLE I
EFFECT OF VARIATIONS IN BREAD FORMULATIONS ON BREAD QUALITY OF
LACTOSE-CONTAINING MIXES

| No. | TREATMENT | BREAD CHARACTERISTICS | | | | | | | Total Score |
|-----|--|-----------------------|-------------|----------|---------------|-------|-------------|---------|-------------|
| | | Loaf Score | Crust Color | Symmetry | Break & Shred | Grain | Crumb Color | Texture | |
| | (Maximum score) | 20 | 10 | 10 | 10 | 20 | 10 | 20 | 100 |
| 1 | Control | 18 | 4 | 8 | 9 | 15 | 9 | 15 | 78 |
| 2 | 5.0% Sucrose | 18 | 8 | 8 | 8 | 15 | 10 | 16 | 83 |
| 3 | 4.0% Nonfat dry milk | 17 | 7 | 6 | 7 | 16 | 9 | 14 | 76 |
| 4 | 5.0% Sucrose, 4.0% nonfat dry milk | 19 | 9 | 9 | 7 | 19 | 10 | 18 | 91 |
| 5 | 4.0% Nonfat dry milk, 0.05% FLA ^a | 17 | 8 | 7 | 8 | 17 | 10 | 16 | 83 |
| 6 | 4.0% Nonfat dry milk, 0.25% FLA | 18 | 9 | 8 | 8 | 18 | 9 | 16 | 86 |
| 7 | 5.0% Lactose | 17 | 7 | 6 | 5 | 16 | 10 | 14 | 75 |
| 8 | 5.0% Lactose, 0.05% FLA | 18 | 9 | 8 | 7 | 17 | 10 | 16 | 85 |
| 9 | 5.0% Lactose, 0.25% FLA | 18 | 9 | 9 | 9 | 18 | 9 | 17 | 89 |
| 10 | 4.0% Dry whey | 18 | 8 | 7 | 8 | 16 | 10 | 16 | 83 |
| 11 | 4.0% Dry whey, 0.05% FLA | 19 | 9 | 9 | 9 | 18 | 9 | 17 | 90 |
| 12 | 4.0% Dry whey, 0.25% FLA | 18 | 9 | 8 | 9 | 18 | 10 | 17 | 89 |

^a FLA = fungal lactase A.

duced acceptable bread. Higher levels could be used but would probably be economically prohibitive. The main value of using lactase would seem to be to maintain sufficient fermentable sugar in doughs during proofing. In addition, the reducing sugars would contribute to crust browning during the baking stage. A straight-dough formula was used in this study. The use of lactase in sponge-dough procedures or in baking involving long fermentation schedules probably would be more advantageous than in short-term straight-dough formulations.

The rate of hydrolysis of lactose did not appear sufficiently high to allow yeast to ferment glucose at an optimal rate. Increasing proof time (from 45 to 60 minutes) of doughs containing lactose and lactase resulted in an increase in loaf volume of 5 to 8%. Increasing proof time probably is not economically feasible. The lactase activity is higher at more elevated temperatures (6). Higher hydrolysis rates are attained at 40°-45°C. Panary fermentation temperatures of about 30°C. are common. Occasionally, however, temperatures as high as 45°C. are used in proofing. As the rate of lactose hydrolysis is slow, it is feasible that a hydrolyzed skimmilk powder, or the use of lactase in pre-ferments, rather than adding lactase to the dough, would be advantageous.

TABLE II

EFFECT OF SOURCE OF LACTOSE ON HYDROLYSIS BY LACTASES IN BREADMAKING

| No. | TREATMENT | BREAD CHARACTERISTICS | | | | | | | |
|-----|------------------------|-----------------------|-------------|-----------|---------------|-------|-------------|----------|-------------|
| | | Loaf Score | Crust Color | Sym-metry | Break & Shred | Grain | Crumb Color | Text-ure | Total Score |
| | (Maximum score) | 20 | 10 | 10 | 10 | 20 | 10 | 20 | 100 |
| 1 | 0.5% Malt | 17 | 4 | 8 | 8 | 15 | 9 | 15 | 76 |
| 2 | 5.0% Sucrose, | | | | | | | | |
| | 0.5% malt | 17 | 7 | 9 | 8 | 15 | 9 | 17 | 82 |
| 3 | 5.0% Sucrose, 0.5% | | | | | | | | |
| | malt, 5.0% nonfat | | | | | | | | |
| | dry milk | 17 | 8 | 8 | 7 | 18 | 9 | 19 | 86 |
| 4 | 5.0% Sucrose | 17 | 6 | 7 | 6 | 12 | 9 | 14 | 71 |
| 5 | 5.0% Sucrose, | | | | | | | | |
| | 5.0% galactose | 16 | 9 | 8 | 7 | 14 | 9 | 15 | 78 |
| 6 | 10.0% Lactose | 10 | 5 | 3 | 2 | 10 | 7 | 8 | 45 |
| 7 | 10.0% Lactose, | | | | | | | | |
| | 0.20% FLA ^a | 15 | 6 | 5 | 6 | 14 | 8 | 13 | 67 |
| 8 | 10.0% Lactose, 0.20% | | | | | | | | |
| | FLA, 0.5% malt | 16 | 7 | 7 | 8 | 17 | 9 | 17 | 81 |
| 9 | 5.0% Lactose | 10 | 5 | 3 | 2 | 10 | 7 | 8 | 45 |
| 9A | 5.0% Lactose, | | | | | | | | |
| | 0.10% FLA | 12 | 9 | 7 | 6 | 10 | 6 | 10 | 60 |
| 10 | 5.0% Lactose, 0.10% | | | | | | | | |
| | FLA, 0.5% malt | 17 | 7 | 6 | 8 | 15 | 9 | 17 | 79 |
| 11 | 5.0% Nonfat dry | | | | | | | | |
| | milk (NFDm) | 9 | 5 | 3 | 2 | 10 | 8 | 8 | 45 |
| 11A | 5.0% NFDm, | | | | | | | | |
| | 0.05% FLA | 11 | 7 | 5 | 6 | 10 | 6 | 10 | 55 |
| 12 | 5.0% NFDm, 0.05% | | | | | | | | |
| | FLA, 0.5% malt | 16 | 7 | 9 | 8 | 17 | 9 | 17 | 83 |
| 13 | 5.0% Dry | | | | | | | | |
| | whole milk | 8 | 5 | 3 | 2 | 8 | 7 | 8 | 41 |
| 14 | 5.0% Dry whole | | | | | | | | |
| | milk, 0.05% FLA | 9 | 6 | 9 | 8 | 13 | 8 | 16 | 69 |
| 15 | 5.0% Dry whey | 9 | 5 | 3 | 2 | 10 | 6 | 8 | 43 |
| 15A | 5.0% Dry whey, | | | | | | | | |
| | 0.05% FLA | 11 | 7 | 6 | 5 | 11 | 6 | 9 | 56 |
| 16 | 5.0% Dry whey, | | | | | | | | |
| | 0.05% FLA, | | | | | | | | |
| | 0.5% malt | 15 | 6 | 7 | 8 | 14 | 7 | 15 | 72 |
| 17 | 5.0% Buttermilk | 8 | 5 | 3 | 2 | 10 | 8 | 8 | 44 |
| 17A | 5.0% Buttermilk, | | | | | | | | |
| | 0.05% FLA | 11 | 7 | 6 | 5 | 11 | 6 | 9 | 55 |
| 18 | 5.0% Buttermilk, | | | | | | | | |
| | 0.05% FLA, | | | | | | | | |
| | 0.5% malt | 18 | 6 | 9 | 9 | 17 | 9 | 16 | 84 |
| 19 | 5.0% Lactose, | | | | | | | | |
| | 0.5% malt, | | | | | | | | |
| | 1.0% BLA ^b | 18 | 9 | 8 | 9 | 16 | 10 | 18 | 88 |
| 20 | 5.0% Lactose, | | | | | | | | |
| | 0.5% malt, | | | | | | | | |
| | 1.0% BLB ^c | 18 | 8 | 6 | 8 | 14 | 10 | 16 | 80 |

^aFLA = fungal lactase A.^bBLA = bacterial lactase A.^cBLB = bacterial lactase B.

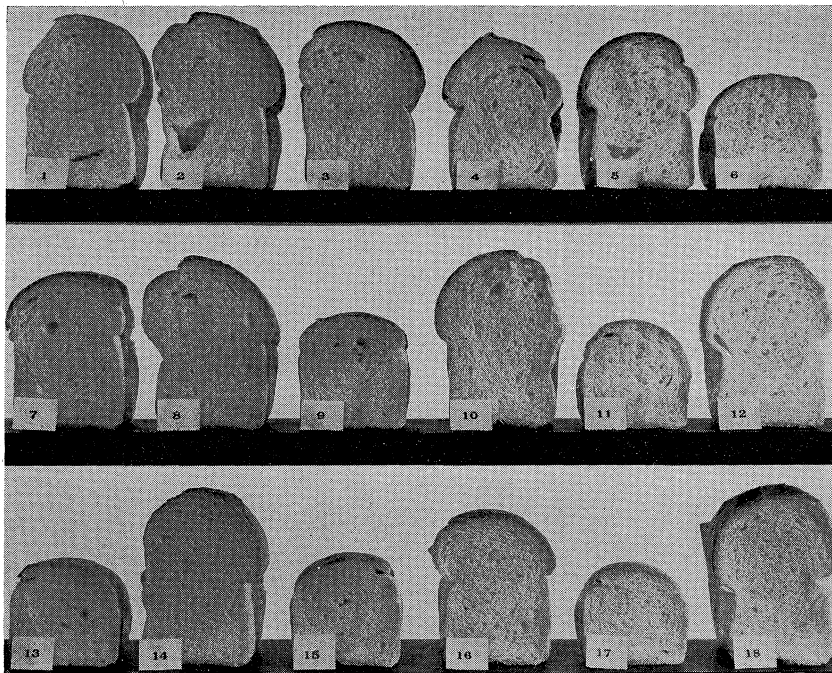


Fig. 4. Bread made with lactose or various milk products and employing both malted and unmalted flour. The numbers correspond to those given in Table II.

The results of these preliminary investigations are limited to a survey of appropriate sources of lactase and their compatibility with panary fermentation under experimental baking conditions. The results seem to warrant a broad investigation regarding possible use of lactase in bread manufacture.

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³Mention of firm or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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