RESEARCH PAPERS

Properties of β-Galactosidase of *Saccharomyces lactis* in Milk and Milk Products

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ABSTRACT

Lactase (β-galactosidase) from *Saccharomyces lactis* was tested on skim milks and wheys to determine optimum conditions for converting lactose to monosugars. Emphasis was on parameters of interest to processors. Lactase effectively can hydrolyze lactose in skim milk, whey, and a 41% total solids concentrate of skim milk; the hydrolysis rate for milk concentrates is 15% lower than that of unconcentrated skim milk. The optimum pH for lactose hydrolysis is 6.5, which is close to the pH of milk. Heating lactase for 1 min results in 97% inactivation at 60 C and complete inactivation at 70 C. Some inactivation occurs at 40 C over 1-h incubation. Lactose can be hydrolyzed in 22 h at 5 C as effectively as in 2 h at 31 C. Potassium, magnesium, and manganese ions accelerated slightly lactase activity in fluid milks while sodium and calcium ions inhibited the reaction significantly.

INTRODUCTION

Treatment of milk with lactase (β-D-galactoside/galactohydrolase; EC 3.2.1.23) converts lactose to glucose and galactose, thus making the milk more suitable for lactose intolerant individuals (2). In addition, enzymatic hydrolysis of lactose increases the stability of frozen 3:1 concentrated whole milks (6, 14) and reduces sandiness in ice creams (1, 5, 6, 11). Recently, lactase has been used in the processing of milks for yogurt (7), cottage cheese (7), and cheddar cheese (13). In frozen products hydrolysis of lactose prevents crystallization of the milk sugar because of the greater solubility of the monosaccharides. In preparation of cultured products, advantages include increased rate of acid development, increased yield of cottage cheese, and reduced aging time for cheddar cheese. The potential benefits of hydrolyzing lactose in preparing dairy products are numerous.

For feasible processing of dairy products, a lactase preparation of high activity and bland taste is required. Commercial preparations of lactases produced by microorganisms have become available for possible use for foods. Kosikowski and Wierzbicki (8) report that as little as 50 to 100 mg of lactase isolated from *Saccharomyces lactis* will hydrolyze 90 to 95% of the lactose in 1 liter of skim milk incubated 48 h at 4 C. No change in flavor other than sweetness was observed. The manufacturers of this enzyme suggest adding 300 mg of lactase per liter of whole milk and incubating for 2 h at 30 C to achieve 90% hydrolysis. The pH for optimum activity of the enzyme, 6.5 to 6.8, coincides with the pH of normal milk (3). It is recommended that pasteurization precede lactase treatment for optimum activity; this is especially important for cheese wheys in which high levels of starter organisms may decrease the pH to below optimum for lactase activity.

This study was undertaken to determine the properties of *S. lactis* lactase in skim milks and cheese wheys and optimum conditions for converting lactose to monosugars. This type of effort is valuable in defining the limits of enzyme activity for useful processing application.

MATERIALS AND METHODS

Materials

The *S. lactis* lactase ("Maxilact"[2] brand of 40,000 ONPG) was obtained from Gist-Brocades, Delft, Holland through the Enzyme Development Corporation of New York, NY. Raw and pasteurized (77 C, 16 s) fluid and

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[2] Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

concentrated skim milks were obtained from the Dairy Products Laboratory, USDA, Beltsville. Dried powders were prepared from pasteurized sweet whey and cottage cheese whey and reconstituted to 7% total solids.

Enzyme Assay

The *S. lactis* lactase was suspended in distilled water at a 3% concentration. The lactase was stored at 5 C for no longer than 3 days and retained over 97% of its activity during this period. Aliquots of the enzyme were added to pretempered fluid or concentrated milks at pH 6.6. At selected intervals, the reaction was stopped by adding 19 ml .15% $CuSO_4 \cdot 5H_2O$ to a 2 ml aliquot of the reaction mixture. Following the addition of 2.25 ml of .05 N NaOH, the contents were diluted to 100 ml and filtered. Aliquots were assayed for monosaccharides in the presence of lactose by the method of Tauber and Kleiner (12). Lactose in untreated milk was determined colorimetrically by the Folin and Wu method (4) with lactose as a standard. Results were expressed as the percentage lactose converted to monosaccharides (glucose and galactose).

Total Solids

Total solids of concentrated skim milks were determined by the Mojonnier procedure (9).

RESULTS

Effect of pH

Since casein is insoluble below pH 6, sweet whey rather than skim milk was used to determine the optimum pH for lactase activity. Lactase activity was optimum in reconstituted fluid whey at pH 6.5 (Fig. 1), the normal pH of milk. Below pH 5.9 enzymatic activity dropped rapidly.

Effect of Temperature on Inactivation of Lactase

The effect of heat on lactase activity in cottage cheese whey was measured to find the range of the temperatures needed to inactivate the enzyme. Heating lactase in whey (adjusted to pH 6.6) above 50 C for 1 min markedly reduced its activity (Fig. 2). Complete inactivation was evident when lactase was heated to 70 C for 1 min. Over 95% of the lactase activity

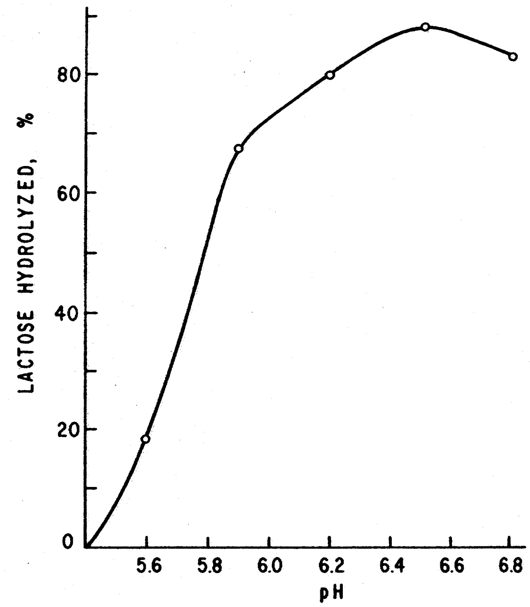

FIG. 1. Effect of the pH of reconstituted sweet whey on lactose hydrolysis by 300 mg *S. lactis* lactase/kg whey. The whey was adjusted to the indicated pH values with 2 N KOH or lactic acid. The lactase was incubated with the whey for 2 h at 30 C.

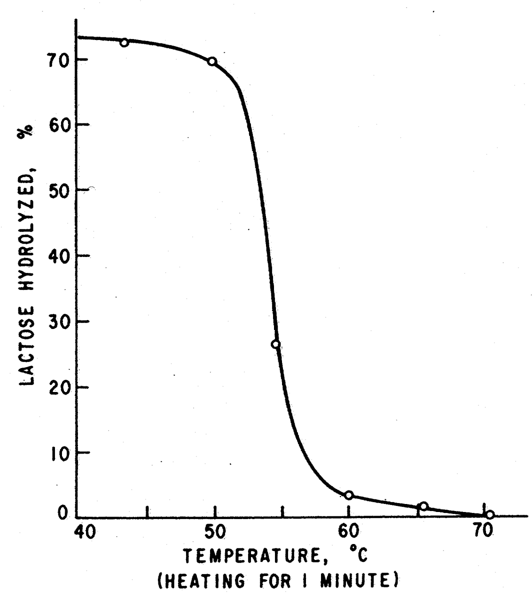

FIG. 2. The effect of heat treatment on the activity of 300 mg *S. lactis* lactase/kg reconstituted cottage cheese whey adjusted to pH 6.6 with 2 N KOH. The enzyme was added to 50 ml of whey; the reaction mixture was immediately and rapidly heated for 1 min, cooled, and incubated for 23 h at 7 C. The unheated control whey had 71% hydrolyzed lactose.

was destroyed by heating at 60 to 70 C for 1 min.

Effect of Temperature of Incubation and Time of Reaction

The effect of incubation temperature on lactase activity in skim milk is in Fig. 3. The optimum temperatures were 30 to 35 C for 2-h incubation, 35 C for 1-h, and 35 to 40 C for .5 h. The optimum temperature for lactase activity decreased when the incubation time was increased from 30 min to 2 h. Lactase was inactivated by temperatures as low as 40 C over incubation of 1 h or more. Inactivation of lactase at relatively low temperatures confirms the findings of Woychik et al. (15), who observed loss of lactase activity at 30 C.

Effect of Pasteurization

Pasteurization of skim milk increased the susceptibility of lactose to enzymatic hydrolysis (Table 1). Milk pasteurized for 30 min at 63, 74, and 83 C was hydrolyzed to a greater extent than the unpasteurized controls. Heating or pasteurization of the milk is recommended by the enzyme supplier, particularly for whey with added starter organisms (3). Our experiments show that pasteurization of skim milk increases lactose hydrolysis and corroborates the studies of Kosikowski and Wierzbicki (8).

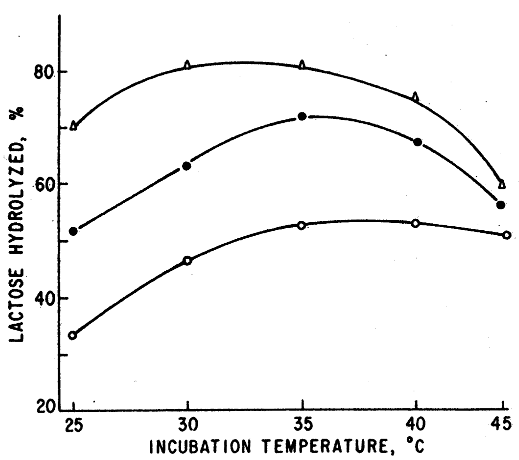

FIG. 3. Effect of temperature on lactose hydrolysis by 300 mg *S. lactis* lactase/kg pasteurized skim milk. The incubation times were .5 h (○—○), 1 h (●—●), and 2 h (△—△).

TABLE 1. Effect of pasteurization temperature on lactose hydrolysis by 300 mg *S. lactis* lactase/kg skim milk incubated at 32 C.

Pasteurization temperature (maintained for 30 min)	% Lactose hydrolyzed	
	45 min	90 min
Control	51.5	67.0
63 C	52.5	71.0
74 C	54.0	73.0
85 C	55.0	73.0

Effect of Enzyme Concentration and Time of Reaction on Skim Milk

The effect of enzyme concentration on percent of lactose hydrolyzed in skim milk is in Fig. 4. When the lactase concentration was increased, more lactose was hydrolyzed. However, the rate of lactose hydrolysis did not increase in direct proportion to a higher enzyme to lactose ratio. For example, in a 2-h reaction only 80 mg lactase/kg were required to hydrolyze 40% of the lactose whereas 300 mg were required to hydrolyze 80% of the lactose. Similarly, doubling the incubation time did not double the amount of lactose hydrolyzed. An

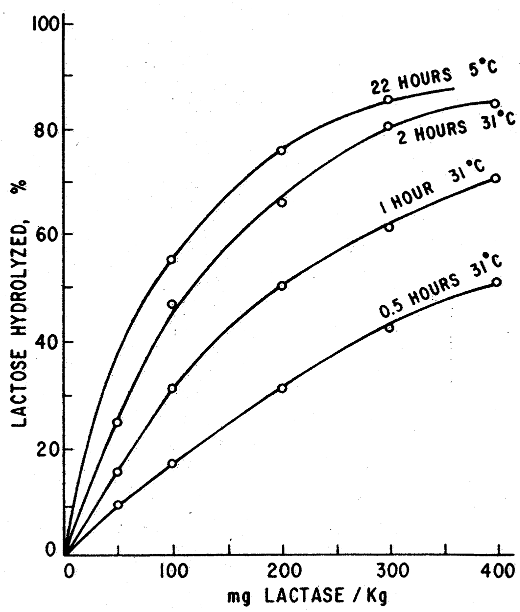

FIG. 4. Effect of *S. lactis* lactase concentration on lactose hydrolysis in pasteurized skim milk.

enzyme concentration of 300 mg/kg skim milk hydrolyzed 40% of the lactose in .5 h but only 60% in 1 h. The same amount of lactase hydrolyzed slightly more lactose in 22 h at 5 C than in 2 h at 31 C. Enzyme activity at this lower temperature is useful and advantageous in large processing operations where holding temperature is important.

Effect of Enzyme Concentration on Concentrated Skim Milk

Figure 5 shows the effect of lactase concentration on the hydrolysis of lactose in a 41% total solids concentrate of skim milk incubated for 2 h at 30 C. The curve is qualitatively similar to the one for fluid skim milk under the same conditions (Fig. 4). Larger amounts of enzyme were used to compensate for the higher concentration of lactose in the condensed product. However, in a comparison of hydrolysis results based on enzyme to lactose ratio, the enzyme was approximately 15% less active in concentrated milk.

A considerable amount of lactose in skim milk concentrate was hydrolyzed at 5 C for 3 to 6 days with as little as 50 to 60 mg enzyme/kg, which on an equal enzyme to lactose basis corresponds to 11 to 14 mg lactase/kg of fluid milk (Fig. 6).

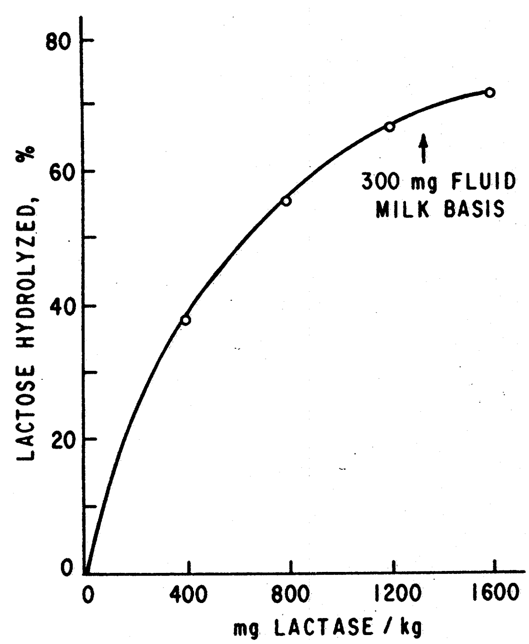

FIG. 5. Effect of *S. lactis* lactase concentration on lactose hydrolysis in a skim milk concentrate with 41% total solids held at 30 C for 2 h.

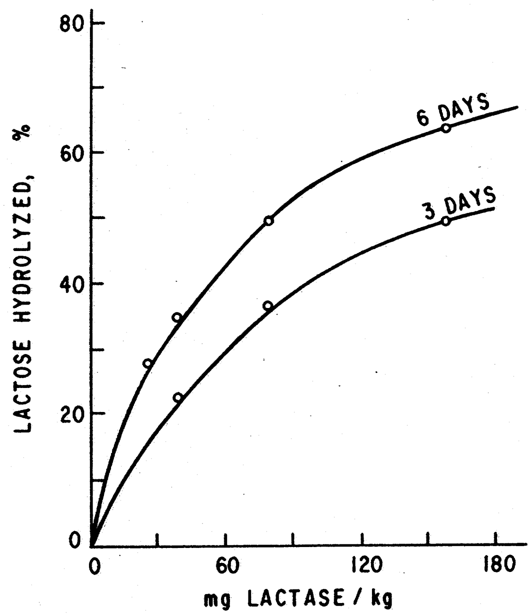

FIG. 6. Effect of *S. lactis* lactase on the lactose hydrolysis of a skim milk concentrate with 42% total solids held at 5 C for 3 days and 6 days.

Effect of Cations

Potassium, magnesium, and manganese ions accelerated slightly the activity of *S. lactis* lactase whereas sodium and calcium ions significantly depressed lactase activity by 29% and 14% (Table 2).

TABLE 2. Effect of cations on the hydrolysis of lactose by 300 mg *S. lactis* lactase/kg skim milk held at 30 C.

Molarity of cation added	% Lactose hydrolyzed in 90 min
None (control)	69.0
4×10^{-2} KCl	72.0
4×10^{-2} NaCl	49.0
8.5×10^{-3} $CaCl_2$	59.5
4×10^{-3} $MgCl_2$	76.0
1×10^{-3} $MnCl_2$	74.0

DISCUSSION

This investigation showed the various conditions under which commercial *S. lactis* lactase hydrolyzed the lactose of skim milk or milk products. The information supplied by the manufacturer was verified and observations were added.

Our study demonstrated that *S. lactis* lactase is unstable to heat and becomes inactivated at relatively low temperatures. Of the activity 75% was destroyed after heating for 1 min at 55 C in a whey medium. Measurable inactivation was also evident in 1-h hydrolysis at 40 C. Other investigators have observed inactivation at 30 C (15). Thus, it is important to hydrolyze the lactose at low temperatures to maintain the full enzyme potency. Incubation for 22 h at 5 C produced even higher lactose hydrolysis than at 31 C for 2 h. Milk processed with *S. lactis* lactase in refrigerated tanks overnight would be ready for further processing the following day.

The lower activity of the *S. lactis* enzyme in concentrated skim milk compared with that in fluid milk may be attributed to formation of oligosaccharides in the concentrates (10), inhibition by Ca^{2+} and Na^{+}, or the slightly lowered pH of the media.

The rate of enzymatic reaction rapidly falls off with time as lactose progressively is hydrolyzed. The decline could be explained by the increase in galactose which inhibits the enzyme as the reaction proceeds (15) or by the decrease in substrate concentration.

Metal ions equivalent in amount or even less than those in fluid milk significantly affected the activity of lactase. For example, Mg ions exerted an accelerating effect whereas Ca ions were inhibitory. Because milks contain both these ions, their kinds, amounts, and solubility state must be factors influencing the rate of the lactase reaction.

When skim milk concentrates with 40% total solids were held at 5 C for 5 to 6 days with 50 to 60 mg enzyme/kg, lactose hydrolysis was sufficient to prevent crystallization of lactose which otherwise would have occurred. In ice cream products, concentrates in which lactose had been hydrolyzed may be used to reduce lactose crystallization (5, 6). Also, in such ice cream products the sugar can be lowered because of the increased sweetening effect of the monosaccharides from lactose.

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