ABSTRACT

A method was standardized for determining lactase activity in cultured and acidified dairy products such as cottage cheese, sour cream, and yogurt. Cottage cheese and sour cream prepared by both the cultured and acidified processes and yogurt prepared by the direct acidification process did not possess lactase activity. However, cultured yogurt possessed considerable enzyme activity mainly due to lactase as an endoenzyme in the yogurt culture (Lactobacillus bulgaricus and Streptococcus thermophilus). Enzyme in yogurt increased with time of incubation, reaching a maximum of 8 orthonitrophenol β-d-galactopyranoside units per gram of yogurt in 4 h. S. thermophilus contained approximately three times more lactase than did L. bulgaricus. Also, an in vitro digestion process appeared to enhance the release of lactase from the composite yogurt culture. It was felt that cultured yogurt would be beneficial to individuals suffering from lactose intolerance not only because of reduced lactose but also because of lactase.

INTRODUCTION

Considerable attention has been focused on the problem of lactose intolerance, a condition that is believed to arise from the deficiency of lactase (β-galactosidase) in the intestinal microvilli, particularly in individuals of certain ethnic groups (6, 9, 13). Kretchmer (9) in a study of various Nigerian tribes discovered that a tribe involved in milk production and consumption was also lactose tolerant. Those villagers that could not digest milk were able to digest a partially fermented milk product with reduced lactose content. In the manufacture of fermented milk products, some of the lactose is converted to lactic acid by microorganisms. Vakil and Shahani (14) demonstrated lactase in Streptococcus lactis. Citti et al. (3) reported that the lactase of S. lactis was inducible. More recently, a survey of 15 microorganisms revealed that among others, Streptococcus thermophilus and Lactobacillus bulgaricus were able to hydrolyze 50 to 60% of lactose in acidified cheese whey within 5 h at 50 C (15).

S. lactis, S. thermophilus, and L. bulgaricus commonly are used as lactic cultures in the manufacture of yogurt, sour cream, and cottage cheese. Recent technological advances have evolved processes to obtain these products by direct acidification. Instead of relying on bacteria to produce acid, acidulants such as gluconodelta-lactone, hydrochloric acid, and citric acid are employed.

The purpose of this investigation was to study lactase in cultured and acidified dairy products, namely, yogurt, cottage cheese, and sour cream. The behavior of the composite yogurt culture, L. bulgaricus and S. thermophilus, in skim milk and broth systems was determined. Also, the effect of an in vitro gastric digestion of yogurt on lactase was determined.

MATERIALS AND METHODS

Manufacture of Products

Cultured yogurt, cottage cheese, and sour cream were prepared by conventional methods as described by Kosikowski (8) and Emmons and Tuckey (4). Acidified yogurt was prepared as described by Reddy and Shahani (12). The acidified cottage cheese samples were prepared by a patented process (5) and acidified sour cream was produced by a technique described by Little (10).

Received December 3, 1975.

1Published as paper No. 5020, Journal Series, Agricultural Experiment Station, Lincoln, NE. Research was conducted on project 16-026. This work was supported in part by grants from Dairy Research Inc. (DRINC) and Dannon Products Inc.
Preparation of Samples for Lactase Assay

Ten grams of the product were macerated with water in a Waring blender and the final volume was made to 100 ml in a volumetric flask. Aliquots of the diluted sample were subjected to sonic disintegration by a Biosonik III cell disruptor (Bronwill Scientific Co., Rochester, NY) at a frequency of 12 KHz. One-milliliter portions of the sonicated solution were used to determine enzyme activity.

To study the effect of incubation time on the enzyme activity of yogurt, 100-g samples of milk were each inoculated at 4% with an active yogurt culture. This composite inoculated milk was apportioned in 10-g batches and incubated at 43°C for periods up to 8 h. Samples were taken at hourly intervals for determining titratable acidity, pH, and lactase activity. Titratable acidity was determined by titrating a 9 g sample with .1 N sodium hydroxide to a phenolphthalein end point. The lactase assay is described later in this section.

Culture Studies

Pure cultures of *L. bulgaricus* and *S. thermophilus* were isolated from a commercial sample of yogurt. The isolates were examined for purity by conventional methods (1) and then maintained in sterile skim milk and Difco All-Purpose Tween (APT) broth. Cultures were transferred successively three times before use.

Batches of sterile skim milk (100 ml) were inoculated with 1% active cultures of *L. bulgaricus* and *S. thermophilus* and incubated at 43°C for 16 h. Also, the composite yogurt culture (*L. bulgaricus* plus *S. thermophilus*) was inoculated at 4% into sterile skim milk and incubated at 43°C for 4 h. At the end of the incubation period, a 10-g portion of the cultured skim milk was removed and assayed for lactase. A 50-g portion of the same cultured milk was subjected to an in vitro gastric digestion process described by Breslaw and Kley (2). Aliquots of the digest were withdrawn at 0, 1, 2, and 3 h intervals and analyzed for thier lactase content.

The isolates maintained in broth were grown separately in APT broth (Difco) containing glucose or lactose as the carbohydrate source. Preliminary trials revealed that it was not possible to attain a one to one ratio between *L. bulgaricus* and *S. thermophilus* in a broth system. When a composite yogurt culture of *L. bulgaricus* and *S. thermophilus* was grown in APT broth, *S. thermophilus* appeared to dominate the flora. Therefore, these studies dealt with single organisms only. One hundred milliliters of broth were inoculated with a 1% active culture and incubated at 43°C for 16 h. At the end of the incubation period, cells were harvested by passing the broth through a Millipore filter. The resulting cell paste was diluted to 10 ml with water. A 5-ml aliquot of the cell suspension was divided into two portions. One portion of the sonicated suspension was used directly for the assay of lactase and the other portion was centrifuged at 10,000 x g to obtain a cell free supernatant which also was assayed for lactase. The lactase activity of the former fraction indicated the total enzyme while the latter reflected the free enzyme. The difference between the total and free enzyme was taken as the enzyme bound to the cell wall. Dry weights of cell suspensions were determined by drying samples at 110°C for 3 h.

Assay of Lactase

The release of o-nitrophenol from the hydrolysis of o-nitrophenol-β-D-galactopyranosidase (ONPG) was used as the parameter for measuring lactase activity (3). Solutions of ONPG at 1.66 x 10^{-3} M were prepared in .1 M phosphate buffer, pH 7.0, and used as the substrate. To 5 ml of the substrate was added 1 ml of the test solution and the reaction carried out at 30°C for 10 min. The reaction was stopped by the addition of 2 ml of 1.0 M sodium carbonate. The absorbancy at 420 nm was measured in a Beckman Model 25 spectrophotometer. A standard curve was prepared from known activities of pure *E. coli* lactase (Worthington Biochemicals, Freehold, NJ). One unit of enzyme activity was defined as the amount needed to hydrolyze 1 μmole of ONPG/min at 30°C and pH 7.0.

RESULTS AND DISCUSSION

Initial qualitative examination of several dairy products revealed that only cultured yogurt contained detectable amounts of lactase. The cottage cheese, sour cream, and acidified yogurt samples, and the milk from which these products were made failed to yield the characteristic yellow color with ONPG even after prolonged reaction times of 2 h, indicating that...
these products did not contain any detectable amounts of lactase. Cultured yogurt, on the other hand, gave a positive reaction with ONPG. Therefore, subsequent studies were focused only on cultured yogurt. The choice of ONPG as a substrate was necessitated by the fact that glucose and galactose resulting from lactose hydrolysis are metabolized rapidly to lactic acid and other intermediates of the Embden-Myerhoff-Parnas pathway (14). Hence, measuring the glucose or galactose in the product would not be an accurate index of the lactase activity.

Since the milk used in the manufacture of cultured yogurt did not show any lactase activity, the enzyme activity of this product was attributed to the yogurt culture. Studies were made to determine the effect of sonication on the release of lactase. Yogurt samples were sonicated at 12 KHz for periods up to 10 min. The sonicated samples were reacted with ONPG and the resultant color recorded at 420 nm. Maximum color was noticed after 7 min of sonication (Fig. 1). Sonication for periods longer than 7 min resulted in less color. In all subsequent studies involving milk cultures, a sonication time of 7 min at 12 KHz was used.

**Effect of Incubation Time on Enzyme Production in Yogurt**

The production of lactic acid and other metabolites was affected by the length of incubation during the manufacture of yogurt (Fig. 2). Lactic acid reached a maximum of 1.5% after 6-h incubation and remained at that upon prolonged incubation. The pH, on the other hand, declined gradually to 4.5 in 7 h. The acidity of yogurt after 6-h incubation may be considered excessive, however, after only 4-h incubation, it was in the optimal acidity range of 1.0 to 1.2%. Lactase activity increased steadily up to 4 h of incubation, reaching a maximal value of 8 units/g. Further incubation resulted in a marked lowering of the enzyme activity to about 3 units/g before leveling off. The decrease in enzyme activity between the 4th and 6th h of incubation was believed due to the increase in titratable acidity or the decrease in pH. Since 4-h incubation periods are commonly employed in the manufacture of yogurt, the lactase content would be near maximum in such a product.

**Culture Studies**

From the above experiments it was uncertain whether a single species of bacterium was
TABLE 1. Lactase content of *L. bulgaricus*, *S. thermophilus*, and yogurt culture in milk.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>L. bulgaricus</em></th>
<th><em>S. thermophilus</em></th>
<th>Composite yogurt culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsonicated (control)</td>
<td>.40</td>
<td>.45</td>
<td>.81</td>
</tr>
<tr>
<td>Sonicated</td>
<td>1.52</td>
<td>2.41</td>
<td>3.84</td>
</tr>
</tbody>
</table>

(responsible for the lactase activity or whether the two organisms, *L. bulgaricus* and *S. thermophilus*, exerted a synergistic effect. Pette and Lolkema (11) reported that a composite yogurt culture exhibited a synergistic effect upon growth and acid production. Rods, cocci, and the composite yogurt culture were growth separately and their enzymic activities determined. Table 1 shows the effect of sonication on the release of lactase from individual cultures. The unsonicated cultures of *L. bulgaricus* and *S. thermophilus* contained nearly the same amounts of lactase activity. However, on sonication *S. thermophilus* had 1.59 times more lactase activity than *L. bulgaricus*. One gram of the unsonicated composite yogurt culture contained .81 u of lactase. Since one gram of the composite yogurt sample consisted of .5 g each of *L. bulgaricus* and *S. thermophilus*, the total activity should have been (.40 + .45) + 2 = .425 u/g of composite sample. However, the observed value was .81 u/g which seemed to suggest a synergistic relationship between the cultures grown together.

Since lactase is an inducible enzyme in certain microorganisms, experiments were designed to study the production of lactase by these cultures in a broth system. The cultures differed from one another in such a system. The lactase of *S. thermophilus* was an inducible enzyme since cells grown in lactose containing APT broth (LAPT) showed an activity of 12,000 units of lactase whereas cells grown in glucose containing APT broth (APT) showed only 4900 units/g (Table 2). However, *L. bulgaricus* grown in LAPT broth did not show any increase in activity. A substantial part of the lactase was bound to the cell-debris. In either case the enzyme was an endoenzyme since the cell-free medium did not possess any enzyme activity. A decrease in total lactase activity of *L. bulgaricus* grown in LAPT broth compared with the activity of cells grown in APT broth could have been due to inhibition of the enzyme by galactose (16).

**Effect of In Vitro Digestion on Release of Lactase**

Since substantial amounts of lactase were bound to bacterial cells, gastric digestion may aid in the release of this enzyme. Trials performed by subjecting 16-h-old cultures of *L. bulgaricus* and *S. thermophilus* and 4-h-old

<table>
<thead>
<tr>
<th>Organism and growth medium</th>
<th>Bound</th>
<th>Free</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. thermophilus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose broth (APT)</td>
<td>2927</td>
<td>1962</td>
<td>4889</td>
</tr>
<tr>
<td>Lactose broth (LAPT)</td>
<td>8427</td>
<td>3655</td>
<td>12,082</td>
</tr>
<tr>
<td><em>L. bulgaricus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose broth (APT)</td>
<td>646</td>
<td>144</td>
<td>790</td>
</tr>
<tr>
<td>Lactose broth (LAPT)</td>
<td>641</td>
<td>3</td>
<td>644</td>
</tr>
</tbody>
</table>
TABLE 3. Effect of in vitro gastric digestion on the release of lactase from cultures.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L. bulgaricus (Units of lactase per gram culture)</th>
<th>S. thermophilus (Units of lactase per gram culture)</th>
<th>Composite yogurt culture (Units of lactase per gram culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control^a</td>
<td>.40</td>
<td>.45</td>
<td>.85</td>
</tr>
<tr>
<td>Digestion control^b</td>
<td>3.4</td>
<td>3.9</td>
<td>4.0</td>
</tr>
<tr>
<td>After 1-h digestion</td>
<td>2.3</td>
<td>2.5</td>
<td>4.1</td>
</tr>
<tr>
<td>After 2-h digestion</td>
<td>2.2</td>
<td>1.9</td>
<td>4.5</td>
</tr>
<tr>
<td>After 3-h digestion</td>
<td>2.0</td>
<td>1.1</td>
<td>4.9</td>
</tr>
</tbody>
</table>

^a No treatments.
^b Zero time sample, i.e., culture + acid + enzymes.

composite yogurt culture to an in vitro gastric digestion process revealed that lactase was affected by digestion. While the enzyme in individual cultures declined with time of digestion, lactase in the composite yogurt culture seemed to show an increase (Table 3). This evidence can be corroborated with the observations of Goodenough and Kleyn (7), who stated that yogurt microflora may contribute to the successful metabolism of lactose.

A cultured milk product, such as yogurt, may be more digestible by lactose intolerant individuals because of a reduced lactose content and the presence of the enzyme lactase. In a milk system a synergistic effect between rods and cocci on lactase activity was observed. In broth cultures the lactase of S. thermophilus was inducible. In vitro digestion also was helpful in releasing lactase from the composite yogurt culture.

ACKNOWLEDGMENT

Skillful technical assistance of Clara Zoz in performing some of the analyses is appreciated.

REFERENCES


