

## A COMPARISON OF SOME CHEMICAL PROPERTIES OF YOGURTS MADE FROM CONTROL AND LACTASE-TREATED MILKS

### ABSTRACT

Certain properties of yogurts made from regular commercial skim milk and from milk which had been treated with lactase enzyme so that 70–75% of the lactose was hydrolyzed to glucose and galactose were evaluated. Initially faster rates of acid production in lactase-hydrolyzed milks (LH) during fermentation resulted in somewhat shortened processing times, although a decrease in the rate of acid production occurred earlier during fermentation in the LH milk so that final product pH was similar for both the control and LH yogurts. Control yogurts contained 5.0% lactose and 0.2% galactose while LH yogurts contained 1.6% glucose, 1.5% lactose and 2.1% galactose. Appreciably more lactic acid was produced in LH yogurts and this effect has been related to fermentation by the starter culture organisms of a greater proportion of the available sugar as glucose.

### INTRODUCTION

RECENT INVESTIGATIONS have shown that a significant proportion of the world's population is unable to digest the disaccharide lactose due to a deficiency of lactase in the intestinal mucosa. According to a statement released by the Food and Nutrition Board of the National Academy of Sciences in 1972, 60–90% of non-Caucasian peoples have low lactase activity. The inability to digest lactose may result in the manifestation of symptoms such as stomach cramps, flatulence and diarrhea when milk and certain other dairy products are consumed (McCracken, 1971). In a survey of the etiology of lactose intolerance, McCracken (1971) noted that fermentation breaks down the lactose in milk and, although the lactose never completely disappears, the proportion of lactose in a fermented product may be reduced enough to make a product compatible to intolerant people. However, commercial yogurts made in the United States have been found to contain appreciable amounts of lactose due to the practice of fortifying the yogurt mix with nonfat milk solids. Goodenough (1975) reported lactose values for commercial yogurts ranging from 3.3–5.75%. Even higher values have been reported elsewhere in the literature (Acott and Labuza, 1972). Thompson and Gyuricsek (1974) developed a process for the manufacture of a yogurt product low in lactose. Their procedure involves pretreatment of milk with lactase in order to convert the milk lactose to glucose and galactose. They reported that yogurt manufactured by their process set more rapidly than yogurt made by conventional methods and had good acceptability. This study was undertaken to define the microbial and chemical properties of yogurts derived from lactase-hydrolyzed milks and to gather information concerning the types of changes which might occur in other cultured dairy products manufactured from lactase-treated milks.

### MATERIALS & METHODS

#### Bacterial cultures

The culture employed for yogurt manufacture was a mixed yogurt starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (LBST<sub>4</sub>) supplied by the Marshall Div. of Miles Laboratories, Madison, Wisc. in cryogenic form. Cryogenic cultures which were kept stored in liquid nitrogen were thawed and then inoculated into sterile skim milk. Transfers in sterile skim milk were made daily.

#### Preparation of hydrolyzed lactose milk

Skim milk fortified 4% w/v with nonfat milk solids was incubated

with 300 µg/ml of *Saccharomyces lactis* β-galactosidase ("Maxilact," Enzyme Development Corp., N.Y.) to hydrolyze the lactose to glucose and galactose. Incubation was overnight at 4°C with constant stirring and resulted in hydrolysis of 70–75% of the lactose.

#### Preparation of yogurts

Fortified control and lactase-hydrolyzed (LH) milks were heat treated at 105°C for 15 min and then cooled to 43°C prior to inoculation. The milks were inoculated at a level of 2% with the mixed yogurt starter culture which had previously been grown at least twice for 24 hr at 30°C in sterile skim milk. The inoculated milks were incubated in a water bath at 44°C and acidity in the milks was allowed to develop until the pH decreased to 4.6. Finished yogurt products were refrigerated at 4°C.

#### Enumeration of starter culture organisms

Enumeration of the starter culture organisms was made by plate count using Hansen's Yogurt Agar (Porubcan and Sellars, 1973) prepared as follows: 15.0g Bacto-agar (Difco), 1.0g beef extract and 10.0g proteose peptone #3 (Difco) were dissolved in 900 ml of distilled water with boiling and then autoclaved at 121°C for 15 min. To the sterile agar cooled to 46–47°C was added 2.5g glucose, 2.5g galactose and 5.0g lactose per liter in the form of sterile 10% solutions of the sugars sterilized by Millipore filtration using a 0.22µ pore size Millipore filter (Millipore Corp., Bedford, Mass.). Pour plates were incubated at 37°C for 48–72 hr and then counted. The starter culture organisms were easily differentiated from each other on this medium since *L. bulgaricus* formed diffuse low mass colonies while *S. thermophilus* formed discrete high mass colonies. Identity of the two types of colonies was confirmed by microscopic examination.

#### Acid development

Acid development was followed by measuring pH during the fermentation period using a Radiometer pH Meter Model 22 (Radiometer; Copenhagen, Denmark).

#### Sugar analyses

One-ml samples of the yogurt mix or the final yogurt product which had been well mixed prior to sampling were placed in centrifuge tubes containing 1.0 ml of 1.0M acetate buffer pH 4.6 and well mixed. (Reproducibility of the volumetric sampling procedure for yogurt was investigated. The sampling procedure was found to have a variance of 0.0007 and a standard deviation of 0.026.) Precipitated casein was removed by centrifugation at 30,000 × G for 15 min at 4°C in a Servall Superspeed RC-2 Centrifuge. Aliquots of the clear supernatants were analyzed for glucose, galactose and lactose.

Glucose was determined using the Salomon and Johnson reagent as described by Jasewicz and Wasserman (1961) but with the following modifications. To 0.1 ml of sample containing 15–150µg of glucose were added 2.0 ml of water and 1.5 ml of Salomon and Johnson reagent. The color was allowed to develop at room temperature for at least 1 hr and then the absorbancies of the solutions were read at 635 nm using a Zeiss Model PMQ II Spectrophotometer.

Lactose was determined by measurement of the glucose released following hydrolysis with lactase as follows. A 3.0-ml aliquot of Maxilact lactase (Enzyme Development Corp., N.Y.) in 0.1M phosphate buffer at pH 7.0 (5 mg/3 ml) was added to 0.1 ml of sample and incubated for 3 hr at 30°C. The enzyme reaction was terminated by placing the samples in a boiling water bath for 5 min and the solutions then clarified by centrifugation. One tenth ml aliquots were analyzed for glucose by the procedure previously described. Lactose concentrations were calculated by multiplying the glucose concentrations by 1.92.

Galactose measurements were made by an enzymatic method utilizing galactose dehydrogenase. The method used was a modification of a procedure described in the Boehringer-Mannheim catalogue (Boehringer-Mannheim Corp., N.Y.). The assay medium consisted of 0.84 ml 0.1M Tris-HCl buffer pH 8.6; 0.05 ml β-NAD, 5 mg/ml (Sigma Corp.,

St. Louis, Mo.); 0.10 ml sample and 0.01 ml  $\beta$ -galactose dehydrogenase (Sigma Corp.) diluted to approximately 3 U/ml. The assay solutions were incubated at 25°C for 75 min and then read at 340 nm using a Zeiss Model PMQ II Spectrophotometer.

#### Other analyses

Yogurt samples having a pH of 4.6 were centrifuged at 30,000  $\times$  G for 15 min in a Servall Superspeed RC-2 Centrifuge at 4°C to sediment the insolubilized casein. The supernatants were used for lactic acid, acetaldehyde and diacetyl determinations. Lactic acid determinations were made by the procedure of Lawrence (1970). Acetaldehyde was determined by the method of Lindsay and Day (1965) and diacetyl was determined by the method of Pack et al. (1964).

All experiments were carried out in duplicate and each experiment was repeated twice.

## RESULTS & DISCUSSION

### Acid development

Curves illustrating the decrease in pH which took place in LH and control milks during the preparation of yogurts are presented in Figure 1. Less time was required for the pH to decrease to 4.6 in LH milk than in the control. Faster acid development in milk cultures to which lactase has been added has been reported previously. Gilliland et al. (1972) reported that acid production by lactic streptococci in milk was stimulated by the addition of  $\beta$ -galactosidase while Thompson and Gyuricsek (1974) reported that acid development was increased and set time decreased when yogurts were prepared from lactase-hydrolyzed milks. In our experiments faster acid development in yogurts prepared from LH milk was primarily due to an acceleration in the initial rate of acid production when the lactose was prehydrolyzed. This effect diminished, however, as acidity built up in the product, probably as a result of inhibition of the starter culture organisms due to the rapid production of large amounts of acid. Further decreases in pH took place during storage at 4°C. After 24 hr of cold storage, the pH of control and LH yogurts had declined to 4.39 and 4.42, respectively. During an additional 3–4 wk of storage at 4°C, there was a further decrease in pH to about 4.1 in both products. Decline in pH of yogurts during cold storage has been reported by Tramer (1973) who related this effect to the ability of the starter culture organisms to carry out metabolic processes at cold storage temperatures.

### Growth of starter culture organisms

Growth curves of a mixed starter culture consisting of *S. thermophilus* and *L. bulgaricus* in control and LH yogurts are presented in Figure 2.

Patterns of growth of the starter culture organisms were similar in both yogurts. *S. thermophilus* predominated in the early stages of the yogurt fermentation due to a rapid rate of growth which was initiated almost immediately after inoculation into the milk medium while *L. bulgaricus* did not begin to multiply rapidly until the latter stages of fermentation after *S. thermophilus* had reached the stationary growth phase. Similar growth patterns were reported by Bautista et al. (1966) in a discussion of the associative growth of yogurt starter culture organisms. Bautista et al. (1966) reported that the lactobacilli stimulate the growth of the streptococci by liberating essential amino acids from the milk proteins. However, the lactobacilli are more acid tolerant than are the streptococci and thus can grow during the latter stages of the fermentation when growth of the streptococci has been inhibited by low pH. Thus our results show that prehydrolysis of the milk lactose had no effect on the symbiotic growth relationships of the starter culture organisms used in this study.

### Sugar concentrations

Concentrations of glucose, galactose, and lactose in LH and control milks prior to fermentation and in the finished yogurt products after 24 hr of storage at 4°C are given in Table 1. There was a 30% decrease in the lactose content of control

milk during fermentation and a slight accumulation of galactose. The mixed starter culture appeared to be unable to metabolize all the galactose released through intracellular hydrolysis of lactose and the galactose which was not utilized was released into the medium. Gilliland et al. (1972) observed an accumulation of galactose in milk cultures during growth of *S. lactis* C<sub>10</sub>. They suggested that *S. lactis* C<sub>10</sub> was unable to

Table 1—Sugar concentrations in control and LH milks prior to fermentation and in the finished yogurt products after 24 hr of storage at 4°C.

	Control		LH	
	Initial	Final	Initial	Final
Glucose (%)	0.0	0.0	2.6	1.6
Galactose (%)	0.0	0.2	2.3	2.1
Lactose (%)	7.1	5.0	2.1	1.5

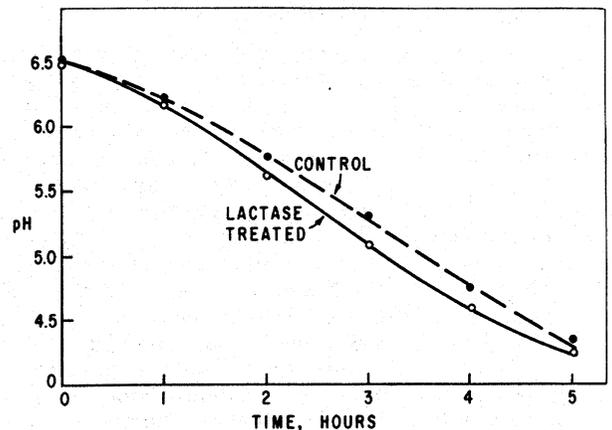


Fig. 1—Changes in pH which occurred during the fermentation of control and lactase-hydrolyzed milks by a mixed yogurt starter culture.

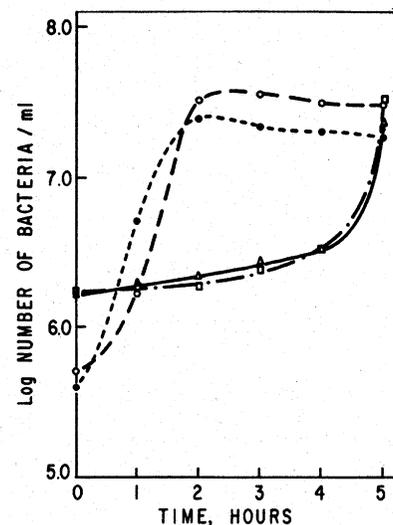


Fig. 2—Growth of a mixed culture of *S. thermophilus* and *L. bulgaricus* in control and lactase-hydrolyzed milks. □, *L. bulgaricus* LH milk; △, *L. bulgaricus* control milk; ○, *S. thermophilus* LH milk; ●, *S. thermophilus* control milk.

utilize all the galactose resulting from catabolism of lactose in milk. Thus it appears that at least certain of the lactic acid bacteria are unable to maximally utilize galactose which is bound in the form of lactose.

Glucose levels in LH milk decreased by 38% during fermentation. In addition, there was a 29% decrease in the lactose content. These results indicate that levels of lactose remaining in lactase-treated milk will be even further decreased during the yogurt manufacturing process. Galactose concentrations, however, showed little or no change during the fermentation process, indicating that free galactose which was made available by prehydrolysis of the lactose was not fermented by the starter culture organisms.

Finished yogurt products prepared from control milk contained about 3.5 times as much residual lactose as did the LH yogurt, i.e., 1.5% in the LH product as compared with 5.0% in the control product. This substantial reduction in residual lactose levels was achieved both by the prehydrolysis treatment and microbial fermentation of some of the remaining lactose. Thus, partial enzymatic hydrolysis of the milk lactose is satisfactory since a 70% hydrolyzed milk yielded a product in which the lactose had been decreased by 80%.

#### Yogurt flavor compounds

Concentrations of some typical yogurt flavor compounds in pH 4.6 yogurts prepared from control and LH milks are given in Table 3. The LH yogurt contained appreciably more lactic acid than did the control yogurt at pH 4.6. However, acetaldehyde concentrations were about the same while no diacetyl was found in either product. The end products of glucose fermentation by the homolactic lactic acid bacteria have been reported to be lactic, acetic, and formic acids, CO<sub>2</sub>, ethanol, glycerol, biacetyl, acetoin, and 2,3-butanediol (Platt and Foster, 1958). Acetaldehyde is also an end product of microbial metabolism in milk cultures (Keenan et al., 1966). Steele et al. (1954) reported that there are marked quantitative but not qualitative differences in the end products produced by *Streptococcus pyogenes*, *Streptococcus faecalis* and *Lactobacillus casei* when these organisms are grown on galactose as opposed to glucose. They found that when galactose serves as the energy source, a much smaller proportion of the sugar is converted to lactic acid and a proportionately greater amount is converted to acetic acid, formic acid, and ethyl alcohol than when glucose serves as the energy source.

Calculations of the total amounts of glucose and galactose

Table 2—Concentrations of some typical yogurt flavor compounds in control and LH yogurts at pH 4.6.

	Lactic acid (%)	Acetaldehyde (ppm)	Diacetyl (ppm)
Control	0.47	2.5	0
LH	0.61	2.4	0

Table 3—Amounts of glucose and galactose catabolized by a mixed yogurt starter culture in control and LH milk during yogurt preparation

	Control			LH		
	Tot. CHO (%)	Glu. (%)	Gal. (%)	Tot. CHO (%)	Glu. (%)	Gal. (%)
Initial	7.10	3.55	3.55	7.00	3.65	3.35
Final	5.20	2.50	2.70	5.20	2.35	2.85
Amount utilized	1.90	1.05	0.85	1.80	1.30	0.50

catabolized by the yogurt starter culture organisms in control and LH milks in our experiments are presented in Table 3. Calculations of the amount of galactose catabolized in LH milks are based on the amounts of lactose consumed. Our calculations show that almost twice as much galactose was metabolized in control milk as in LH milk. Thus, our data indicate that the greater quantities of lactic acid produced by the yogurt starter culture organisms in LH milk may have been due to an alteration in the patterns of metabolites produced resulting from the utilization of a greater proportion of the total available sugar in the form of glucose.

In flavor evaluations of the two yogurts by a sensory panel, the LH yogurt was scored significantly higher than was the control product due to the substantially sweeter character of the former resulting from the presence of free glucose and galactose. Although some panel members commented that there appeared to be some other flavor differences between the two yogurts in addition to the difference in sweetness, the substantially greater sweetness of the LH yogurt was the overriding factor in evaluation of this particular product.

Our results have shown that more lactic acid is produced by a mixed yogurt starter culture consisting of *S. thermophilus* and *L. bulgaricus* in yogurts prepared from LH milk. In addition we have observed that a greater proportion of the total available carbohydrate is catabolized in the form of glucose in LH milk. Thus our data indicate that lactase treatment of milk to be used in the manufacture of cultured dairy products may result in changes in the flavor profiles of the resulting products. Such changes may occur as a result of alteration in the types or amounts of metabolic end products produced by the starter culture organisms when the lactose in milk is prehydrolyzed so that a greater proportion of the carbohydrate available for fermentation is metabolized in the form of glucose.

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