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Use of Lactase in the Manufacture of Dairy Products

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The potential for lactase application in the manufacture of dairy products has been recognized for many years. Lactase (β-D-galactosidase) hydrolyzes milk lactose into its constituent monosaccharides, glucose and galactose. Chemical and physical changes that occur as a result of lactose hydrolysis provide the rationale for its application. The principal changes are reduced lactose content, increased carbohydrate solubility, increased sweetness, higher osmotic pressure, reduced viscosities, and more readily fermentable sugar. Enzymatic hydrolysis of lactose in dairy foods would improve product quality and provide low-lactose products for the lactose intolerant segment of the population.

Available Lactases

Efforts to utilize lactases for the manufacture of hydrolyzed lactose (HL) products have been restricted primarily by the lack of suitable, commercially available, enzymes. Lactases are found in plants, animals and microorganisms (Table I), but only microbial sources can be used commercially. The pH optima of the microbial lactases are quite varied. The two enzymes of commercial value are isolated from the fungus, *Aspergillus niger* (1), and the yeast, *Saccharomyces lactis* (2), and differ widely in their properties, particularly in pH optimum (Table II). The *S. lactis* enzyme (pH optimum of 6.8-7.0) is ideally suited for the hydrolysis of lactose in milk and sweet whey (pH 6.6 and 6.1, respectively); lack of stability below pH 6.0 precludes its application to acid whey (pH 4.5). The *A. niger* lactase (pH optimum 4.0-4.5) has good pH stability, but the fall-off in relative activity at pH values above 4.5 limits its usefulness primarily to acid whey. Both of these lactases are available commercially in purified form.

Table I
Lactase Distribution

Plants	
	Phaseolus vulgaris, kefir grains, almonds, tips of wild roses, and seeds of soybeans, alfalfa and coffee.
Animal	
	Intestinal brush border (pH optimum 5.5-6.0).
Microbial	
	Bacteria (pH optimum 6.5-7.5)
	Fungi (pH optimum 2.5-4.5)
	Yeasts (pH optimum 6.0-7.0)

Table II
Properties of *A. niger* and *S. lactis* Lactases

	A. niger	*S. lactis*
pH Optimum	4.0-4.5	6.8-7.0
Temperature Optimum	55°C	35°C
pH Stability	3.0-7.0	6.0-8.5
Half-life (Days) at 50°C		
Whole Acid Whey	8	
5% Lactose	100	

Purity, availability, and cost of lactases are important considerations in any full-scale enzymatic process for lactose hydrolysis. Consequently, although lactose hydrolysis can be achieved most simply by the addition of soluble enzyme to milk or whey, immobilized enzyme technology has been evaluated with lactases in an effort to improve the economics of lactose hydrolysis (3,4,5,6). While efforts have been made to develop a satisfactory immobilized process for the S. lactis lactase (2), its stability following immobilization has not been sufficient to warrant its use. Problems associated with protein adsorption to a variety of enzyme support systems and maintaining acceptable column sanitation levels working with nutritive substrates, such as milk or sweet whey, further limit its usefulness. It would appear that batch treatment with soluble enzyme would be the method of choice for HL dairy products made with S. lactis lactase.

In contrast, the A. niger lactase has proven to be adaptable to use in immobilized forms and has been bound to a wide variety of solid supports (3,4,7,8,9). Depending on the substrates used and operating conditions, operational half-lives from 8 to 100 days have been obtained. Although some operational problems still exist, there is little doubt that the A. niger lactase can be used successfully in immobilized systems.

Applications

The hydrolysis of lactose in whole or skim milk, best accomplished by batch treatment with S. lactis lactase, can be achieved by incubation with 300 ppm lactase at 32°C for 2.5 hr (10), or at 4°C for 16 hr (11). Hydrolysis levels of 70-90% are obtained and the milk can be processed into a variety of products or used directly as a beverage. Some of the advantages and disadvantages in application of lactases in the manufacture of dairy products and processes follow.

Fluid and Dried Milk Products

Beverage Products. In recent years, numerous studies (12, 13,14) have established a pattern of lactose intolerance among non-Caucasian children and adults that is attributable to low intestinal lactase levels. Flatulence, cramps, diarrhea, and possibly a general impairment in normal digestive processes may accompany lactose intolerance. The inability of individuals to hydrolyze lactose can have serious implications in nutrition programs based on milk products.

The HL milk prepared in our laboratory for beverage use was evaluated in terms of its physical and organoleptic properties. The fact that HL milk is sweeter than normal milk posed some problems in defining its flavor in taste panel testing. Based on flavor scores (Table III) it was evident that a reciprocal relationship existed between the amount of lactose hydrolyzed in the

product and its flavor score. It was clear that judges treated the marked sweetness as a "foreign" flavor. Additional taste panel studies showed that hydrolysis of 30, 60 and 90% lactose had the same effect on flavor of milk as the addition of 0.3, 0.6, and 0.9% sucrose. Paige et al. (15) reported that Negro adolescents found milk with 90% of its lactose hydrolyzed quite acceptable to drink, although the majority of the group surveyed judged it sweeter than control milk.

Table III
Flavor Scores of Hydrolyzed-Lactose Milk

% Lactose Hydrolyzed	Flavor Score
0	37.0
30	37.0
60	36.7
90	36.2

In addition to overcoming the problems of the lactose intolerant, hydrolysis of lactose in milk is advantageous for the preparation of milk concentrates. A highly attractive method of preserving whole milk is freezing a 3:1 concentrate. The products keep much longer than fluid milk under normal refrigeration and, when reconstituted, have a flavor virtually indistinguishable from fresh milk. However, concentrates prepared from normal milk have a tendency to thicken and coagulate on standing. This protein destabilization results from crystallization of lactose which has been brought to its saturation point in the concentration process. Early research (16) showed that lactose hydrolysis led to improvement in the physical stability of the concentrates during storage. However, hydrolysis of 90% of lactose alone did not increase storage stability by more than one month over the control (Figure 1). HL samples heat treated at 71°C for 30 min after canning showed only a moderate rise in viscosity after 9 months storage (lower curve). Organoleptic evaluation showed no difference in flavor score of the reconstituted concentrate with 90% of its lactose hydrolyzed and a fresh whole milk control with sucrose added. Similarly, lactose crystallization was avoided when skim milk concentrates were prepared from HL milk and stored at cold temperatures.

Dried Products. No problems were encountered in the manufacture of milk powder from HL whole milk. However, HL skim milk powder, with a major portion of its lactose hydrolyzed, had a tendency to stick to the hot metal surfaces of the dryer at temperatures significantly lower than regular skim milk sticks (60 vs 75°C) at comparable moisture levels. Thus, the surfaces of the powder collecting apparatus need to be held below 60°C to avoid the sticking problem.

Cultured Products. Cultured products have long been thought to contain low levels of lactose because of its fermentative utilization. However, while the lower levels of lactose may make these products more compatible to the lactose intolerant, the practice of fortifying yogurt with lactose results in residual levels as high as 3.3-5.7% (17). Gyuricsek and Thompson (18) reported that among yogurts prepared from lactase treated milk in which more than 90% of the lactose was hydrolyzed, the HL yogurts set more rapidly than controls did and had good acceptability. Advantages claimed for the application of lactase in yogurt manufacture include accelerated acid development, which reduces the required set-time, and a reduction of "acid" flavor by the glucose and galactose, which made the plain yogurt more acceptable to consumers.

Supplying starter culture organisms with glucose and galactose, rather than lactose, as an energy source can be expected to result in altered carbohydrate utilization patterns and metabolite production. O'Leary and Woychik (19,20) undertook a study to define the microbial and chemical changes that might occur in cultured dairy products manufactured from HL milk. They reported that typical sugar concentrations in the HL milk for yogurt are glucose, 2.6%; galactose, 2.3%; lactose, 2.0%. Decrease in pH accompanied fermentation in the control and HL milk; less time was required to reach pH 4.6 in the HL milk than in the control (Figure 2).
The faster acid development in the HL milk is a result of a higher initial rate of fermentation. These findings support the decreased set-times reported by Gyuricsek and Thompson (18). The growth curves of the mixed starter culture consisting of *Streptococcus thermophilis* and *Lactobacillus bulgaricus* shown in Figure 3 demonstrate that lactose hydrolysis has no effect on the symbiotic growth relationships of the starter organisms. The carbohydrate utilization patterns by the cultures in the two yogurts were different. The values in Table IV show that almost twice as much galactose was catabolized in the control milks as in HL milk. At the same time, the total amount of lactic acid produced by the starter organisms was greater in the HL milk. These findings reflect altered patterns of metabolite production resulting from the utilization of a greater proportion of the total available sugar in the form of glucose.

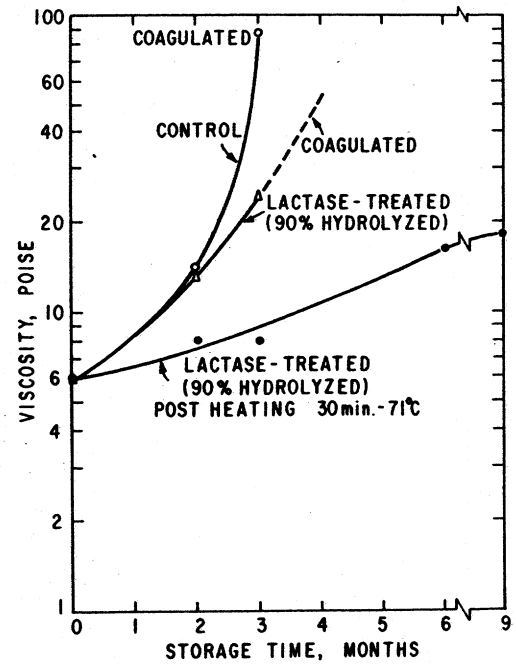
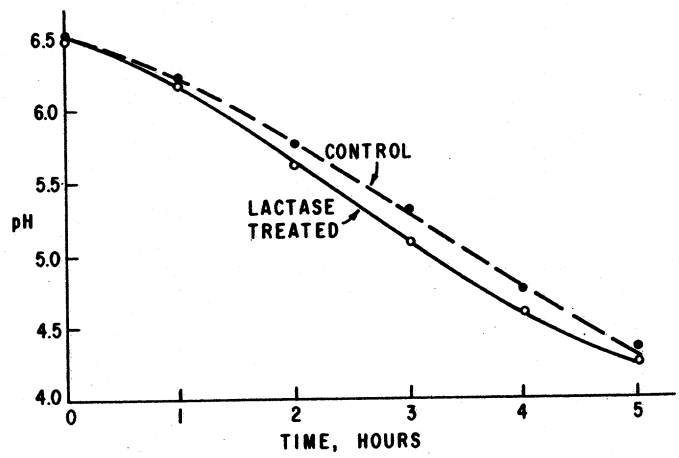


Figure 1. Effect of storage on viscosity of frozen 3:1 pasteurized whole milk concentrates



	Control		HL	
	CHO (%)	Galactose (%)	CHO (%)	Galactose (%)
Initial	7.11	3.50	7.01	3.30
Final	5.23	2.73	5.20	2.95
% Utilized	1.88	0.77	1.81	0.35

The accelerated acid production by yogurt culture organisms in HL milk was also observed by Gyuricsek and Thompson (18) with cultures used in cottage cheese manufacture. Advantages of using HL milk reported (18) include reduced setting-time, less curd shatter and reduced loss of fines, a more uniform curd, and use of lower cook-out temperatures.

With the exception of improved starter cultures and more automated equipment, few modifications have been developed for improving the manufacture or accelerating the ripening of Cheddar cheese. Thompson and Brower (11) extended the use of HL milk to the manufacture of Cheddar cheese and found that lactose hydrolysis modified the process considerably. The faster acid development reduced renneting times and significantly reduced curd cooking and cheddaring times. With equivalent times in cure, the HL Cheddar was superior to control cheeses in flavor, body, and texture and more rapidly developed these characteristics closer to that of older control cheeses. The improved qualities were attributed to the effects of lactose hydrolysis. The body and texture changes may be the result of increased osmolarity accompanied by reduced casein solvation. The accelerated ripening may be attributable to increased levels of enzymes released into the cheese by higher bacterial populations when glucose is available as a growth substrate.

Whey Utilization

The nation's cheese industry annually produces over 32 billion pounds of whey, of which little more than half is utilized. In the case of lactose, this excess production over utilization represents approximately 600 million pounds which could conceivably be converted into salable products. Modification of whey by lactase hydrolysis could offer new avenues for whey

utilization. Two of the most promising processes appear to be conversion to lactose-derived sirups and fermentative utilization.

Sirups. The low sweetness level of lactose relative to sucrose does not allow much direct competition; however, a considerable increase in sweetness results following hydrolysis of lactose. Relative sweetness is dependent on several factors, the principal one being concentration. Table V lists the sweetness of several sugars at 10% concentrations relative to sucrose.

Table V
Relative Sweetness of 10% Aqueous Sugar Solutions

Sugar	Sweetness (%)
Sucrose	100
Lactose	40
Glucose	75
Galactose	70

Thus, the availability of HL wheys increases the potential for producing lactose-derived sirups. HL sirups have been prepared by Guy (21) using both hydrolyzed wheys and lactose solutions. Deproteinized and demineralized sirups concentrated to 65% total solids exhibited good solubilities, showed no mold growth, and had good humectant properties. A ready application for these sirups was found in caramel manufacture where the HL sirup showed the same stabilizing effect against sucrose crystallization as did "invert" sugar. Further applications for these sirups await development by food technologists.

Fermentation. Utilization of whey as a fermentation substrate, especially for single cell proteins (22,23,24) and alcohol production (25), has been studied extensively. A major obstacle to broader utilization of whey as a fermentation medium has been the fact that relatively few organisms are able to utilize lactose. Several yeasts were evaluated for alcohol production in whey (26) and, although *Kluyveromyces fragilis* was found to be the most efficient, only 55% of the lactose was converted to alcohol. The low conversion level has been attributed

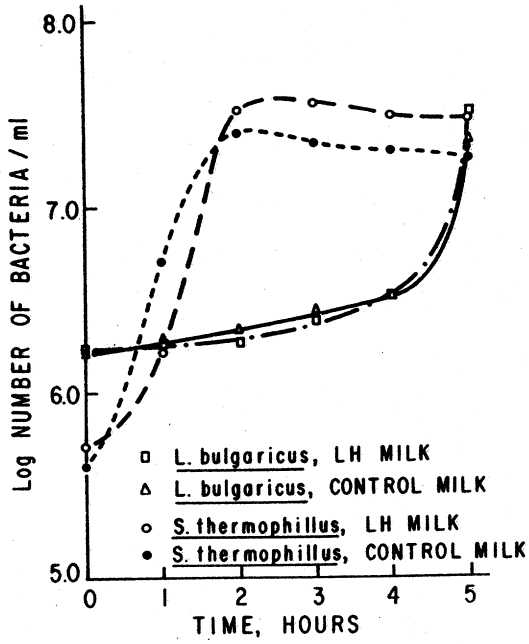


Figure 3. Growth of yogurt cultures in control and HL milks

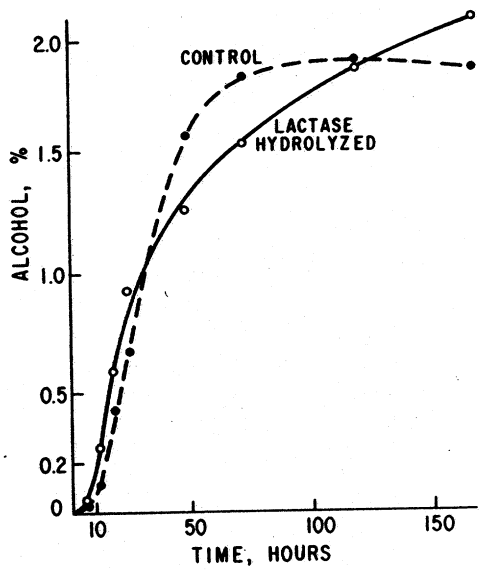


Figure 4. Alcohol production by K. fragilis *in control and HL acid whey*

to a possible inability of the yeast to tolerate high alcohol concentrations possibly because of sensitivity of the yeast lactase to alcohol. The availability of whey having its lactose hydrolyzed to its constituent monosaccharides permitted an evaluation of nonlactose fermenting organisms such as Saccharomyces cerevisiae, which tolerate high alcohol concentrations, for alcohol production. O'Leary et al. (27) evaluated HL wheys using S. cerevisiae and K. fragilis for comparison. Curves illustrating alcohol production by glucose-pregrown K. fragilis in control and HL wheys are shown in Figure 4. Ethanol production was more rapid in HL whey during the first 24 hr fermentation, but later decreased so that the rate of production was less than that in the control whey. The total yield of ethanol was similar in both cases and averaged 1.9%. Reducing sugar levels decreased at the same rate in the first 24 hr fermentation, but less utilization occured in HL wheys in the later stages of fermentation. The pattern of sugar utilization in the HL wheys (Figure 5) showed that although glucose disappeared rapidly during the first 24 hr fermentation, there was little change in the galactose concentration during this period. Galactose utilization began only after 24 hr fermentation and is typical of a diauxic pattern of sugar utilization. A longer fermentation period was required in the HL whey due to the diauxic phenomenon.

S. cerevisiae, a nonlactose fermenting yeast, is commonly used in wine and beer production because of its ability to withstand relatively high alcohol concentrations. Growth curves of S. cerevisiae, pregrown on glucose and on galactose, showed that similar cell populations existed in HL whey for the first 48 hr growth, but declined thereafter, except that those pregrown on galactose increased significantly after the intial decline. Alcohol production by these organisms in the HL wheys showed a similar pattern in that comparable levels of alcohol were attained after 48 hr (Figure 6). Alcohol production by the glucose pregrown S. cerevisiae began to decrease after 48 hr, whereas the galactose pregrown cells continued their alcohol production which reached a level twice that of the glucose-pregrown cells. Sugar utilization closely paralleled alcohol production. S. cerevisiae pregrown on glucose utilized glucose rapidly but did not utilize galactose. S. cerevisiae pregrown on galactose similarly utilized glucose but also utilized galactose after 96 hr. These studies indicated that although lactose hydrolysis in whey permits alcohol production by organisms unable to ferment lactose, the galactose released by hydrolysis is not efficiently utilized. In model system studies, the efficiency of converting sugar to alcohol by K. fragilis was as follows: glucose > lactose > galactose.

Conclusions

The dairy industry today has available existing technology for the application of enzymatic hydrolysis of lactose in milk

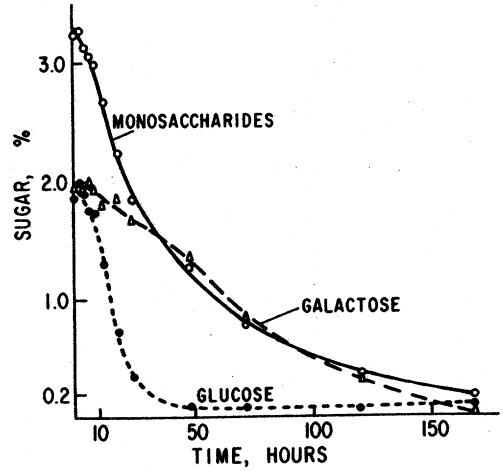


Figure 5. Change in sugar concentrations during fermentation of HL acid whey by K. fragilis

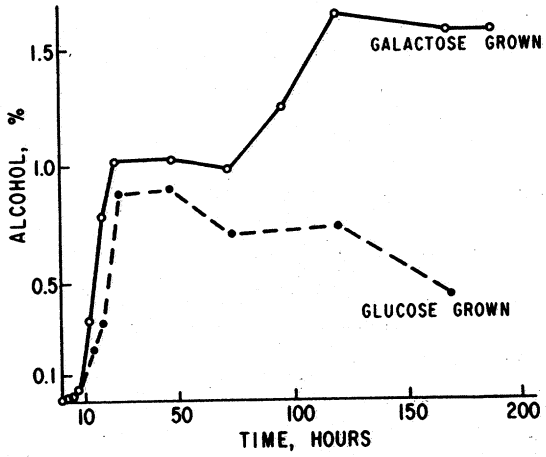


Figure 6. Alcohol production in HL acid whey by glucose-pregrown and galactose-pregrown S. cerevisiae

and milk products. Utilization of the lactase enzyme, in either "free" of immobilized systems, can result in quality improvements in a number of products and yield processing economies in others.

The application discussed in this report should be reviewed and assessed for potential benefits by dairy product manufacturers in their specific operations. Whey, in particular, with its ever increasing production, poses a special problem whose solution can be attained only through application of unconventional technology. It is believed that application of lactase offers a potential for innovative whey utilization.

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