

# Microbial technologies in the production of low-lactose dairy foods

## Tecnologías microbiológicas para la elaboración de productos lácteos con bajo contenido en lactosa

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Low-lactose milk products with 70% or more of the lactose hydrolysed by food grade  $\beta$ -galactosidase enzymes of yeasts or fungi have become widely accepted for alleviating the symptoms of lactose maldigestion. This condition limits the intake of nutritious dairy foods by large segments of the world's population. Alternative approaches recently proposed for dealing with lactose maldigestion include the supplementation of milk with dormant dairy cultures, treatment of milk with sonicated or permeabilized cultures as food-grade sources of  $\beta$ -galactosidase and the use of cold-active enzymes to hydrolyse lactose in milk under refrigerated storage conditions.

*Keywords:* lactose, dairy products,  $\beta$ -galactosidase, maldigestion

Los productos lácteos con bajo contenido en lactosa, hidrolizada en un 70% o más mediante enzimas de levaduras  $\beta$ -galactosidasa o de hongos, cada vez gozan de mayor aceptación y consumo para disminuir los síntomas de la mala digestión de la lactosa, condición que limita la ingestión de productos lácteos por determinados grupos de la población. Recientemente se han propuesto soluciones alternativas a este problema como son la adición a la leche de cultivos lácteos inactivos, el tratamiento de la leche con cultivos que sirvan de fuente de  $\beta$ -galactosidasa y el uso de enzimas que actúan a baja temperatura para hidrolizar a la leche en refrigeración.

*Palabras clave:* lactosa, productos lácteos,  $\beta$ -galactosidasa, indigestión

## INTRODUCTION

Lactose is a natural disaccharide found in milk of mammals. It is an important source of energy for the newborn and it also stimulates the intestinal absorption of calcium (Hourigan, 1984). Lactose digestibility is dependent on the presence of  $\beta$ -galactosidase (lactase) enzyme in the epithelial cells of the small intestine. After infancy, the  $\beta$ -galactosidase titre in the intestines gradually diminishes which, according to the best estimates, results in  $\beta$ -galactosidase

deficiency in 70% of the world's human population (National Dairy Council, 1985). The frequency of lactose maldigestion varies between different populations (Table 1).

Incomplete lactose digestion may result in unpleasant symptoms in humans (diarrhoea, bloating, flatulence) collectively referred to as lactose intolerance. The anticipated experience of distress compels many adult consumers to exclude from the daily diet some of the nutritionally most complete dietary items made available by the food processing industry.

Lactose intolerance may be alleviated in several ways. These include consuming reduced amounts of milk several times during the day, replacing milk with

**Table 1.** Geographical distribution of lactose maldigestion in human populations (Andersen and Barfoed, 1977).

**Tabla 1.** Distribución geográfica de las poblaciones que sufren de indigestión de lactosa (Andersen and Barfoed, 1977).

Area	Incidence of lactose maldigestion (%)
Russia	2
Scandinavia	4-14
British Isles	24
USA: Caucasian	6-25
African	45-81
Native	50-75
Hispanic	47-74
Asian	65-100
Africa	100
Japan	100

low-lactose dairy foods (cheeses), consuming fermented dairy foods (yoghurt, kefir) with a somewhat reduced lactose content brought about by microbial activity, or using essentially lactose-free dairy products in which lactose has been mostly eliminated by prior treatment with exogenous  $\beta$ -galactosidase enzymes.

This paper briefly reviews procedures currently in use for alleviating lactose maldigestion but focuses mainly on alternative approaches based on viable or permeabilized microbial cells that have been proposed by various research groups. More detailed information on broader aspects of lactose hydrolysis for food and non-food uses may be found elsewhere (IDF, 1993; Rao, 1996; Renner, 1996).

## CURRENT PALLIATIVE APPROACHES TO LACTOSE INTOLERANCE

### Consumption of fermented dairy foods

Dairy cultures such as *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* metabolize lactose for energy production and may reduce the lactose content of milk by as much as 30% in the course of dairy fermentations (Goodenough and Kleyn, 1976). Several research groups proposed that  $\beta$ -galactosidases present in yoghurt cultures may release their enzymes upon coming into contact with intestinal bile salts, thus further reducing the lactose content of the ingested dairy food (Kilara

and Shahani, 1976; Gilliland and Kim, 1984). Tests on human subjects confirmed that consumption of cultured yogurt actually reduced breath hydrogen levels in humans and treatment of yoghurt samples with bile increased the measurable levels of  $\beta$ -galactosidase activity (Alm, 1982; Gilliland and Kim, 1984). Thus, the simplest way of dealing with mild cases of lactose intolerance while assuring accessibility to milk's excellent nutritive components appeared to be the consumption of small amounts of fermented products like yoghurt. The potential advantages and limitations of treating lactose malabsorption with fermented dairy foods were reviewed by Savaiano and Levitt (1987).

However, for millions of consumers the relatively limited reduction in lactose content by natural fermentation may not be adequate to palliate the symptoms of lactose intolerance. Therefore, the production of milk or milk-based products with a high degree of lactose hydrolysis is fully justifiable, and may be achieved by pretreatment with exogenous  $\beta$ -galactosidases.

### Low-lactose milk production with exogenous $\beta$ -galactosidases

Microbial sources of  $\beta$ -galactosidases available for lactose hydrolysis include bacteria, yeasts and moulds (Table 2). As expected, each enzyme is characterized by a special set of requirements for optimum activity (e.g. pH, temperature, cofactor) which implies that not all  $\beta$ -galactosidases may be suitable for industrial applications. Enzymes used in food processing also must be free of contaminants that may lower product quality (e.g. proteases, lipases) and meet the legal requirements for food use (i.e. the enzyme and its source must qualify for the 'generally recognized as safe' or GRAS status specified by the Food and Drug Administration). In spite of the still growing list of  $\beta$ -galactosidases, relatively few have found application on an industrial scale.

Current industrial practice for reducing the lactose content in milk involves the addition of free, sterile  $\beta$ -galactosidase preparations derived primarily from *Kluyveromyces marxianus* (formerly *K. lactis*) or *Aspergillus niger* to milk at concentrations to cause 70% or greater hydrolysis of lactose under a variety of holding conditions (Modler *et al.*, 1993). Alternatively, lactose hydrolysis in milk may be accomplished more economically with  $\beta$ -galactosidases immobilized on suitable supports (Gikas and Lopez-Leiva, 1981; Greenberg and Mahoney, 1981; Bakken *et al.*, 1992). More recently, the addition of liposome-encapsulated  $\beta$ -galactosidase to milk was suggested;

**Table 2.** Microbial sources of  $\beta$ -galactosidase.**Tabla 2.** Fuentes microbiológicas de la  $\beta$ -galactosidasa.

Species	Reference
<i>Alternaria alternara</i>	Macris, 1982
<i>Aspergillus niger</i>	Jackson and Jelen, 1989
<i>Bacillus circulans</i>	Iida <i>et al.</i> , 1980
<i>B. coagulans</i>	Long and Lee, 1982
<i>B. subtilis</i>	Anema, 1964
<i>B. stearothermophilus</i>	Goodman and Pederson, 1976
<i>Bifidobacterium bifidum</i>	Dumortier <i>et al.</i> , 1994
<i>Fusarium moniliforme</i>	Macris and Markakis, 1981
<i>Kluyveromyces fragilis</i>	Mahoney and Whitaker, 1978
<i>K. marxianus</i>	Dickson <i>et al.</i> , 1979
<i>Lactobacillus bulgaricus</i>	Itoh <i>et al.</i> , 1980
<i>Neurospora crassa</i>	Comp and Lester, 1971
<i>Pediococcus</i> sp.	Bhowmik and Marth, 1990
<i>Streptococcus thermophilus</i>	Somkuti and Steinberg, 1979
<i>Thermus aquaticus</i>	Cowan <i>et al.</i> , 1980

this delays lactose hydrolysis and overcomes consumer objection to the sweeter flavour of low-lactose milk (Rao and Chawan, 1996).

Hydrolysed milk products prepared with yeast or fungal  $\beta$ -galactosidases are now widely available to consumers in supermarkets. In addition, enzymes are also available in tablet form for home use. In the USA, low-lactose milk products currently represent a \$78 million business and their market share continues to expand.

## ADVANCES IN THE UTILIZATION OF $\beta$ -GALACTOSIDASES OF DAIRY FERMENTATION MICROBES

Over the past few years several groups of researchers have advocated making use of the indigenous  $\beta$ -galactosidases present in dairy fermentation microbes for lactose reduction in dairy foods. The advantage of using these enzymes is that the cultures already enjoy a 'food grade' status as their biomass is ingested as a component of the finished products. Thus,  $\beta$ -galactosidases from dairy microbes may not need to be purified extensively. Another way to make low-lactose milk is by the use of dairy cultures directly as enzyme microcarriers, in which the  $\beta$ -galactosidase was made more accessible by cell permeabilizing techniques. These alternative approaches are summarized below.

### Supplementation of milk with dormant dairy cultures

Since dairy cultures ferment lactose to derive energy, it was a logical assumption that once ingested as components of fermented dairy foods, intracellular  $\beta$ -galactosidases of these microbes may contribute to the further degradation of lactose in the digestive tract (Kilara and Shahani, 1976). This notion later gave rise to studies on the supplementation of milk with non-growing dairy cultures.

The first microbe tested in the dormant state in unfermented milk to palliate symptoms of lactose maldigestion was *Lactobacillus acidophilus* (Payne *et al.*, 1981; Newcomer *et al.*, 1983; Kim and Gilliland, 1983). However, the results on the efficacy of ingested 'acidophilus milk' as an aid in lactose digestion were variable because of the differences among microbial strains, cell concentrations used, and levels in  $\beta$ -galactosidase activity (Johnson *et al.*, 1987).

In 1991, Lin *et al.* suggested that selected yoghurt starter cultures (*L. bulgaricus*, *S. thermophilus*) with high  $\beta$ -galactosidase activity may be added to milk to improve lactose digestibility in humans. The recommended cell density was  $1 \times 10^8$  colony forming units (cfu)/ml, corresponding to that usually attained in yoghurt fermentation. Under refrigeration conditions the cultures remained physiologically dormant in milk for up to 14 d without loss of enzyme activity. After ingestion, the  $\beta$ -galactosidase released by the cells in the intestinal tract contributed significantly to the hydrolysis of lactose, which was manifested by a

reduction in breath hydrogen concentrations in end alveolar breath samples. This approach requires the use of bile-sensitive strains to facilitate enzyme release by microbial cells in the intestinal tract.

The concept of supplementing milk with dormant yoghurt cultures may become more attractive and find practical applications with the selection or genetic development of  $\beta$ -galactosidase hyperproducing strains of *L. bulgaricus* and *S. thermophilus*. It is a convenient way of supplying reduced lactose milk to consumers who find the flavour of yoghurt-like fermented products unacceptable.

### Recovery of $\beta$ -galactosidase from autolysing cultures

The availability of dairy cultures undergoing autolysis under controllable conditions would obviously facilitate the convenient recovery of intracellular  $\beta$ -galactosidase from these food-grade microorganisms. Strains of *S. thermophilus* with autolytic properties were reported by Thomas and Crow (1983) and were subsequently used for enzyme production, characterization, and lactose hydrolysis in milk and whey, in both free and fibre-entrapped forms (Smart *et al.*, 1985; Chang and Mahoney, 1989; Yang *et al.*, 1993).

While the use of autolysing dairy cultures is an attractive solution to producing food-grade  $\beta$ -galactosidase, fluctuations in the level of autolytic response and enzyme yields remain a problem. Work is in progress on the characterization of the lytic mechanism in *S. thermophilus* that may lead to standardized cultures more suitable for enzyme production (R. Mahoney, personal communication).

### Lactose hydrolysis by sonicated microbial cells

The use of sonicated microbial cells for lactose hydrolysis was advanced by Jelen and coworkers (Shah and Jelen, 1991; Jelen, 1993) and was based on the earlier observations of Kilara and Shahani with sonicated yoghurt (1976) and Toba *et al.* with sonicated fermented milk (1990). Sonication causes extensive cell destruction or membrane destabilization resulting in release of intracellular  $\beta$ -galactosidase. With sonicated and unsonicated cells of *L. bulgaricus* ATCC 11842 at an initial cell density corresponding to  $1 \times 10^8$  cfu/ml, Jelen's group reported 85% and 25% hydrolysis of lactose in milk, respectively, after a 16 h incubation period at 55 °C. The sonication of biomass induced a ten-fold increase in measurable  $\beta$ -galactosidase activity per cell density unit and resulted in a lower drop in medium pH (6.3) in comparison with unsonicated control cells (pH 5.9).

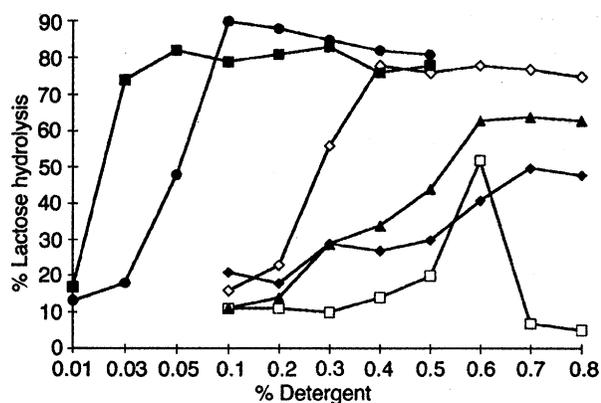
Research is currently in progress on more productive cell lines, improvement of the efficiency of cell disintegration and the selective release of  $\beta$ -galactosidase without contamination by proteases (P. Jelen, personal communication).

The apparent advantage of the sonication technique is the elimination of the need for enzyme purification. It is also possible that this approach may be extended to potentially useful  $\beta$ -galactosidases of thermophilic microbes (e.g. *Bacillus stearothermophilus*), which would permit milk processing at higher temperatures and minimize undesirable enzymic side reactions.

### Lactose hydrolysis by permeabilized cells

Permeabilization of microbial cells with organic solvents and detergents for the purpose of selectively releasing intracellular or membrane-bound enzymes has been used successfully with a variety of microorganisms under industrial conditions (Schutte and Kula, 1990). Disadvantages of these treatments include the possible denaturation of enzyme sought, contamination of product streams with chemicals and the need for fire hazard-free conditions. The main advantage of detergent treatment over other chemical treatments is the apparently selective release of certain proteins or enzymes from cells without the concomitant release of nucleic acids (Schutte and Kula, 1990).

The use of chemical permeabilization to dairy lactic cultures to enhance the accessibility of exogenous lactose to intracellular  $\beta$ -galactosidase has been investigated in our laboratories (Somkuti and Steinberg, 1990, 1993; Somkuti *et al.*, 1996). The permeabilization procedure apparently inactivates the lactose permease system normally responsible for the active transport of the disaccharide into the cell's interior. As a result, lactose freely passes into treated cells and is hydrolysed at a high rate to glucose and galactose, after which the hydrolytic products freely exit from permeabilized cells into the environment. The treatment of *S. thermophilus* cells with an acetone/toluene mixture or a variety of compounds with detergent activity allows full expression of intracellular  $\beta$ -galactosidase but without causing cell lysis and the loss of enzyme activity (Somkuti and Steinberg, 1994). Among compounds tested, sodium salts of deoxycholic and chenodeoxycholic acids, Triton X-100 and sodium dodecyl sulfate (SDS) showed the highest permeabilizing activities, each characterized by an optimum concentration range (Figure 1). Cholic acid and conjugated forms of all three bile salts were less effective. Tween 80 and Brij 35 failed to permeabilize *S. thermophilus* cells.

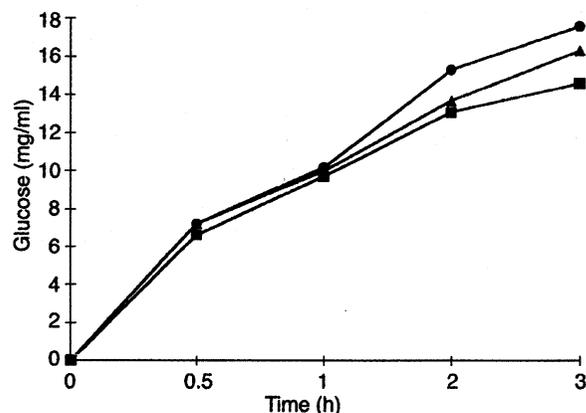


**Figure 1.** Lactose hydrolysis by *S. thermophilus* ( $1 \times 10^8$  cfu/ml) after 20 min treatment with: SDS (●); Triton X-100 (■); Na-cholate (□); Na-deoxycholate (▲); Na-glycodeoxycholate (◇); and Na-taurodeoxy-cholate (◆). Substrate: 5% lactose (pH 7.0); incubation time, 10 min.

**Figure 1.** Hidrólisis de lactosa con *S. thermophilus* ( $1 \times 10^8$  cfu ml<sup>-1</sup>) después de un tratamiento durante 20 min con SDS (●), Triton X-100 (■), Na-cholate (□), Na-deoxycholate (▲), Na-glycodeoxycholate (◇) and Na-taurodeoxy-cholate (◆). Substrate: 5% lactose (pH 7.0). Tiempo de incubación: 10 min.

We have also shown that some detergents suppressed culture growth for several hours, long enough to permit the *in situ* use of permeabilized *S. thermophilus* biomass for lactose hydrolysis in milk, without concern about concomitant production of lactic acid and the souring of the product that would normally occur in dairy fermentations (Somkuti and Steinberg, 1995). Figure 2 shows a time study on lactose hydrolysis in aqueous solution, whey and milk by SDS-treated cells of *S. thermophilus*. At comparable cell densities, similar results were obtained with agarose-entrapped cell preparations. Permeabilized cell preparations could be stored in the frozen state for 6 months or longer and at refrigeration temperature (6 °C) for up to 3 months without the loss of  $\beta$ -galactosidase activity. Immobilization of treated *S. thermophilus* cells was possible by entrapment in agarose films that could be used repeatedly for lactose hydrolysis in milk or whey without loss of activity. Another cost-saving aspect of cell permeabilization with detergent compounds is that the same detergent solution may be reused several times without reduction in efficacy.

The main advantages of using permeabilized cell suspensions of *S. thermophilus* for lactose hydrolysis in milk include: full expression of intracellular



**Figure 2.** Lactose hydrolysis in: 5% lactose (■); whey (▲); and skim milk (●) by *S. thermophilus* cells ( $1 \times 10^8$  cfu/ml) treated with 0.1% sodium dodecyl sulfate.

**Figura 2.** Hidrólisis de lactosa con *S. thermophilus* cells ( $1 \times 10^8$  cfu/ml) en presencia de dodecil sulfato sódico al 0,1%: 5% lactose (■), suero (▲) y leche desnatada (●).

$\beta$ -galactosidase activity, a relatively high (50–55 °C) processing temperature; absence of contaminating nucleic acids and extraneous enzyme activities in the product; prolonged cessation of culture growth which precludes the formation of acidic metabolites during treatment; and the suitability of the technique to both batch and continuous processing of milk or whey. Further improvement in the process will be made by selecting strains with increased levels of intracellular  $\beta$ -galactosidase activity and the development of more stable and reusable supports for immobilizing the permeabilized biomass.

## COLD-ACTIVE $\beta$ -GALACTOSIDASES

Enzymes, including  $\beta$ -galactosidase, with high catalytic activities below 20 °C may have important applications in food processing. Cold-active  $\beta$ -galactosidase may be suitable for reduction of the lactose content of refrigerated milk to benefit human subjects inconvenienced by lactose malabsorption, and to hydrolyse lactose in cheese industry whey effluents to glucose and galactose to facilitate bioconversion and biodegradation, thus alleviating environmental pollution.

Recently, two reports have been published on cold-active  $\beta$ -galactosidases. The enzyme of *Bacillus subtilis* KL88, a strain adapted to grow at low temperatures, was optimally active between pH 6.0 and 8.0 and at

50 °C but retained over 50%  $\beta$ -galactosidase activity at 10 °C. The degree of lactose hydrolysis in milk was concentration dependent and up to 80% of lactose could be hydrolysed during an 8 h incubation period (Rahim and Lee, 1991; Torres and Lee, 1995).

Another cold active  $\beta$ -galactosidase was recently characterized in an apparently novel psychrotrophic strain of *Arthrobacter* sp. which was recovered from fields sprayed with cheese whey at wintertime (Loveland *et al.*, 1994). This organism could grow at 0 °C and its enzyme required pH 7.2, 40 °C and 1 mM Mg<sup>2+</sup> for optimum activity, and retained 25% of lactase activity at 10 °C (Trimbur *et al.*, 1994).

The discovery and characterization of more cold-active bacterial and fungal  $\beta$ -galactosidases may permit the development of yet another approach to lactose reduction in milk under refrigerated storage conditions. These enzymes and their biotechnological applications will continue to receive attention in the future.

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