Dairy Products Modified in their Lactose Content

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Abstract: Lactose intolerance is a problem suffered by a large part of the world population. The simplest solution is to remove (partially or completely) dairy products from diet. However, a more convenient alternative from the nutritional viewpoint would be to replace the intake of regular dairy products by others in which the lactose content has been modified. In effect, the dairy products with reduced-lactose and lactose-free contents are examples of added-value products, whose production is targeted to a specific group of consumers. The increased manufacture of these dairy products is driven by the increasing knowledge we have on the lactose intolerance problem.

There are different technological methods to modify the lactose content in dairy products, such as enzymatic hydrolysis, ultrafiltration and chromatography. The lactose hydrolysis using β-galactosidases is the strategy most widely used in the industry, making it possible to obtain products with low lactose levels. A wide range of this type of products such as milks, creams, ice-creams and fermented milks, are commercially available around the world. In Latin America, which records a very high incidence of deficient-lactase individuals, there are delactosed products available such as fluid and powder milks and some fermented milks. The consumption of fermented dairy products has undergone a rapid increase in Argentina, where the yogurt is the most popular product. In this context, we are working on different aspects on the production of lactose-hydrolyzed yogurt, as this product is still not available in the Argentinean market.

The purpose of this contribution is to review the current knowledge on the lactose intolerance problem and the reduced-lactose dairy products, with special emphasis on the applied technological processes. Preliminary results obtained by our research group are also included.

Keywords: Lactose intolerance, technological process, lactose-modified dairy products.

1. INTRODUCTION

The problem of lactose intolerance is quite widespread in most part of the world. In effect, it has been estimated that over 70% of the world population suffers from the inability to use lactose. This is caused by the lack of lactase activity in the brush border of the small intestine. Consequently, these individuals suffer from severe intestinal disorders such as cramps and diarrhea. Under normal conditions, the lactose is easily absorbed from the intestine after hydrolysis to its monosaccharide moieties glucose and galactose [1-5].

Milk is a natural and essential food, as a source of valuable proteins, vitamins, minerals and lactose [6]. An effective and acceptable solution to allow the consumption of dairy products for those with lactose intolerance would be to remove or reduce the lactose content from milk [7, 8]. In fact, the interest of dairy industries in the development of reduced-lactose and lactose-free products has progressively increased, stimulated mainly by the knowledge acquired on the lactose intolerance problem [7, 9]. Various technologies are employed for their production, such as enzymatic hydrolysis of lactose with soluble or immobilized enzymes, and the physical processes of lactose separation (ultrafiltration and chromatography) [7].

The industry of fermented milks is one of the most dynamic sectors since it is in a continuous development of innovative products as a response to the growing demand of consumers of natural, fresh and healthy products [9]. In South America, where the prevalence of lactose intolerance is high, there are delactosed products available such as fluid and powder milks and some fermented milks. Only UHT and powder delactosed milks are commercially available in Argentina. In this context, we are working on different aspects about obtaining the lactose-hydrolyzed yogurt.

The purpose of this contribution is to review the current knowledge on the lactose intolerance problem and the reduced-lactose dairy products available in the market, with special emphasis on the applied technological processes including preliminary results obtained by our research group.

2. CHARACTERISTICS AND NUTRITIONAL SIGNIFICANCE OF LACTOSE

Lactose (β-D-galactosyl-D-glucose) is a unique disaccharide because it occurs exclusively in the milk of mammals. Its concentration is variable, human milk has the highest content (of about 7g per 100mL) while cow milk has a lower quantity of 4.5-5g per 100mL, approximately [1, 10].

As far as it is known, the lactose is the most important source of energy during the first year of human life, providing almost half the total energy requirement of infants [11].

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2212-3881/12 $58.00+.00

It also has important biological functions such as stimulating the growth of bifidobacteria in the gut and supplying galactose, an essential nutrient for the formation of cerebral galactolipids [8]. The lactose, as well as some other sugars, acts as carrier of minerals through ligand-formation, being able to facilitate their absorption [1].

To be absorbed, lactose must necessarily be hydrolyzed into its constituents - glucose and galactose. This is carried out mainly by the β-galactosidase enzyme (β-D-galactoside galacto-hydrolase, E.C. 3.2.1.23), also called by its trivial name: lactase. This enzyme is anchored to the brush border membrane of the small intestine mucosa [1, 2, 5], showing maximum activity in the jejunum and a lower level in the duodenum and ileum [3].

The monosaccharides are actively absorbed from the small intestine after lactose splitting; then, they are transported to the liver *via* the portal vein and finally utilized as an energy source or building bricks in the body. After absorption, galactose turns into glucose in the liver by Leloir pathway, which involves three enzymes (galactokinase, galactose-1-phosphate uridyltransferase and uridine-diphosphogalactose-4-epimerase). A deficiency in one of these enzymes can lead to metabolic disturbances known as lactose intolerance and galactosemia [1].

3. LACTOSE INTOLERANCE

Lactose intolerance describes the presence of gastrointestinal symptoms such as diarrhea, abdominal pain and cramps, bloating, gas formation (flatulence), nausea, and loss of appetite resulting from maldigestion or malabsorption of lactose. ***Lactose malabsorption*** simply refers to the incomplete digestion of lactose, since it is not fully absorbed in the small bowel and thus delivered to the colon where it can be utilized as a fermentable substrate by bacterial flora producing fatty acids and gaseous by-products. Besides this, undigested lactose in the colon increases the osmotic load resulting in a higher water flow toward the lumen [2, 4, 11].

Although both terms, 'lactose malabsorption' and 'lactose intolerance', are often employed as synonyms, the demonstration that an individual cannot properly absorb lactose does not necessarily mean that the individual will develop clinical symptoms [2, 4]. The appearance of these symptoms after ingestion of lactose varies from person to person and it depends on many factors. The amount of lactose consumed, the dietary components co-ingested with lactose (meal effect), the lactase activity degree of the mucosa, the rate of gastric emptying, the gastrointestinal transit time, the pattern of the flora in the large intestine and psychological factors of individuals that are not still known [4, 11-13], are some of these factors.

In addition to the malabsorption of lactose, a loss of calcium and other minerals occurs, which represents an important factor in the decalcification process of the skeleton [14].

Sometimes, lactose intolerance is confused with cow milk intolerance because the symptoms are often similar; however, these problems are not related. The intolerance to cow milk results from an allergic reaction to milk protein triggered by the immune system, while lactose intolerance is a problem caused by the digestive system as was mentioned above [15].

Several methods have been developed to objectively diagnose lactose intolerance in humans. The most widely employed are the blood glucose and the breath hydrogen tests. Both methods indicate the ability of a person to digest the lactose rather than the individual expression of lactase [2].

In the first test, a baseline measurement of blood glucose is taken before ingestion of a lactose load and then at time intervals during a period of 2 hours. An increase in blood glucose concentration indicates lactose digestion, since lactase hydrolyzes the lactose into glucose and galactose, allowing its absorption into the bloodstream and its subsequent detection. A small increase or no increase in blood glucose is indicative of a low activity of lactase or lack of activity, respectively; consequently, malabsorption or intolerance to lactose may be present [2, 3].

The breath hydrogen test is less invasive, more accurate, sensitive, specific and popular than the former. It measures the hydrogen production by colonic bacteria. If lactose meets the enzyme lactase in the small intestine, no change in breath hydrogen is observed and the diagnosis is lactose tolerant. On the contrary, in lactose maldigesters, the lactose passes through the small intestine escaping digestion, reaches the colon and is fermented by the bacterial flora producing gases, mainly hydrogen. Part of this hydrogen is absorbed into the bloodstream and, when it passes through the lungs the gas exchange takes place and the hydrogen is exhaled into the breath. The amount of hydrogen released is proportional to the hydrogen produced, which is related to the quantity of unabsorbed lactose. A baseline measurement of breath hydrogen is taken previously to the lactose ingestion; further readings are taken at 30 minutes intervals during the following 3 hours [2]. A rise in hydrogen concentration as a function of time after consumption is used as an indicator of malabsorption or intolerance to lactose [3].

On the other hand, lactase activity may be low immediately after birth and increases independently of milk intake, but soon it begins to decrease considerably after weaning. Lactose intolerance may develop in children after two years of age, gradually increasing to childhood. In the same way as mammals, humans lose their ability to digest lactose in adult age because the β-galactosidase synthesis is reduced. This fact is a genetically controlled trait that varies among populations; a genetic mutation present in a few populations causes the enzyme level of childhood to remain unchanged throughout adulthood [2, 3, 8]. Populations of Northern and Central Europe, Australia and New Zealand have a low incidence of intolerance, but the prevalence is above 50% in South America, Africa and Asia reaching almost 100% in some Asian countries. In Japan and China, about 70-100% of population suffers of lactose intolerance. In the Unites States, the prevalence varies from 15% among whites to 80% in the black population [4, 9, 11].

3.1. Alternatives to Alleviate the Symptoms of Lactose Intolerance

As there is no treatment to increase the body ability to produce lactase, the only solution to this problem would be to partially (or completely) remove the milk or dairy products from the diet [2]. Accordingly, the risk of consuming

low levels of calcium and other nutrients (vitamins, phosphorus, etc.) that milk conveniently provided would be present [16, 17]. In fact, milk is a natural, essential, well-balanced and generally accepted food [6], so it would be very difficult to find a suitable substitute.

Several alternative approaches were reported in the management and treatment of lactose intolerance [18].

It is usually recommended for lactase-deficient individuals to learn to regulate the amount of milk consumed at one time without symptoms. Most individuals are able to digest small quantities of milk (one glass of milk of about 250 mL) without experiencing symptoms, especially if taken with a meal [3, 19]. Studies have shown that lactose digestion improves with the yogurt ingestion compared with milk. Although the mechanisms involved in this aspect are not clear, it is believed that this greater absorption is due to in part to the production of β-galactosidases by the live bacteria of starter culture added in yogurt production, and by probiotic bacteria when the yogurt formulation requires it [11, 20-26]. Other mechanism suggests that the pH reduction caused by the production of lactic acid during fermentation induces a finer suspension of protein matrix that promotes a better digestibility of yogurt [24].

On the other hand, pharmaceutical preparations of β-galactosidase derived from fungi or yeast have been developed for the treatment of this problem. Oral preparations in tablet form are ingested together with food intake [8, 11, 19]. Furthermore, the consumer himself can prepare milk low in lactose at home by adding the enzyme in capsules or in liquid form, which is then left in the refrigerator overnight [7]. Although there is evidence that this procedure increases lactose digestion and alleviates symptoms, the different preparations available commercially seem to vary in their effectiveness [11].

Nowadays, the consumption of lactose-modified dairy products constitutes an attractive way to get nutritional rich milk with a reduced level of lactose and results in a more convenient alternative to treat the lactose intolerance problem [8, 16]. Indeed, the dairy industry has developed both reduced-lactose dairy products and lactose-free dairy products, which are increasingly available on the retail market around the world. Fluid milks, such as pasteurized and UHT milks, fermented milks, milk powders, creams, and ice-creams [5, 7, 9, 13] are more widespread. The most popular lines of products in the US and Europe markets include Lactaid, Dairy Ease, Mootopia, HYLA® of Valio Ltd., Emmi, Kaiku, and Lacto-free of Arla [7]. The first products were launched 30 years ago (in 1980s), as sales began to grow slowly [27]. In Latin America, fluid and powder milks and some fermented milks are examples of such products. Particularly in Argentina, only one dairy company (www.laserenisima.com.ar/productos) produces UHT and powder milks with a lower content of lactose than the regular milks.

4. INDUSTRIALLY APPLICABLE METHODS TO REDUCE LACTOSE CONTENT

Enzymatic hydrolysis is one of the most important biotechnological processes, in which soluble or immobilized β-galactosidases are employed. Other technological approaches are based on the physical separation of lactose by ultrafiltration and chromatography [7, 8].

4.1. Enzymatic Hydrolysis Process

4.1.1. General Aspects of the β-galactosidase Enzyme

Food processing using biological agents is historically a well-established procedure. The trend for the design and implementation of processes, including enzymes, has steadily increased in the food industry. The enzymes are generally considered as natural products, which have the ability to operate at mild conditions of pH, temperature and pressure with high enzymatic activity and biodegradability. Besides this, the amount of by-products and energy requirements are relatively low, but some limitations for their commercial scale application include the high cost, low productivity and stability, the inhibition by the final product and the kinetic parameters of the reaction. Of all enzyme classes applied in food area, hydrolases are the most frequently used [28, 29].

β-galactosidases are a widespread family of glycoside hydrolase enzymes (or glycosidase), capable of catalyzing the hydrolysis of the glycosidic linkage of lactose to generate two smaller sugars. The lactose is a specific substrate for lactases, and while all lactases can be classified as β-galactosidases, the converse is not true [13].

The reaction outcome depends on the relative abundance of the hydroxyl acceptors [30].

In general, glycosidases can transfer the glycosyl moiety of a substrate to hydroxyl acceptors. Hydrolysis represents a special case where water serves as the hydroxyl acceptor [30].

The mechanism of β-galactosidase action on lactose is not completely elucidated, but the hydrolytic reaction can be described as follows [5]:

$$E + L \longrightarrow E\text{-}L \text{ (complex)}$$

$$E\text{-}L \longrightarrow E\text{-}Gal^{+} + Glu$$

$$E\text{-}Gal^{+} + H_2O \longrightarrow E + Gal$$

Where E: β-galactosidase enzyme, L: lactose, E-L: enzyme-lactose complex, $E\text{-}Gal^{+}$: enzyme-galactose intermediate, Gal: galactose, Glu: glucose.

The lactose hydrolysis reaction rarely leads to complete conversion of both monosaccharides, due to feedback inhibition of the reaction by galactose as well as to the concurrent side reactions that produce oligosaccharides [5, 30]. The synthesis of oligosaccharides occurs by the transferase activity of enzyme through a reaction called transglycosylation. The enzyme-galactose intermediate reacts with another sugar molecule (e.g. lactose, glucose or galactose) instead of water [5]. While initially the oligosaccharides, mainly composed by galacto-oligosaccharides (GOS), were considered undesirable by-products of lactose hydrolysis, recent investigations have suggested that these compounds are resistant to digestive enzymes and they have a beneficial effect on human intestinal health by promoting the growth of bifidobacteria (bifidogenic factor) [1, 5, 30-32].

The factors that govern the hydrolysis process are the lactose concentration in the raw material, and the reaction conditions such as temperature, pH and the time of contact between lactase and the substrate [7]. The secondary reaction of transgalactosylation can be minimized by choosing a suitable enzyme and proper working conditions [30].

β-galactosidases are widely distributed in nature [5, 33]. They have been detected in plants (peach, apricot, almond [33], chicken bean [34], orange [35], tomato [36], and strawberry [37], among others) and animals (mammalian intestinal tract [30]) and microorganisms [5, 13, 33]; however, those of microbial origin including bacteria, fungi and yeasts are preferred because of their higher performance in the production of these enzymes. In recent years, marine environment has also been considered as a source for enzymes from either microbial or the higher organism origin [28, 30, 38].

Although numerous microbial β-galactosidases have been purified and characterized, those employed in the dairy industry are isolated from a rather small number of microorganisms with GRAS status (generally recognized as safe). β-galactosidases with a great commercial significance originate from yeasts: *Kluyveromyces lactis*, *K. fragilis* and *Candida pseudotropicalis*, from fungi: *Aspergillus niger* and *A. oryzae*, and *Streptococcus thermophilus*, *Bacillus subtilis* from bacteria [5, 8, 39].

Characteristics such as specificity, structure, molecular weight, amino acid sequence, position of the active site, and the pH and temperature optimum differ significantly according to the microbial source of the β-galactosidase [8, 30]. This enzyme is usually intracellular in bacteria and yeasts, but it can be intra- or extracellular in fungi. The temperature and pH optimum of the lactases are the predominant factors determining the potential area of application. In effect, the lactases from *Kluyveromyces* with a neutral optimum pH (6.5-7.5) are usually used to hydrolyse lactose in milk at a moderate temperature (30-40°C) because they are thermolabile [5, 8, 30]. These working conditions can encourage microbial growth. Fungal β-galactosidases with an acid optimum pH have the advantage of being more thermostable than those from yeasts and they can be used at temperatures up to 50°C [5, 30]. The higher temperatures and acidic environment are effective in minimizing microbial growth [5]. To avoid microbial contamination, thermostable and cold-active β-galactosidases are increasingly used in lactose hydrolysis [28, 30, 40].

Depending on the β-galactosidase origin, the presence of ions may have a positive or negative effect on the enzymatic activity. In addition, the magnitude of this effect also differs depending on whether the enzyme is used in whey, milk or buffered lactose [13, 41]. In general, divalent cations such as Mn^{2+} and Mg^{2+} can improve the activity while monovalent cations such as Na^{+} and K^{+} can produce a variable effect depending on the enzyme origin; heavy metals inhibit the activity of all β-galactosidases [8, 13]. In relation to calcium, as it is an important component of milk [52], several studies have evaluated its effect on the enzymatic activity.

In fact, β-galactosidases from *Kluyveromyces lactis* require Na^{+}, K^{+} [42, 44] and Mn^{2+} [43] and those from *K. fragilis* need Mn^{2+}, Mg^{2+} and K^{+} [43]. In contrast, the fungal β-galactosidases do not need ions to act [8, 13].

Some studies have been performed to describe the effect of ions on the activity of β-galactosidases from lactic acid bacteria (LAB). The activity of β-galactosidases from *Lactobacillus delbruekii* ssp. *bulgaricus*, *L. casei*, *Lactococcus lactis* ssp. *lactis*, *Streptococcus thermophilus*, *Bifidobacterium bifidum* was enhanced by Mg^{2+}, while the effect of K^{+} and Na^{+} differed from strain to strain [45]. Rhimi *et al.* [46] found that Mn^{2+} and Co^{+2} enhanced the β-galactosidase activity from *L. bulgaricus*, while Cu^{+2} caused a decrease in the activity. Variable results were found regarding the effect of calcium, which depended on the calcium concentration used. No activity loss was observed at low concentrations (1 – 4mM) [46, 47], whereas an inhibition of the enzymatic activity was reported at higher levels [47-50]. Nevertheless, the enzyme retains a high percentage of its original activity at the calcium concentration found in milk [47, 50-52], allowing the production of reduced-lactose dairy products.

4.1.2. Hydrolysis Methods

The enzymatic process can be accomplished in different ways. The most popular methods involve the use of purified β-galactosidases, both *free* or *immobilized*. A more recent technique with potential application in lactose hydrolysis utilizes cell cultures that provide the β-galactosidases without further purification.

The method that employs the soluble or free enzyme is the most widely applied because of its feasibility of implementation at an industrial scale. Purified β-galactosidase is added in solution and is discarded after a single usage, which represents a disadvantage. The technology of immobilized enzyme, in which the enzyme is physically localised on a solid matrix retaining its catalytic activity, has been developed to better control the reaction and to allow the reuse of enzyme. The soluble enzyme is normally used for batch process but the immobilized form has the advantage of being used in batch wise as well as in the continuous operation [28, 39, 40].

As mentioned above, the microbial contamination is another problem associated with the enzymatic hydrolysis process, which leads to the necessary implementation of intermittent sanitation steps in the continuous processing of milk on a large-scale [29, 30].

β-Galactosidases are one of the most studied enzymes in terms of their immobilization; various types of support matrices (cotton, alumina, glass beads, silanized porous glass, phenol formaldehyde resin, corn grits, sepharose, fructogel derivates [53], silica, cellulose beads, graphite slabs [39]) and immobilization methods have been performed [39]. Although many studies describe the effective immobilization of β-galactosidase isolated from *Escherichia coli*, its application in food industry is an obstacle because this microorganism is not GRAS [13, 30].

The immobilization methods include physical adsorption, entrapment/(micro)encapsulation, covalent binding [54], cross-linking or the combination thereof [28, 39]. *Physical adsorption* is the oldest method of immobilization. It is based on the physical interactions between the biocatalyst and the support with hydrogen bonding, hydrophobic inter-

actions and van der Waals forces [39]. In the *entrapment/(micro)encapsulation method*, the enzyme is located within a given structure such as a network of natural or synthetic polymers or a gel, a membrane as a hollow fiber or a microcapsule [28, 39]. In the *covalent bonding method*, a strong bonding between the enzyme and the support occurs through the functional groups that are not essential for the enzyme catalytic activity [39, 54]. The *cross-linking method* utilizes multifunctional compounds, which act as reagent for intermolecular cross-linking of the enzyme. This approach is often employed in combination with any of the methods mentioned above.

Each method has its own advantages and drawbacks [39]. The first two methods are the simplest, but have certain limitations such as desorption and leakage of the enzyme, which could lead to low stability and catalytic activity. In the covalent bonding method, the enzyme activity could diminish as a result of damage to its active site and distortion of its native structure [53, 54]. To minimize this problem, Song *et al.* [54] studied the pretreatment of β-galactosidase from *K. lactis* with lactose to prevent the formation of covalent bonds near the active site during the immobilization process. This approach improved the enzyme activity, which was significantly higher than that of the immobilized enzyme without pretreatment. Moreover, the treated enzyme showed a better reusability because a high level of activity was retained after 10 reuses.

The fact that immobilization processes have not been implemented widely in the industry is due to the increased chances of microbiological contamination since long time at a high temperature and high cost of such process [5, 13, 40].

Finally, the method of whole cell culture is a convenient and inexpensive strategy to obtain lactose-hydrolysed products, even for the small dairy plants [55]. This method provides β-galactosidases without purification; it includes the growth of a culture from bacteria or yeasts (with GRAS status) with high β-galactosidase activity, the concentration of the cell biomass, and the disruption or permeabilization of the concentrated culture. Cell disruption implies the breakage of the cell envelope and the release of all intracellular constituents into the surrounding medium. Cell permeabilization involves the loss of the cell membrane ability to control the active transport of solutes, consequently allowing the passive passage of low molecular weight solutes in and out of cells, including lactose and its products of hydrolysis [27, 55-58].

Different procedures of disruption have been studied, such as sonication [59], bead milling [60], and microfluidizer [61], among others. In this respect, Bury *et al.* [62] compared sonication, high-pressure homogeneization and bead milling with respect to the release of β-galactosidase from *Lb. delbrueckii* ssp. *bulgaricus*, and they found that the last two procedures were the most effective, for being suitable for their industrial application in milk.

In the permeabilization treatments, differents compounds such as ethanol, synthetic detergents (SDS, Triton) and bile salt preparations (deoxycholate, Oxgall) have been studied as permeabilization agents, in order to obtain a biomass with high β-galactosidase activity for direct application to hydrolyse lactose. High levels of lactose hydrolysis were reached in milk and aqueous solutions using a permeabilized biomass of bacteria (*S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*) or yeast (*Kluyveromyces marxianus*) [57, 58, 63, 64].

4.1.3. Production of Lactose-Hydrolyzed Milks

In general, the hydrolysis process for industrial production of lactose-hydrolyzed milks can be described as follows: Milk is pasteurized and cooled to the required incubation temperature, e.g. 5°C [5], the enzyme is added and the milk is left overnight in the hydrolysis tank. When the desired degree of hydrolysis is reached (typically 70% or 100%), the milk is pasteurized again to inactivate (denaturalize) the enzyme and is then packed in retail containers [5, 29]. Pasteurization conditions before and after the hydrolysis step are 72-75°C/15s, alternatively the hydrolysis can also be done after thermization at 68°C/15s, followed by a pasteurization at 72-75°C/15s [29]. Sterilized milks with lactose reduced content can be obtained applying the UHT treatment before (post-hydrolysis) or after (pre-hydrolysis) the hydrolysis step. In the first case, a small dosage of sterilised enzyme (for example, 10 mg/kg of milk) is injected into UHT milk pack before sealing and the hydrolysis is produced during the storage of product at room temperature (for example, 7-10 days until the near complete hydrolysis) [5, 9, 65, 66]. The advantage of this procedure is that it uses a small amount of enzyme reducing processing costs, but it presents the drawback that the enzyme must be very pure and free of protease activity [5, 66]. In the second process, the milk is hydrolyzed in a tank with a higher amount of enzyme (e.g. at 5-10°C for 24 h) before UHT treatment and then packed [66, 67].

The intensity and sequence of thermal and hydrolytic treatments produce a decrease in the quality of this type of milks [7, 65]. In addition, other factors such as quality of raw milk and storage temperature have an impact on the shelf life of hydrolyzed milks [66].

The occurrence of the Maillard reaction is increased in the hydrolyzed milk, especially in UHT milk. The extent of this change will depend on the percentage of hydrolysis reached [7]. The Maillard reaction is a nonenzymatic browning reaction between reducing sugars and free amino groups of proteins; the ε-amino group of lysine is the functional group most reactive in milk proteins. In effect, in hydrolyzed milks the molar quantity of reducing sugars is doubled, besides the glucose and galactose being more reactive than lactose [68, 69]. Once the Maillard reaction starts, it continues easily to further stages during storage. The furosine content is one of the parameters used to evaluate the Maillard reaction.

Tossavainen *et al.* [68] analyzed the effect of pasteurization temperature (60-90°C during 15s) on furosine formation. They found that the furosine level was higher in hydrolyzed milks than in unhydrolyzed milks, even at the lowest temperature of pasteurization. Results obtained by Messia *et al.* [65] confirmed the high reactivity of UHT-hydrolyzed milks to the Maillard reaction and their more limited chemical stability when they are stored for 4 months at 20°C, by monitoring the glycidic fraction and thermal treatment markers (furosine, lactulose, fructose). Tossavainen *et al.* [69] evaluated the furosine formation in pre-hydrolyzed UHT

milks during storage for 8 weeks at 8°C. Once again, the furosine content in these milks was higher than in unhydrolyzed milks.

Moreover, some commercial preparations of β-galactosidase have several side activities and among these, the proteolytic ones are common [66]. The proteolytic enzymes are highly heat stable and may not be inactivated by pasteurization or even UHT treatment, resulting in poor shelf life of hydrolyzed milks caused by protein coagulation during storage [7]. Tossavainen *et al.* [66] found a significantly higher proteolysis in hydrolyzed UHT milks compared with unhydrolysed milks during 3 months of storage at different temperatures (5, 22, 30 and 45ºC). The proteolysis was similar in pre- and post-hydrolyzed milks, even taking into account that the dosage of enzyme was much higher in the former. This fact points out that a large part of the proteolytic activities was destroyed during the UHT treatment of the pre-hydrolyzed milk. In relation to the behaviour during storage, the results showed that the proteolytic activities of the remaining enzyme were active during storage at 22, 30 and 45 ºC. The degree of proteolysis was highest at 45°C, while it was non-existent at 5°C.

On the other hand, hydrolyzed milks have a total carbohydrate content and calories similar to the unhydrolyzed products. Besides, they are generally characterized by a greater level of sweetness than regular milks as they have a higher content of glucose and galactose [7, 27, 70]. This sensorial characteristic is often disliked by consumers [7] and perceived as unnatural [27]. This fact is its main drawback and has constrained the sale of these products, causing some subjects to change to soy or oat "milks" [7].

4.1.4. Lactose-Hydrolyzed Yogurt

Yogurt is defined as the fermented milk obtained by lactic acid fermentation due to the presence of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which grow in synergy in milk. The final product might contain a significant quantity of these live and active cultures. These bacteria metabolise a part of the lactose into lactic acid, producing a change in the consistency of milk in that of yogurt [71, 72].

From a nutritional viewpoint, yogurt has been recognized as an excellent source of vitamins [73-75], minerals [76, 77] and high-quality protein [78-80]. More recently, the attention has been focused on the nutritional role of certain functional compounds such as GOS [81-83], bioactive peptides [84, 85] and conjugated linoleic acids (CLA) [86-88], which can be generated *in situ* in the food matrix under certain conditions or added as additives.

Although it has been postulated that the consumption of yogurt improves the digestion of lactose by lactose intolerants, contradictory results have been published depending on the amount of lactose contained in the yogurt and the degree of intolerance, among other factors [11, 18, 71, 89, 90].

Nowadays, there are few publications related to the development of hydrolysed yogurts. Toba *et al.* [91] studied the effect of two commercial preparations of β-galactosidases on the hydrolysis of lactose simultaneously added with starter culture. As was expected, the extent of lactose hydrolysis depended on the amount of enzyme added, reaching the complete hydrolysis with the higher level of enzyme assayed at 6 hours of incubation. Ismail *et al.* [92] evaluated the general quality of hydrolyzed yogurts which were produced hydrolyzing the lactose during the fermentation process. There were no differences in the sensorial characteristics, where texture, consistency and flavor were evaluated, between yogurts made with the minor and intermediate levels of lactase and controls. In contrast, a sweet taste was recorded in yogurts prepared with the higher level of lactase in comparison to controls.

More recently, Nagaraj *et al.* [93] studied different levels of lactase to hydrolyze the lactose in a previous step to the fermentation process. The percentages of hydrolysis were between 50 and 90%, depending on the level of lactase employed. Yogurts with 50% and 70% of hydrolyzed lactose had higher scores of flavor, body, texture and overall acceptability than controls; this was attributed to the increased content of monosaccharides that are more soluble and impart a softer body and creamier texture. The best flavor characteristic in hydrolyzed products may be due to a higher amount of available glucose for the aromatic compounds production. In contrast, yogurts with 90% of hydrolyzed lactose had lower scores for those sensorial attributes than the controls. The authors suggest that the weaker body, texture and higher degree of whey separation may be caused by the higher amount of monosaccharides that reduce the viscosity. The poor flavor, instead, could be a consequence of the intense sweet taste and a bitter after-taste.

In our institute, we are working on obtaining a lactose-hydrolyzed yogurt, since this type of product is not yet available in the Argentinean market; preliminary results are presented.

For this purpose, yogurts with a reduced concentration of lactose (experimentals) and yogurts with normal lactose content (controls) were made at laboratory-scale employing the classical procedure [71]. β-galactosidase from *Kluyveromyces lactis* (GODO YNL-2, Japan) was used in the experimental yogurts.

Different variables such as the initial pH of milk (6, and normal pH of milk, 6.7-6.8), addition of enzyme before or simultaneously with starter culture, level of enzyme (0.15, 0.25, 0.40 g/L) and the incorporation of sucrose, were studied. The residual lactose concentration was measured indirectly through the quantification of glucose by enzymatic-colorimetric method and the percentage of lactose hydrolysis was obtained [94].

The milk was standardized at 3% of fat content and sucrose was added (in the appropriate case). Then, the mix was heated at 85 ºC for 30 minutes and cooled at 41-43 ºC (optimum temperature of the enzyme activity and the fermentation process). At this point, the β-galactosidase enzyme was added in experimental yogurt using two different procedures: incubation of the enzyme for 30 minutes before adding the starter culture and the addition of the enzyme simultaneously with the starter. Starter culture containing *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* was employed and the fermentation process was carried out until the pH reached a value of 4.7-4.8 that occurred at approximately 3.5-4 hours. After that, yogurt was

rapidly cooled at 25 ºC in an ice water bath and then stored at 4 ºC for 28 days.

In relation to the initial pH of milk, the levels studied were selected according to the range of the optimum pH (6-8) of the enzyme in lactose solution (according to the manufacturer's information). The percentages of hydrolysis obtained in milk at pH = 6 were very poor (< 70%). The best results (percentages higher than 70%) were obtained at the normal pH of milk.

Regarding the moment of enzyme addition, there was not significant difference in the lactose content between yogurts made with pre-incubated milk before starter addition, and yogurts in which the hydrolysis and fermentation processes were carried out simultaneously. The pre-incubation step was performed adding the lactase to milk (pH = 6.7-6.8) and then incubating the mix for 30 minutes at 41 - 43ºC. Therefore, the selected method for subsequent experiments was to add the lactase together with the starter; as it reduced the processing time and decreased the chances of microbial contamination.

As expected, the lactose hydrolysis in experimental yogurt increased progressively to approximately 150 minutes and then remained constant until the end of the process. Yogurts made with enzyme levels of 0.25 and 0.40 g/L had a percentage of hydrolysis higher than 80%, while yogurts with the lower level of lactase (0.15 g/L) showed values close to 70% of hydrolysis (Table **1**). Argentinean legislation (Código Alimentario Argentino) establishes that reduced-lactose food must not contain more than 30% of lactose of the normal content [95]. Therefore, all the enzyme levels assayed were suitable for this type of product, as the hydrolysis of lactose was close to or higher than 70%. Although the presence of sucrose was associated with slight decreases in the percentages of hydrolysis, the values obtained were still appropriate.

Table 1. Ranges of Hydrolysis Percentages Obtained at Final Stage of Production

Enzyme Level (g/L of milk)	Yogurts without Sucrose (%)	Yogurts with Sucrose (%)
0 (controls)	13 – 17	9 – 14
0.15	76 – 81	72 – 79
0.25	83 – 87	79 – 85
0.40	88 – 95	85 – 90

The residual concentration of lactose in all experimental yogurts ranged from 0.4 to 1.4 %. Similar results were obtained by Ismail *et al.* [92] and Nagaraj *et al.* [93].

The fermentation time was slightly lower in experimental yogurts than in controls. In reduced-lactose yogurts, the higher availability of easily fermentable sugar (glucose) probably favored a faster growth and the acidification of starters [5, 91]. The development of acidity during storage (post acidification) was observed in all yogurts; the pH values decreased approximately by 0.3 units. These results are in accordance with those obtained by Birollo *et al.* [96].

The index of syneresis [97] of all yogurts increased progressively during storage; however, a slightly higher level of whey separation was observed in hydrolyzed products in comparison to controls. Similar results are consistent with those obtained by Nagaraj *et al.* [93]. Syneresis is considered an important defect that adversely affects the sensory quality of yogurt, being able to be reduced with the addition of dairy powders or thickeners as pectin and starch, among others [71].

On the other hand, the adequate level of sucrose in hydrolyzed yogurt was evaluated in experiments at pilot scale, for which the intermediate level of enzyme (0.25 g/L) was employed, adding the lactase together with starter at normal pH of milk. A group of panelists evaluated the level of sweetness and found that hydrolyzed yogurts made with 10% of sucrose (quantity commonly employed in the yogurt production) were sweeter than controls. By reducing the amount of sweetener (of about 25%), did not reveal any difference in the level of sweetness in comparison to controls.

Although the results presented are preliminary, they are considered as satisfactory. In accordance to the Argentinean legislation, the concentration of residual lactose in yogurts was appropriate.

Even if the presence of GOS in fermented milks has been associated with the β-galactosidase activity of starter culture LAB [98-101], the use of exogenous β-galactosidase to hydrolyze lactose also leads to the synthesis of these bioactive compounds. As there is little information on this aspect, we will perform further studies to evaluate the formation of GOS in the hydrolyzed yogurt production, due to the potential capability of lactase to synthesize these compounds.

4.2. Ultrafiltration Process

Membrane techniques such as ultrafiltration (UF) are used to concentrate the milk and whey, produce whey protein, modify the content of lactose in milk and dairy products and to produce other products with high added value [7, 102]. Recently, UF has acquired great interest because it is a process that saves energy and reduces transport costs [70]. Besides, the advances in material science have led to the development of membranes more robust and easier to clean [102].

Ultrafiltration refers to a membrane separation technique that uses semi-permeable polymeric or ceramic materials. These membranes have the function of separating the different components of a fluid mixture, based on molecular size and chemical interactions between the membrane and the components. The pressure applied is the main driving force of the process. Typical operating pressures vary from 0.3 to 0.8 MPa and the working temperatures are in the range of 25-50°C for milk. UF membranes have pore sizes between 10^{-1} – 10^{-3} μm and molecular weight cut-off (MWCO) in the range of 1-100 kDaltons. The performance of the process is affected by different parameters such as feed flow rate, temperature, pH and feed concentration [70, 103, 104]. This process is used to selectively concentrate some milk macromolecular components, e.g. proteins (caseins and soluble

proteins), fat and fat-soluble vitamins, while water, lactose, minerals, non-protein nitrogen, urea, amino acids and water-soluble vitamins can easily pass through the membrane [7, 70]. An exception is constituted by those components that are linked and retained completely, such as minerals and ions attached to proteins like calcium, magnesium, phosphate and citrate, and protein-bound vitamins [7]. The streams obtained are called retentate or concentrate (particles bigger than the membrane pores) and permeate or filtrate (particles smaller than the membrane pores). The retentate is considered milk concentrated, which is suitable for cheese and yogurt production [68].

In relation to traditional production of set- and stirred-yogurt, the total milk solids must be increased (until 15-17%) to obtain a final product with good viscosity and texture [71]. The fortification with skim milk or whey powders is the method most widely employed, although the use of membrane technology has also been reported [102, 105]. The latter strategy is a suitable alternative to obtain a yogurt with higher nutritional value than yogurt produced with dairy powders, because the damage to proteins is lower in yogurts produced by ultrafiltration. In effect, dairy powders are products that have undergone at extreme heat treatments so some of their constituents may have altered. In addition, the yogurt obtained by UF has special properties, as the molecule of lactose is relatively small and passes through the membrane, so that this product will be beneficial to the lactase-deficient individuals [102].

Kosikowski [105] obtained low lactose yogurts from the retentates of skim milk by UF using a membrane with a cut-off of 20 kDa and operating at 50°C and 3.4kg/cm^2. Retentates were diluted with water about 5-6 times to vary protein content between 3.5-4.5% and the fat content was standardized with cream between 1.4% to 2.6%. These mixtures were homogenized and pasteurized (75°C/30 min), and then the culture starter was added. The fermentation was carried out at 43°C for 3.5-4 h. The yogurts contained < 0.6% of lactose and the sensorial characteristics of firmness and viscosity were maintained, although they had a lower amount of mineral elements than those of the standard, commercial yogurt.

More recently, Rinaldoni *et al.* [102] studied the physicochemical, microbiological and organoleptic characteristics of yogurts manufactured from concentrated milks by UF. The concentrates were obtained at 22°C and 1.5 bar using a polysulfone membrane with a cut-off of 10kDa; they were subjected to thermal treatment (80°C/60 min) and then the starter culture was added to yogurt production. The yogurts showed an increase in the protein content of 30%, a reduction in lactose content of 50% and presented typical sensory characteristics with respect to the regular commercial product.

4.3. Chromatographic Process

In 2001, the Finnish dairy company Valio developed a chromatographic process to specifically remove the lactose from milk. Although the occurrence of lactose intolerance in Finland is relatively low (nearly 20% of the total population) considering the international context, the problem is significant due to the high consumption of milk in this country (approx. 140 L per person annually) [7, 9, 27].

Solid/liquid chromatography is based on the absorption/desorption of components present in the feedstock, to/from specific (functional) groups present on the pore surface of a resin [104].

The patent-protected process of lactose removal from skim milk by the ion-exclusion chromatography allowed obtaining a “lactose-free milk drink”. In this process, milk is split into two streams, which are finally combined. In one of them, the lactose is hydrolyzed by means of a traditional enzymatic procedure, while in the other one the lactose is removed by chromatography. In the chromatographic separation, the charged resin placed in a column allows the separation of proteins and other charged ions found in milk from lactose. The proteins and ions bind to resin with opposite charge, while lactose as a non-ionized molecule does not attach and passes directly through the system. The lactose is eluted at the bottom of the bed, while the protein and mineral fraction is eluted in another stream [7, 27, 106].

The final product has a similar composition to that of low fat milk, except for its very low lactose content (approx. 0.01%). Besides, it has the additional advantage that contains lower content of the total carbohydrate, providing a less caloric value but the same taste and sweetness of fresh milk [27]. The main disadvantage of this technology is its complexity and time-consumption, and it cannot be directly applied to conventional dairy industries without expensive equipment investments [7].

5. CONCLUSION

The potential market for dairy products specifically targeted for lactose intolerant or lactase-deficient individuals is enormous and has been estimated to be about 50 million consumers. It is widely accepted that the problem of lactose intolerance should not be a reason to discourage milk and dairy product consumption.

Thereby, the development of new dairy products based on the modification of cow milk composition could open up new markets for the dairy industry and even help to generate new technologies.

At this moment, there are a wide range of reduced- or lactose-free dairy products available commercially in some countries, which are obtained by different technological procedures including enzymatic and physical processes, the former being the most common. However, in our country (Argentina), this type of modified products is scarce and only hydrolyzed milks are available. For this reason, we are working to obtain a reduced-lactose yogurt; so far the results obtained are considered satisfactory.

ACKNOWLEDGEMENTS

Declared none.

CONFLICT OF INTEREST STATEMENT

Declared none.

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Received: March 09, 2011 Revised: December 30, 2011 Accepted: January 02, 2012