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A novel strategy for improved digestibility: Designer milk nutrition

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ABSTRACT

Most people lose some lactase activity in the small intestine after childhood. Many traditional dairy products, such as ripened cheeses, are naturally lactose-free. Over the last few decades, new technologies have been developed to produce lactose-free fresh dairy products to meet the needs of people with lactose intolerance. This article reviews recent advances and technological developments in producing lactose-free milk in the dairy industry.

Keywords: Lactose, Membrane separation, Acid hydrolysis, Immobilization, Free enzyme

Introduction

Milk is a complex fluid rich in fat, carbohydrate, protein, minerals, and vitamins. Lactose is a major carbohydrate in bovine milk, with concentrations ranging from 4.5 to 5.0 g/100 ml. The small intestine absorbs it and hydrolyzes it into glucose and galactose using β -galactosidase on the mucosal membrane of enterocytes. Many people cannot digest lactose properly because they lack intestinal β -galactosidase, and they may have difficulty consuming milk and dairy products (Jurado *et al.*, 2002) [1]. Excessive lactose in the large intestine can cause tissue dehydration due to osmotic effects, poor calcium absorption due to low acidity, and fermentation by microflora, resulting in diarrhea, bloating, flatulence, and watery stools (Puri *et al.*, 2010) [2]. In fact, more than 70% of the world population cannot use lactose or lactose-containing products because of lactose intolerance caused by a lack of β -galactosidase activity (Harju, 2003; Jelen *et al.*, 2003; Vasiljevic *et al.*, 2003) [3, 4, 5]. Lactose is digested by an enzyme that is present during infancy, but a genetic deficiency in β -galactosidase activity in the small intestine of adults leads to lactose intolerance (Jackson *et al.*, 2001) [6]. However, most Asians and Negroes, including some Caucasians, become deficient in digesting lactose due to gradual loss of lactase after weaning (Kim, 1994; Boey, 2001; Jackson *et al.*, 2001) [8, 7, 6]. Thus, consuming more than 20-50 g of lactose on an empty stomach causes lactose intolerance symptoms in lactase-deficient individuals.

Industrially, milk lactose is reduced by enzymatic hydrolysis to glucose and galactose, using a β -galactosidase isolated from several sources. The final product (low lactose milk or lactose-hydrolyzed milk) is slightly sweeter and easier to absorb in the intestinal tract than untreated milk. Moreover, lactose-reduced milk, yogurt, and ripened cheeses are the best sources for people with lactose intolerance. It is a source of bioavailable calcium, and deficiency can lead to osteoporosis (Bannan *et al.*, 1996) [9]. Lactose-hydrolyzed milk and dairy products have received much attention since the 1970s, when the first β -galactosidases enzyme preparation became commercially available. Lactases were first proposed for dairy applications in 1950 (Van Dam *et al.*, 1950) [10]. Nowadays, lactase is one of the most important enzymes used in food processing (Panesar *et al.*, 2006) [11]. It is commercially available for consumers with lactose intolerance (Tossavainen *et al.*, 2003) [12] and is marketed successfully by Valio Ltd. (Mattila-Sandholm *et al.*, 2003) [13].

Lactose hydrolysis

Lactose hydrolysis is a promising process in the food industry for the development of lactose free products (Pessela *et al.*, 2003) [14]. In addition, it increases product sweetness, which in many cases allows lower added sugar levels. Hydrolyzing 70% of the lactose in milk increases sweetness by an amount corresponding to adding about 2% sucrose (Zadow, 1986) [15]. Furthermore, lactose hydrolysis lowers the freezing point and speeds fermentation in the medium. Commercial lactose-free milk contains less than 0.25 g lactose per 100 g of milk and is manufactured by enzymatic hydrolysis, which breaks down lactose in milk into glucose and galactose. Demand for lactose-free dairy products has increased by approximately 20% per year since 1997 (Jelen *et al.*, 2003) [4]. The high lactose content in milk products such as ice cream, frozen milks, whey spreads, concentrated products, and condensed milk leads to excessive lactose crystallization, resulting in products with a mealy, sandy, or gritty texture. Using β -galactosidase to process such products could reduce

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lactose concentrations to acceptable levels and improve some technological and sensorial qualities of dairy foods, e.g., increasing digestibility, softness, creaminess, etc. (Zadow, 1993) [16]. Also, hydrolyzing this sugar could help develop additives like galacto-oligosaccharides for animal and human diets (Ladero *et al.*, 2000) [17].

Industrial production of lactose-hydrolyzed products

Using hydrolyzed milk in yogurt and cheddar cheese manufacture accelerates acidification because lactose hydrolysis is normally the rate-limiting step, reducing yogurt set time and accelerating the development of structure and flavor in cheese (Puri *et al.*, 2010) [2]. Moreover, monosaccharides formed by lactose hydrolysis ferment more easily than lactose, reducing the time from starter-culture addition to reaching the desired low pH in products such as cottage cheese and yogurt. In addition, glucose and galactose considerably increase product sweetness (about 50%). Therefore, yogurts require less added sweetener, resulting in fewer calories in the final product (Johansen *et al.*, 2002) [18]. Cheese made from hydrolyzed milk ripens more quickly than cheese made from normal milk (Kim, 1997) [19]. In milk powder, a certain amount of amorphous lactose is hygroscopic and crystallizes when stored for a long time in the presence of moisture (Hogan *et al.*, 2013) [20]. Most notably, lactose is the main source of instability in spray-dried milk powder. Lactose hydrolysis is also more desirable at high lactose concentrations in condensed milk and can lead to excessive lactose crystallization, resulting in products with a mealy, sandy, or gritty texture. For this reason, using β -galactosidase before the condensing operation can reduce lactose to a level where it is no longer a problem (Zadow, 1992) [21].

Enzymatic hydrolysis of lactose has been proposed to offer several benefits for industrial application, including developing lactose-hydrolyzed products as one possible approach to reduce lactose intolerance, which affects more than half of the world's population (Suarez *et al.*, 1997) [22]. Galacto-oligosaccharides form during lactose hydrolysis and favor the growth of intestinal bacterial microflora (Mahoney, 1998) [23]. In addition, improvement in the technological and sensory characteristics of foods containing hydrolyzed lactose was observed by Zadow (1992).

Lactose hydrolysis alleviates problems related to lactose intolerance and improves dairy processing. This can be achieved using the following method.

Hydrolysis of lactose

Acid hydrolysis

Acid hydrolysis is viable only for protein-free streams such as ultrafiltration (UF) permeate of milk or whey. pH can be adjusted by direct acid addition or by treating the permeate with an ion exchange resin. Typically, pH is adjusted to 1.2 and temperature to 150 °C for a short period. The hydrolyzed product is brown and requires neutralization, demineralization, and decolorization before use. Corrosiveness of the conditions is a major engineering design challenge in this process. Thus, it has not been commercially adopted to any significant extent (Zadow, 1986) [15]. Several processes based on ion exchange resins have been developed, working typically at pH 1.2 and at 90-98 °C (Thompson *et al.*, 1991) [24]. Browning of the neutralized reaction mixture is the biggest problem, increasing processing costs and creating disposal issues. Industrially, this process has been used only for pure lactose solutions

Hydrolysis with soluble enzymes (β -galactosidase)

Lactase (β -D-galactosidase; β -D-galactoside galactohydrolase, E.C. 3.2.1.23) is widely distributed in nature and can be isolated from sources such as plants (almonds, peaches, apricots, apples), animal organs, yeasts, bacteria, and fungi (Richmond *et al.*, 1981) [25]. It is a hydrolytic enzyme that catalyzes the breakdown of lactose into glucose and galactose, which are sweeter, more soluble, and more di-

gestible than lactose (Haider *et al.*, 2009) [26]. Compared with animal and plant sources, microorganisms produce enzymes at higher yields, lowering β -galactosidase costs (Santos *et al.*, 1998) [27]. It occurs in a variety of microorganisms, including yeasts (mainly *K. lactis* and *K. fragilis*), which have GRAS (generally recognized as safe) status and are commercially important (Harju, 1987) [28]. Enzymatic hydrolysis of lactose produces lactose-reduced milk and derived dairy products that lactose-intolerant people can consume (Haider *et al.*, 2008; Heyman, 2006) [29, 30]. In addition, it catalyzes the formation of galacto-oligosaccharides, which are prebiotic additives also known as healthy foods (Grossova *et al.*, 2008) [31]. The hydrolysis rate of lactose in milk depends on β -galactosidase activity, which in turn depends on substrate concentration, pH, temperature, contact time, enzyme activity, and cost.

Unlike the enzymatic method, the acidic method causes problems such as protein denaturation in the lactose solution, brown color formation, and undesirable by-product production. Therefore, the enzymatic method is a better choice for food applications (Pessela *et al.*, 2003) [14]. In addition, the enzymatic process offers several benefits, including no by-products, no degradation of compounds in dairy products, and no additional off-flavors, odors, or colors. Furthermore, enzyme-treated milk retains its original nutritional value, especially since glucose and galactose are not removed (Ladero *et al.*, 2000) [17]. In addition, it increases the product's sweetness, which in many cases allows reducing added sugar. Hydrolyzing 70% of the lactose in milk increases sweetness by an amount corresponding to adding about 2% sucrose (Zadow, 1986) [15]. Commercial lactose-free milk contains less than 0.25 g lactose per 100 g of milk and is produced by enzymatic hydrolysis, which breaks down milk lactose into glucose and galactose.

Immobilization technique

Immobilization technology is economically important because it can be reused many times (Kallioinen *et al.*, 2008) [32]. Enzyme parameters are the main factors that determine the technology and the relative costs of the lactose hydrolysis process. Because β -galactosidase is expensive, direct addition to the substrate is not economically viable (Mahoney, 1997) [33]. Enzyme immobilization can overcome this problem using various techniques and matrices. Immobilized biocatalysts can be reused several times, which reduces process costs (Genari *et al.*, 2003) [34]. In addition, immobilized enzymes offer several benefits over free enzymes, including easy separation from the reaction solution, no product contamination by the enzyme, operational and long-term stability, continuous processing, and multi-enzyme reaction systems (Dervakos *et al.*, 1991) [35]. Thus, enzyme immobilization is widely used in industrial applications because it enhances stability, improves reusability, reduces inhibition by reaction products, and increases selectivity toward non-natural substrates, making the process cost-effective and viable (Iyer *et al.*, 2008; Mateo *et al.*, 2007) [36, 37]. The application of immobilized β -galactosidase in the dairy industry for lactose hydrolysis has attracted researchers' attention because of its potential benefits (German, 1997) [38]. Immobilized β -galactosidase for lactose hydrolysis is usually used to reduce enzyme and processing costs. β -galactosidases with a neutral pH optimum are not very stable when immobilized with classical techniques such as adsorption or covalent linkage (Mahoney, 1997) [33]. Bakken *et al.*, 1992 [39] studied the use of immobilized *Bacillus circulans* lactase for hydrolysis of skim milk. At hydrolysis temperatures of 20-50 °C, microbial growth could not be avoided, and at higher temperatures, the enzyme was significantly inactivated. The only known commercial application for lactose hydrolysis in milk involved immobilizing a neutral-pH enzyme from *Kluveromyces lactis* by entrapping it on porous cellulose acetate fibers (Dinelli, Marconi, & Morisi, 1976; Mahoney, 1997; Pastore, Morisi, & Zaccardelli, 1974) [33]. Immobilization gave the enzyme a shelf life of 100 days (Morisi *et al.*, 1973) [40].

Membrane separation

Ultrafiltration membranes (UF) having a molecular weight cutoff in the range of 1,000-500,000 Daltons can be used for the separation of lactose from milk. Lactose can easily pass through the membrane and be retained in the permeate, while fat and milk proteins are retained in the retentate. The permeate containing lactose was hydrolyzed with β -galactosidase and returned to the UF retentate to maintain mineral content (Kulkarni *et al.*, 2001) [41]. Diafiltration is a modification of the ultrafiltration process in which lactose can be removed completely from the milk. In diafiltration, water is added to the milk to wash more molecules through the membrane and remove more lactose into the permeate. Combining ultrafiltration and diafiltration produces low concentrations of lactose and salts. This is often the method of choice for manufacturers because, although it increases the price by 20-30%, it produces lactose-free milk with the genuine taste of milk (Shakeel, 2009) [42]. SNAM Progetti produced low-lactose milk using this technology at the Centrale di Latte in Milan (Mahoney, 1997; Panesar *et al.*, 2006) [33, 11]. A batchwise process achieved about 75% lactose hydrolysis. This application has been developed further (Cerlesi, 2000) [43], but the main goal here was not to minimize enzyme costs but rather to keep the end product free from enzyme residues

Conclusion

Lactose hydrolysis can be a promising process in the food industry for developing lactose-free products that people with lactose intolerance can consume. It also helps in preventing lactose crystallization in many dairy products. Immobilization technology is economically important because it allows repeated enzyme reuse. Thus, the application of immobilized β -galactosidase in the dairy industry for lactose hydrolysis has attracted researchers' attention. Membrane technology has also gained more importance because of its energy-saving process. A suitable, economically feasible process for lactose hydrolysis using β -galactosidase may alleviate lactose intolerance in humans and crystallization defects in dairy products.

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