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Review

Lactose: Crystallization, hydrolysis and value-added derivatives

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A B S T R A C T

Lactose, the most abundant component of milk of most mammals, has been thoroughly studied for its physico-chemical properties, crystallization behavior and importance as a fermentation medium. Studies of various approaches to lactose modifications to increase its value as a food ingredient or nutraceutical component are more recent and presently predominate the research interest concerning lactose. This review, while summarizing briefly some physico-chemical properties and older studies concerning crystallization behavior (mutarotatory equilibrium, solubility, crystalline habit and form) focuses also on the modification alternatives to increase the utilization of lactose through value-added products. Various approaches to lactose hydrolysis leading to increased solubility, higher sweetness and expanded availability of milk and dairy products for lactose intolerant consumers are compared with an emphasis on crude enzyme extracts. Principles and processes for conversion of lactose to lactitol, lactobionic acid, lactulose, lactosucrose, and galacto-oligosaccharides are highlighted.

Contents

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1. Introduction

Prior to the seventeenth century, milk was considered to have only three components, curd, fat, and whey (Whittier, 1944). Bartolettus (1633) isolated an "essential salt without nitrogen" from whey in 1633; Ettmueller (1688) isolated lactose from evaporated whey and purified it by recrystallization (both authors as cited by Whittier, 1944).

During the 18th century, lactose became a commercial commodity. The foundation of the present knowledge of lactose—especially regarding its chemistry and molecular structure—was laid during the early 20th century. From this basis over the years, the present concept of understanding of the characteristics and the utility of this unique sugar was developed. The objective of this somewhat historical but mainly forward-looking review is to highlight some of the well-known properties related to the roles of lactose in modern dairy and food industries, bearing on the ongoing quest to find solutions that utilize one of the main dairy by-products—the cheese whey, of which lactose is the main component.

2. Characteristics of lactose

2.1. Occurrence and properties

Lactose is present in the milk of all mammals with only a few minor exceptions. The approximate concentration in mammalian milk is between 2.0% and 10% (Holsinger, 1988; Whittier, 1944). The lactose content of bovine milk ranges between 4.4% and 5.2% averaging at 4.8% anhydrous lactose. This compares with 7% in human milk.

Lactose (4-0-β-galactopyranosyl-D-glucopyranose, $C_{12}H_{22}O_{11}$) is a disaccharide comprising one glucose molecule linked to a galactose molecule. A distinctive feature of lactose is its manifestation in different states and temperature-dependent physico-chemical interrelationships. Lactose in aqueous solutions is present in α and β forms (Fig. 1). Crystals of α-lactose can be prepared as monohydrate by concentrating an aqueous lactose solution to supersaturation and allowing it to crystallize at a moderate rate below 93.5 °C (Drapier-Beche, Fanni, & Parmentier, 1999; Gillis, 1920; Hudson, 1908). Its specific optical rotation in water is $[\alpha]_D^{20} = +89.4^\circ$ (anhydrous weight basis). The melting point is 201.6 °C. The most predominant crystals forms are prisms, pyramids, or "tomahawks" depending on the conditions of crystallization. The crystals are hard and not very soluble. If their size in food products is over 10–16 μm they can be detected sensorically and create a defect called "sandiness".

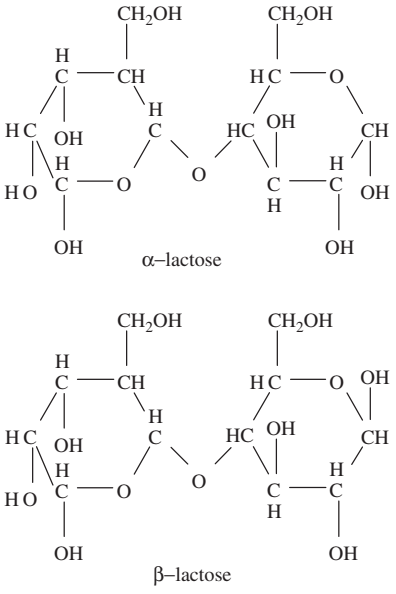

Fig. 1. Structure of lactose molecules in α and β configuration.

The anomer of α-lactose is β-lactose. Since no water is associated with the molecule in this case, its designation is β-anhydride. It crystallizes from supersaturated lactose solutions at temperatures above 93.5 °C. Its specific optical rotation is $[\alpha]_D^{20} = +35.0^\circ$ and it has a melting point of 252.2 °C. The β-anhydride crystals, which are sweeter and considerably more soluble than the α-hydrate, commonly occur as uneven sided diamonds when crystallized from water, and curved and needle-like prisms from alcohol-based solutions.

2.2. Mutarotation

In aqueous solutions, α- and β-lactose are present in equilibrium. Regardless of the form used in preparing a solution, the optical rotation will change the α form into the β form and vice-versa through the process of mutarotation until $[\alpha]_D^{20} = +55.3^\circ$ at the equilibrium (anhydrous weight basis). This is equivalent to 37.3% α-lactose and 62.7% β-lactose; this equilibrium ratio is affected slightly by differences in temperature (Nickerson, 1962), but not by differences in pH.

The rate of mutarotation is greatly influenced by both temperature and pH as well as by other sugars and salts (Haase & Nickerson, 1966; Patel & Nickerson, 1970). The rate is slow at low temperatures but increases 2.8 times with every 10 °C rise in temperature, becoming almost instantaneous at about 75 °C. The rate of mutarotation is at a minimum at about pH 5.0 increasing with changes on either side of this value.

2.3. Solubility and sweetness

The solubility of lactose is low compared with other disaccharides but the effect of temperature on solubility is more pronounced (Sienkiewicz & Riedel, 1990). The solubility of lactose is only about 10% of that of sucrose at ambient temperature (Ryder, 1988). The α- and β-lactose have vastly different solubilities. The β-lactose is much more soluble in the ambient conditions and the α-lactose above 93.5 °C. The mutarotatory equilibrium favoring the α variant at ambient temperature results in the low overall solubility of lactose in these conditions and much higher solubility at temperatures close to 93 °C. The sweetness of lactose solutions at ambient conditions is about 20% that of sucrose. However, the sweetness of lactose in milk is clearly noticeable and a sweet taste following addition of 0.75% lactose to regular skim milk could be detected by trained taste panels (Jelen & Michel, 1999).

3. Lactose crystallization

Lactose can be present in dairy products in two crystalline forms, α-hydrate and β-anhydride, as well as an amorphous "glass" mixture of α- and β-forms. This non-crystalline lactose glass contains the α- and β-forms in the same ratio as in the solution from which it was generated. Solutions of lactose are capable of being highly supersaturated before spontaneous crystallization occurs.

3.1. Crystalline habits

Crystallization in its most basic concept is a two-step process involving nucleation and growth of the nucleus to a macro-size

(Nickerson, 1974). In general, the rate of crystal growth increases rapidly as supersaturation (or precipitation pressure, expressed as ratio of actual concentration to solubility) is increased (Twieg & Nickerson, 1968; van Kreveld & Michaels, 1965). The rate is different for the different faces of the crystals, an effect that alters the shape of the crystals during growth. Crystal surface integration is a critical factor in the formation of crystals, while the diffusion rate of α-lactose to the surface is not rate-limiting (Thurlby, 1976). The rate of lactose crystal growth is not much different from that of sucrose, when considering that the growth of lactose crystals occurs predominantly or solely on only one face—the bottom—of the pyramidal crystal (Bhargava & Jelen 1996; Jelen & Coulter, 1973a, 1973b). Several older investigations, especially in the Netherlands at the Cooperative Condensfabriek Friesland, focussed on the mechanisms and kinetics of the lactose crystal formation and growth in great detail (e.g. van Kreveld, 1969; van Kreveld & Michaels, 1965; Visser, 1983).

3.2. Shapes and crystallization

The pyramid, tomahawk and prism shapes are the most common forms but lactose crystals can be observed in a variety of other shapes, depending on the conditions of crystallization. The principal factor governing the form of lactose crystals is the supersaturation of the solution (Herrington, 1934). High supersaturation forces rapid crystallization and only prisms form. As supersaturation decreases, the dominant crystal form changes to diamond-shaped plates, then to pyramids and tomahawks, and finally to fully developed crystal showing a multitude of faces (Holsinger, 1988; Jelen & Coulter, 1973a; Nickerson, 1979; van Kreveld & Michaels, 1965). More recent studies demonstrated the physico-chemical interrelationships of aqueous lactose solutions at different degrees of supersaturation, temperatures, different stages of crystallization and in the presence or absence of different water-miscible organic solvents (Zeng, Martin, Marriott, & Pritchard, 2000b). Although the majority of lactose crystals were found to be either tomahawk-shaped or pyramidal, at higher concentrations elongated cuboidal crystals were observed, with higher initial lactose concentrations resulting in more elongated particles.

3.3. Effects of acidity, alkalinity, and salts

The pH is an important factor in lactose crystallization (Nickerson & Moore, 1974b). The effect has been attributed to its influence on the rate of mutarotation, as crystallization removes only one anomer from the equilibrium, which may become depleted in crystallizing form if mutarotation is slow. However, Twieg and Nickerson (1968) and Nickerson and Moore (1974b) demonstrated that mutarotation becomes limiting only when crystallization occurs rapidly with a large surface area. Alkaline conditions speed up crystallization but also favor formation of lactose degradation products that may inhibit crystallization. However, the addition of calcium lactate or K_2HPO_4 (both of which increase pH) resulted in opposite effects on crystallization (Bhargava & Jelen, 1996), indicating additional effects of the mineral composition and the ionic strength.

Lactose solubility values in whey UF permeate solutions as well as model systems seem to relate directly to growth rates of lactose crystals although the rates of crystal growth were lower in permeate than in pure lactose solutions (Bhargava & Jelen, 1996). Presence of non-lactose impurities such as riboflavin or KCl could be responsible for this effect (Jelen & Coulter, 1973b; Smart, 1988).

When salts were added specifically to pure aqueous solutions, various salts had very different effects on crystal growth rates (Jelen & Coulter, 1973b; Smart, 1988). Some salts led to significantly higher crystal growth rates, and their effect was concentration dependent. Thus, salt concentrations per se appear to be important factors influencing growth rates, with each salt exerting a different effect. In addition, some salts seem to change the shape of crystals leading to elongations, needle-like shapes or triangular flake forms (Bhargava & Jelen, 1996; Jelen & Coulter, 1973b).

3.4. Other milk components, chemicals and food ingredients

Methanol and ethanol accelerate crystallization by as much as 30–60% even at low (1%) concentrations (Nickerson, 1974; van Kreveld & Michaels, 1965). Alcohols reduce the solubility of lactose and support spontaneous nucleation. The latter effect, accelerating the formation of crystals, may support the step theory (step generation and rapid growth) in lactose crystallization (Michaels & van Kreveld, 1966; Nickerson & Moore, 1974a, b).

In dairy products, the presence of "impurities" or chemicals used as food additives may inhibit or accelerate the growth of lactose crystals, alter crystal form (Jelen & Coulter, 1973b) or have no effect. In some instances, impurities may inhibit the formation of the nuclei. The effects on crystal growth are a function of intricate physico-chemical interactions of available energies, adsorption rate to the various crystal faces, steric hindrance caused by proteins and polysaccharides, tendency to spontaneous nucleation, and the concentration of substances in question (Nasirpour, Scher, Lindner, & Desobry, 2006; Nickerson, 1974). In the presence of impurities, lactose crystals tend to be irregularly shaped and clumped, instead of yielding the characteristic crystals obtained from simple lactose solutions (Nickerson, 1962).

Gelatine is an example of a crystallization inhibitor that reduces the rate by 25–60%. However, gelatine cannot suppress nucleation in highly supersaturated lactose solutions, which explains its ineffectiveness in preventing sandiness in ice cream (Nickerson, 1962). On the other hand, various marine and vegetable gums are now widely used in ice-cream formulations, because they inhibit the formation of lactose crystal nuclei. Carrageenan-containing systems have been shown to inhibit crystallization (Kouassi, Jouppila, & Roos, 2002). Gels can provide a "protective environment", thus presenting a "control" mechanism for the rate of crystallization as well as the uniformity of the crystals (Zeng, Martin, Marriott, & Pritchard, 2000a).

3.5. Industrial process conditions

Lactose crystallization is particularly complicated in complex dairy systems subjected to industrial process conditions, such as spray-drying, freeze-drying, and various storage situations. In these cases, the distinct crystals start to coexist with amorphous lactose. During storage, the rate of crystallization in dry lactose-containing powders increases with increasing relative humidity (Miao & Roos, 2005), and is higher in spray-dried materials than in freeze-dried materials. The crystallization rate was lower in all lactose/protein mixtures than in pure lactose (Haque & Roos, 2004). Proteins have a profound effect on lactose crystallization (Kouassi et al., 2002; Miao & Roos, 2005). Lactose crystallized mainly as α-lactose monohydrate in spray-dried lactose/whey protein isolate and lactose/gelatin mixtures. Anhydrous β-lactose and α-lactose monohydrate crystals formed in freeze-dried lactose/whey protein isolate and lactose/gelatin mixtures, while only α-lactose monohydrate crystallized in both spray-dried and freeze-dried lactose/Na-caseinate mixtures (Miao & Roos, 2005). In freeze-dried skim milk, lactose crystallized as an anhydrous mixture of α- and β-lactose in a molar ratio of 5:3 (Jouppila, Kansikas, & Roos, 1997).

3.6. Amorphous noncrystalline lactose (lactose glass)

When a lactose solution is dried rapidly, its viscosity increases so quickly that crystallization cannot take place and the dry lactose forms an amorphous (noncrystalline) lactose "glass". Lactose in milk powder (spray, roller, or freeze-dried) is noncrystalline and exists in the same equilibrium mixture of α- and β-lactose as existed in the milk prior to drying (Nickerson, 1974; Zadow, 1984). The glass transition temperature of lactose is influenced by its concentration, relative humidity, and the presence of other milk components (Jouppila & Roos, 1994; Roos & Karel, 1992). Stickiness of dairy powders was shown to be related directly to the glass transition temperature (Paterson, Brooks, Bronlund, & Foster, 2005).

3.7. Lactose in food products—quality and industrial applications

The crystallization principles and the factors influencing the growth of lactose crystals have been applied to dairy products to understand the consequences of processing conditions of lactose-containing milk products on their quality. It is generally understood that rapid crystallization produces small crystals (Fox & McSweeney, 1998; Nickerson, 1974). Innovative designs of equipment and appropriate processing techniques (such as seeding a sufficient amount of small crystals) enable the formulation of high quality products even under high-yield process conditions.

One of the most objectionable texture defects in dairy products is sandiness that may occur particularly in ice-cream and the Norwegian whey cheese mysost. Sandiness is caused by lactose crystals which are large enough to be detectable in the mouth but which do not dissolve readily, thus producing a rough or gritty sensation. Lactose glass in spray-dried milk and whey powders is stable if protected from humidity, but is very hygroscopic, causing stickiness and caking (Haque & Roos, 2005; Holsinger, 1988; Paterson et al., 2005).

In the pharmaceutical industry, lactose is commonly used as a bulking agent in pharmaceutical formulations for both human and veterinary medicinal products (EMEA, 2002; Miao & Roos, 2005). Uniformity of crystals, surface smoothness as well as favorable shapes are of critical importance for medical applications. The source and grade of lactose can have a substantial effect on drug delivery. For example, in a dry-powder-inhaler application increasing the elongation ratio of the lactose crystals improved the medical profile of the drug in question significantly. The higher dispersibility and fine particle fraction of the lactose appeared to be responsible factors (Larhrib, Martin, Prime, & Marriott, 2003).

4. Lactose hydrolysis

4.1. Principles and theoretical considerations

The explosive rise in cheese production in the second part of the last century necessitated the search for new alternatives for whey utilization, which means predominantly finding uses for lactose. The uses for traditional crystalline lactose remained static, or even declined as new, cheaper sources of fermentable carbohydrate became available. The theoretically simple but industrially and especially economically challenging process of hydrolyzing lactose for production of sweetening syrups gained prominence in the lactose literature in the latter part of the 20th century. The aims of these industrial developments were two-fold—to use lactose as a sweetening carbohydrate; and to enable fermentation by lactose-negative microorganisms. Hydrolysis of lactose into the much sweeter monosaccharide components glucose and galactose enables its use as sweetening syrups in ice cream and other dairy and non-dairy foods. In addition, lactose hydrolysis increases the availability of dairy nutrients worldwide by alleviating lactose intolerance. *Saccharomyces cerevisiae* and the majority of other yeast species are lactose-negative and the lactose hydrolysis is a prerequisite for whey conversion to baker's yeast, animal feeds based on yeast biomass, ethanol or other value-added conversions by yeasts. Numerous technical reviews (e.g. Geilman, 1993; Gekas & Lopez-Leiva, 1985; Harju, 1987a; Shukla, 1975) can be consulted for details of the various technical approaches to accomplish lactose hydrolysis (Table 1). Thus, the subject will be discussed here only briefly.

Table 1
Technological alternatives for production of sweetening syrups based on lactose hydrolysis

Process	Main characteristics
Acid-catalysed hydrolysis	pH < 1.5
Direct acid addition	Temperature ~90 °C
Ion exchange resin	Temperature ~150 °C
Immobilized enzyme technology	Reactor containing suitably immobilized enzyme
Membrane-based enzyme reactor	Free enzyme hydrolysis with enzyme separation and reuse
Free (soluble) purified enzymes	Single use of the enzyme added to each batch and not recovered

Generally, all that is needed to hydrolyze lactose is an enzyme or a chemical process breaking the bond connecting the two monosaccharides. Young mammals and certain bacteria, yeasts, moulds, and plants exhibit β-galactosidase activity (Pritzwald-Stegman, 1986). The enzyme has been characterized thoroughly (Wallenfels & Weil, 1972), and its properties and potential applicability in the dairy industry described and reviewed (Smart, 1993). Alternatively, a chemical route for hydrolysis of lactose was known since the beginning of the 20th century (Whittier, 1925), requiring extremely acidic conditions (pH < 1.5) and very high temperatures (up to 150 °C). As an alternative to the enzymatic lactose hydrolysis, this approach has attracted renewed interest in Australia, the Netherlands, and New Zealand (e.g., de Boer & Robbertsen, 1981; Haggett, 1976; MacBean, Hall, & Willman, 1979), and was even patented (Block, 1952) but proved to be of limited industrial applicability.

4.2. Industrially applicable alternatives for lactose hydrolysis

The move towards industrial applications of the lactose hydrolysis process gained momentum in mid-1980s. In the International Dairy Federation, a very active Group of Experts under the leadership of Wayne Modler was set up to foster international cooperation on the subject, resulting in production of a valuable technical monograph (IDF, 1993). Major projects in UK, Australia, Finland and several other countries were driven by perceived major market opportunities. Unfortunately, most of them proved to be deceptive as in the case of the Australian initiative that focussed on the Far East (Zadow, 1993). The two major approaches included the "free enzyme" route and the development of immobilized enzyme reactors. Several β-galactosidase preparations were commercialized by large industrial producers in the 1990s, at the peak of these developments.

The free enzyme route appeared initially to be the preferred mode for production of lactose hydrolyzed milk and is still being used today. Although the enzyme preparation cannot be reused, which represents a major economic disadvantage, "lactose-free" milk produced by this method is available in limited quantities on retail shelves in many countries. Over-the-counter preparations of the soluble β-galactosidase such as Lactaid® are sold for use by

lactose-intolerant consumers as pills, providing time-limited relief of lactose intolerance after ingesting lactose-containing foods. The "tetra-lacta" process by the Tetra-Pak company for production of UHT lactose-free milk was based on dosing very small quantities of sterile β-galactosidase preparation into product at the time of aseptic packaging-lactose hydrolysis being accomplished during the unrefrigerated shelf life of the product.

A very successful industrial application of the soluble enzyme approach has been developed recently by the Finnish company Valio, one of the principal industrial pioneers in the field of lactose hydrolysis. The patented process is based on removing most of the lactose from regular milk by a chromatographic process developed by Harju (1987b), followed by hydrolysis of the remaining lactose by a soluble enzyme. Now marketed successfully in several Scandinavian and other European countries, the Finnish process results in comparable taste to the regular cows milk and avoids the uncharacteristic excess sweetness caused by liberated glucose and galactose associated with traditional in situ lactose hydrolysis methods (Jelen & Tossavainen, 2004).

The requirement for enzyme preparations in the free enzyme approach is reduced by membrane reactors in which the soluble enzymes are being recovered for repeated use (e.g., Mehaia, Alvarez, & Cheryan, 1993). However, the practicality and economical feasibility of this technology in industrial applications seems doubtful. Effectiveness of membrane reactors for lactose hydrolysis in milk or whey is affected by several of the typical problems of the membrane processes (membrane fouling, membrane selectivity, effects of some solutes on flux) as well as by the gradual loss of enzyme activity.

At the height of the "lactose hydrolysis euphoria" in the mid-1980s, developments of the immobilized enzyme technology appeared to offer the ultimate solution. The much publicized "Corning process", involving enzyme immobilization on sintered glass beads, seemed to be "winning the race" of the day (Anonymous, 1981, 1984). Unfortunately, even in this very promising case, the unfavorable economic realities and technical difficulties resulted its final demise. Several other commercial processes using the immobilized enzyme approach were tabulated by Harju (1987a) and the most prominent ones described briefly by Sienkiewicz and Riedel (1990). The Finnish development by the Valio company, based on the β-galactosidase immobilization on a special resin, is still being used for production of a successful whey drink (Jelen, personal observation, 2006) as well as for production of some of the many lactose-hydrolyzed dairy products marketed under the HYLA (HYdrolyzed-LActose) brand (Jelen & Tossavainen, 2004).

4.3. Lactose hydrolysis by mechanically disrupted bacteria

The unfavorable economies of either free or immobilized enzyme routes limit the large-scale applications of lactose hydrolysis for many potential uses. In an attempt to find an inexpensive solution to this problem, a recent research project has been carried out within the framework of an NSERC-supported Canadian Research Network centered at the Université Laval. The project aimed at using traditional dairy bacteria, known to be high producers of the β-galactosidase, as an "in-house" source of the enzyme that could be produced by the potential users themselves, similarly to the traditional production of bulk cheese starters in the cheese factories. However, in contrast to starter cultures for use in cheese, the fermentative activities of β-galactosidase producing bacteria in the subsequent lactose hydrolysis process would be detrimental to many such applications. Thus, the second important aspect of this approach is the mechanical disruption of the bacterial cultures, resulting in the inactivation of the bacterial cells and production of "enzyme cocktails" with high β-galactosidase activities. The application of these "in-house" produced cocktails for lactose hydrolysis under carefully selected conditions could result in optimal hydrolysis rates, without significant detrimental effects of other enzymatic processes. Our research, using a high β-galactosidase producer *Lactobacillus delbrueckii* subsp. *bulgaricus* strain ATCC 11842, focussed on the economic (Bury & Jelen, 2000) and technical (Kreft, Roth, & Jelen, 2001) feasibility of the process; optimization of the growth media (Vasiljevic & Jelen, 2001) and of the mechanical disruption process (Bury, Jelen, & Kalab, 2001; Geciova, Giesova, & Jelen, 2002); suppression of competing enzymatic reactions (Vasiljevic & Jelen, 2002); stability of the enzyme during the hydrolysis process (Kreft & Jelen, 2000); and sensory aspects of the final products (Vasiljevic, Wismer, & Jelen, 2003). Clearly, before this approach could become successful, additional research would be needed, including a search for a more effective microbial producer of the enzyme and/or involving genetic engineering leading to significant overproduction of the β-galactosidase and suppression of some of the other enzymes catalyzing potentially undesirable reactions.

Table 2
Current and potential industrial applications of the lactose hydrolysis process in mainstream dairy processing or utilization of dairy by-products

- Lactose hydrolysis in fluid milk products
- Control of lactose crystallization in concentrated dairy products (whey cheeses, ice cream, sweetened condensed milk)
- Whey and permeate applications (food and non-food uses)
- Formulated pet food applications
- Production of oligosaccharides and exopolysaccharides
- Enhancement of starter culture production
- Development of new flavors
- New product ideas combining lactose hydrolysis and flavor developments
- New/modified products (whey cheeses, spreads, drinks, "milk honey", dairy-based confectionery)

4.4. Future prospects for industrial applications of lactose hydrolysis

Lactose hydrolysis as a major industrial process appears to be grossly underutilized today. The amount of lactose available just in the dried whey produced in Europe and North America can be estimated as at least 1.4 MT annually (IDF, 2005). In addition to one of the main uses of lactose hydrolysis for alleviation of the lactose malabsorption problem, other prospective applications both in mainstream processing and as a route for by-products valorization are listed in Table 2. In this regard, lactose hydrolysis can be considered as belonging to the family of enzymatically catalyzed or chemical processes leading to added-value conversion of lactose to other industrially attractive products.

5. Conversion of lactose to value added materials

5.1. Overview

An overview on commercially produced derivatives of lactose is shown in Fig. 2 and their properties and applications are outlined below. Currently, only a small proportion of the lactose is used as feedstock for the chemical, enzymatic or microbiological conversion to lactose derivatives. However, because the market value is substantially higher than lactose, these compounds provide significant opportunities for value-added conversion of lactose to functional food ingredients. In recent years, attention was focussed on prebiotic lactose derivatives. Prebiotics were

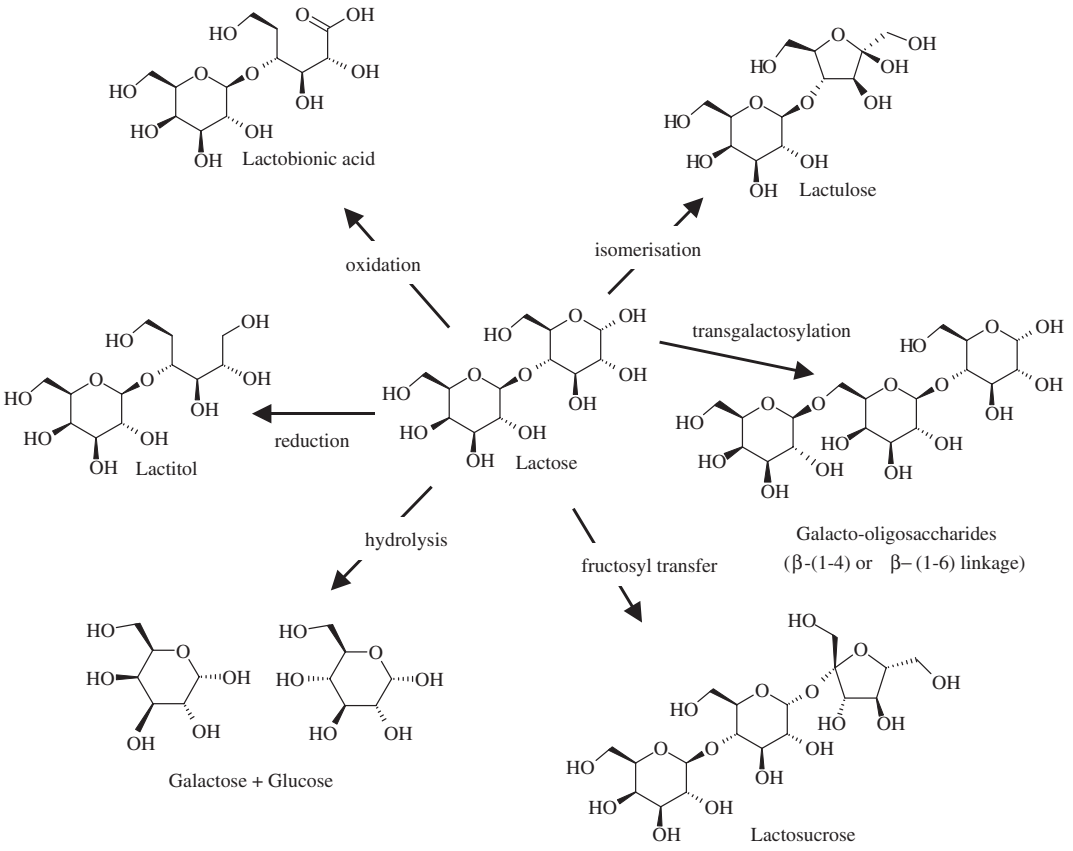

Fig. 2. Overview on commercially produced lactose derivatives.

defined in 1995 as "non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health" (Gibson & Roberfroid, 1995). Prebiotics are frequently referred to as "bifidus factor" in the older literature. Prebiotic compounds are not digested and adsorbed in the small intestine, therefore, they are classified as dietary fibre and contribute to a fibre-rich diet recognized as beneficial to human health (Englyst & Englyst, 2005). The end products of the metabolism of prebiotics by intestinal bacteria are lactate and short chain fatty acids (SCFA) which contribute to the beneficial effects of prebiotics (Topping & Clifton, 2001). Moreover, prebiotics are generally applied to stimulate growth and metabolism of bifidobacteria and the consumption of prebiotics results in an increase in both the occurrence and number of bifidobacteria isolated from fecal material (Böhm et al., 2004; Cummings, Macfarlane, & Englyst, 2001). The establishment of metabolically active bifidobacteria is considered to be beneficial for the host. Beneficial effects may include the regulation of bowel habit, stabilization of the gut mucosal barrier and the prevention of diarrhea, and increased mineral absorption (Cummings et al., 2001; Hopkins & Macfarlane, 2003; Lee, 1999). The application of prebiotics in infant foods is especially relevant to substitute the bifidogenic effect of oligosaccharides that are present in human milk at a level of 1–1.3%, whereas the milk of ruminants has much lower concentrations of oligosaccharides (Kunz, Rudloff, Baier, Klein, & Strobel, 2000). More than 130 different human milk oligosaccharides were identified. Most compounds carry lactose at the reducing end and are substituted with *N*-acetylglucosamine, L-fucose, and/or *N*-acetylneuraminic acid (sialic acid). In addition to the modulation of the microbial colonization of infants, possible functions of these compounds are the stimulation of the immune system, anti-microbial and/or anti-inflammatory protection, and to act as soluble receptors for pathogens (Kunz et al., 2000). While functions other than the prebiotic effects cannot be mimicked by lactose derivatives, the addition of prebiotic oligosaccharides to infant formulae resulted in levels of bifidobacteria in the intestine that was comparable to the one found in breast-fed infants (Veereman-Wauters, 2005).

5.2. Lactulose

As small quantities of lactulose are formed during heating of milk, it has been used as an indicator for milk heat treatment (Elliott, Datta, Amenu, & Deeth, 2005). Lactulose is produced by isomerization of lactose in alkaline solution; boric acid shifts the isomerization equilibrium in favor of lactulose and prevents side reactions. Lactulose, the first lactose derivative that was commercialized (Kozempel, Kurantz, Craig, & Hicks, 1995; Timmermans, 1997), can be produced enzymatically with β-galactosidases and fructose as galactosyl acceptor (Lee, Kim, & Oh, 2004; Mayer et al., 2004). The relative sweetness of lactulose is 0.6 in comparison to sucrose. Lactulose is a non-digestible carbohydrate which is fermented in the human colon (Nilsson & Nyman, 2005) but its specific bifidogenic effects is less pronounced when compared with fructo-oligosaccharides or galacto-oligosaccharides (Bouhnik et al., 2004). Lactulose is widely applied as laxative and administration of lactulose or lactitol is a standard treatment for chronic hepatic encephalopathy, although the scientific basis for the latter therapy is questionable (Als-Nielsen, Gluud, & Gluud, 2004).

5.3. Lactitol

Lactitol is produced by chemical hydrogenation of lactose; the relative sweetness of lactitol is 0.3–0.4 compared with sucrose.

Lactitol hydrolysis to galactose and sorbitol in the human intestine is dependent on the intestinal microflora; most of the lactitol is metabolized to short chain fatty acids by the colonic microflora while a part of the galactose is resorbed (for review, see Dills, 1989). Lactitol is a strong laxative and its metabolism by humans is insulin-independent. Polyols such as mannitol, xylitol or arabitol, lactitol are applied as non-caloric sweeteners in calorie-reduced and diabetic foods. Moreover, lactitol is used as an alternative to lactulose in the treatment of hepatic encephalopathy (Als-Nielsen et al., 2004).

5.4. Lactobionic acid

Lactobionic acid is commercially produced by chemical oxidation of lactose (Gerling, 1997). Alternative methods for the production of lactobionic acid employing a glucose-fructose oxidoreductase from *Zymomonas mobilis* were described (Satory et al., 1997). Lactobionate is a strong chelator of calcium and is used in calcium supplements in pharmaceuticals. The chelating properties of lactobionate also enable applications as ion sequestrant in detergent solutions (Gerling, 1997). Moreover, lactobionate at a concentration of 100 mmol L^{-1} is a key component of solutions used for the cold storage of transplant organs. The cell impermeant lactobionate prevents hypothermically induced cell swelling; in addition, iron chelation by lactobionate is thought to reduce oxidative injury during storage (Southard & Belzer, 1995).

5.5. Lactosucrose

The trisaccharide lactosucrose, derived from transfructosylation of lactose, is commercially produced in Japan. Its sweetness relative to sucrose is 0.3–0.6. Lactosucrose is not resorbed in the upper intestine and is, thus, available for hydrolysis and metabolism by the colonic microflora. Lactosucrose has a bifidogenic effect and its consumption was reported to decrease fecal pH and to inhibit growth of colonic clostridia (Ogata et al., 1993). However, the prebiotic effect of lactosucrose is not as well documented as is the case for fructo-oligosaccharides or galacto-oligosaccharides.

Transfructosylation of lactose is carried out by bacterial or fungal fructosyltransferases using sucrose or raffinose as fructosyldonor. Fructosyltransferases catalyze the hydrolysis of sucrose as well as the transfructosylation to acceptor carbohydrates. Fructosyltransferases catalyze the formation of high molecular weight fructan polymers and the formation of oligosaccharides in addition to sucrose hydrolysis. Depending on the type of polymer formed, fructosyltransferases are referred to as levansucrases or inulosucrases (Tieking & Gänzle, 2005; van Hijum, Kralj, Ozimek, Kijkhuizen, & van Geel Schutten, 2006). The formation of lactosucrose by levansucrase was initially described using an enzyme from *Rhanella aquatilis* (Hestrin & Avigad, 1958; Ohtsuka et al., 1992). Because the spectrum of acceptor-carbohydrates is essentially comparable in all bacterial levansucrases characterized so far (Cote & Ahlgren, 1993; Hestrin & Avigad, 1958; Tieking, Kühnl, & Gänzle, 2005), it can be assumed that the synthesis of lactosucrose from sucrose and lactose is a general property of bacterial levansucrases. Levansucrase activity is frequently found in food fermenting lactic acid bacteria, particularly *L. reuteri*, *L. pontis* and *L. acidophilus*. These organisms have been successfully used for the generation of high levels of oligosaccharides in food fermentations (Tieking, Ehrmann, Vogel, & Gänzle, 2005; Tieking et al., 2005).

5.6. Galacto-oligosaccharides

Galacto-oligosaccharides (GOS) are commercially produced from lactose using fungal β-galactosidases. The β-galactosidases hydrolyze lactose to glucose and galactose and alternatively catalyze the transgalactosylation of lactose to produce galacto-oligosaccharides (Matthews, 2005, Fig. 3). Depending on the source of the enzymes, the oligosaccharides have predominantly β(1-4) and/or β(1-6) linkages. For example, the β-galactosidase of *Kluyveromyces lactis* produced predominantly β-(1-6) oligosaccharides (6′-galactosyl-lactose and β-D-Gal(1-6)D-Gluc), a β-galactosidase of *Sterigmatomyces elviae* produced predominantly 4′-galactosyl-lactose whereas *Bacillus circulans* β-galactosidase forms β-(1-2), β-(1-3), β-(1-4) or β-(1-6) linkages to produce a large variety of oligosaccharides (Asp, Burvall, Dahlqvist, Hallgren, & Lundblad, 1980; Onishi, Yamashiro, & Yokozeki, 1995; Yanahira et al., 1995). Glucose, galactose, mannose, fructose, maltodextrins, *N*-acetylneuraminic acid, glucuronic acid and a number of aromatic compounds have been shown to act as galactose-acceptor for β-galactosidases, providing a virtually unlimited variety of oligosaccharides (Bridiau, Taboubi, Marzouki, Legoy, & Maugard, 2006; Lee et al., 2004; Miyasato & Ajisaka, 2004; Takada, Ogawa, Saito, Murata, & Usui, 1998; Yanahira et al., 1998, Fig. 3). Both 4′-galactosyl lactose and 6′-galactosyl lactose are considered to have prebiotic properties (for review, see van Loo et al., 1999). However, different oligosaccharide preparations may vary with respect to technological benefits such as flavor enhancing properties, sweetness, hygroscopicity, and solubility.

The transgalactosylation reaction of β-galactosidases is favored at high lactose concentrations (Huber, Kurz, & Wallenfels, 1976) and typical oligosaccharide yields range from 10% to 40% (GOS/initial lactose). The effect of lactose concentration on lactose turnover by a β-galactosidase from *L. delbrueckii* spp. *bulgaricus* is depicted in Fig. 4. High temperatures enable increased initial lactose concentration (Boon, Janssen, & van't Riet, 2000; Bruins, Strubel, van Lieshout, Janssen, & Boom, 2003). Moreover, high incubation temperatures strongly favored oligosaccharide formation over lactose hydrolysis by β-galactosidase from *L. delbrueckii* spp. *bulgaricus* and the optimum yield of GOS was achieved under denaturing conditions at 50 °C (Fig. 4, Vasiljevic & Jelen, 2003). Other approaches to optimize oligosaccharide formation by β-galactosidases include screening for enzymes with high preference for trangalactosylation (Boon et al., 2000; Cheng et al., 2006; Vasiljevic & Jelen, 2003), enzyme immobilization to achieve an improved enzyme stability at high temperatures (Albayrak & Yang, 2002), and use of high hydrostatic pressure to improve the yield of oligosaccharides (Kawade, Sakakibara, Nomura, Suzuki, & Kunugi, 1999). Moreover, protein engineering was successfully employed to obtain truncated or modified β-galactosidases with high preference for production of oligosaccharides (Jørgensen, Hansen, & Sougaard, 2001).

The use of lactic acid bacteria (LAB) as producers of β-galactosidase enzymes offers substantial potential for the production of GOS. First, LAB are known to be good producers of extracellular β-galactosidases that enable GOS production from lactose (Garman, Coolbear, & Smart, 1996; Hung, Xia, Hu, & Lee, 2001; Smart, 1991). Second, LAB have a safe tradition in food fermentations and exhibit rapid anaerobic growth on agricultural substrates including waste products such as whey. Therefore, GOS may be produced from crude cellular extracts without costly downstream processing (Vasiljevic & Jelen, 2001, 2003). Moreover, GOS may be produced in situ during food fermentations or by using whey to produce food-grade GOS preparations. *Bifidobacterium* spp. deserve special attention as a relevant source of enzymes suitable for production of galacto-oligosaccharides. The competitiveness of bifidobacteria in the intestinal tract of mammals is linked to their ability to metabolize a large variety of oligo- and polysaccharides. It was estimated that 8% of the genomic information in *Bifidobacterium longum* is dedicated to the metabolism of complex carbohydrates (Ventura, van Sinderen,

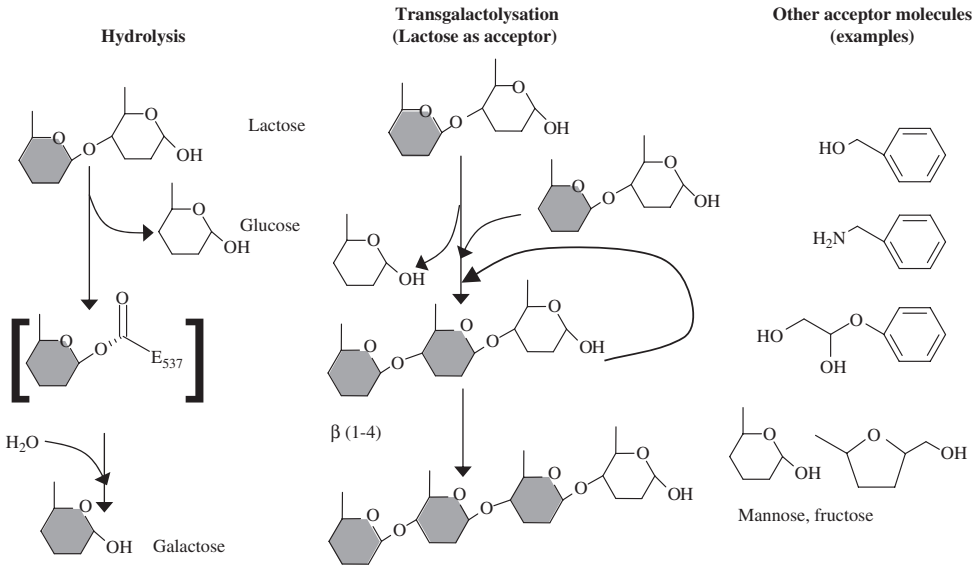

Fig. 3. Schematic overview of the hydrolysis- and acceptor-reaction catalyzed by β-galactosidases. Carbohydrate moieties are drawn to indicate the carbon atoms and the oxygen atoms of interest; galactose moieties are shaded gray. Lactose hydrolysis involved intermediate linkage of the galactosyl moiety to the glutamine residue in the active site of the enzyme (E537 in the β-galactosidase of *Escherichia coli*) and subsequent transfer to H_2O acting as galactosyl acceptor. Transgalactosylation to lactose or galacto-oligosaccharides yields β(1-2), β(1-3), β(1-4) or β(1-6) linked galacto-oligosaccharides (the latter two predominating in most enzymes); other monosaccharides as well as phenolic compounds have additionally been shown to act as galactosyl acceptor.

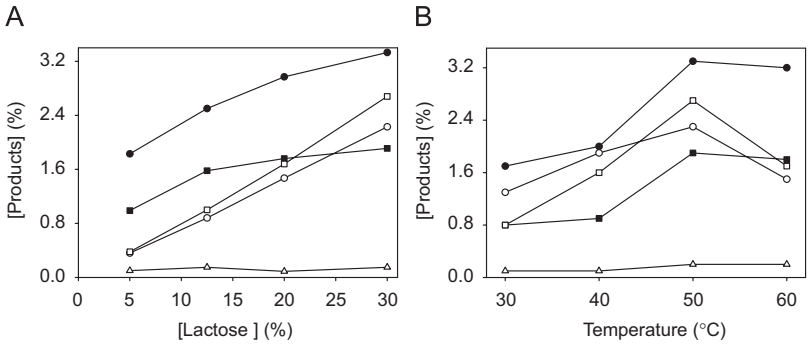

Fig. 4. Lactose turnover by β-galactosidase in crude cellular extract from *Lactobacillus delbrueckii* spp. *bulgaricus*. Panel A: influence of lactose concentration on product composition and Panel B: influence of temperature; ●, glucose; ■, galactose; ○, disaccharides; □, trisaccharides; Δ, tetrasaccharides. Replotted from Vasiljevic and Jelen (2003).

Fitzgerald, & Zink, 2004). Several enzymes with activity on lactose or galacto-oligosaccharides were characterized at a biochemical level. The enzyme β-Gal-II from *Bifidobacterium adolescentis* DSM20083 has a low transgalactosylation activity and a remarkable specificity for hydrolysis of lactose, 4′galactosyllactose and higher β-(1-4) linked galacto-oligosaccharides (Hinz, van den Broek, Beldman, Vincken, & Voragen, 2004). *Bifidobacterium infantis* HL96 harbors two β-galactosidase genes, β-Gal-I and β-Gal-III. Both enzymes are active towards lactose but the oligosaccharide yield of β-Gal-I exceeded that of β-gal-III more than threefold (Hung et al., 2001).

6. Conclusions and future prospects

Lactose is presently a most underutilized dairy component. The continued struggle to find new uses for the cheese whey is tied to finding new uses for lactose, the main whey component. It becomes increasingly difficult to dispose of whey or any other process streams containing high amounts of lactose from dairy and cheese operations as: (i) environmental discharge standards are getting higher and more difficult to comply with, (ii) authorities are becoming more vigilant in all regions, (iii) biological as well as physico-chemical treatments are becoming more complex, and (iv) available waste treatment scenarios are becoming more expensive. Therefore, the desire to convert lactose and lactose-containing components into commercially viable products is increasing, and additional innovative applications in the pharmaceutical and chemical industries are needed.

The traditional view of lactose as a commodity, produced by traditional crystallization or possibly other processes such as spray drying, has not been economically advantageous in the past. Although there are several industrial lactose manufacturers offering a wide array of lactose products for diverse uses (from infant foods to confectionery to pharma lactose), the overall market for the traditional lactose products is relatively static and radically new approaches to lactose utilization are needed to achieve a "quantum leap" in converting this unique carbohydrate to new industrially attractive products. Some of the lactose derivatives discussed above are just a few examples of the new trends in surplus lactose management. Additional major opportunities may arise in using lactose (with or without prior hydrolysis) for various fermentation applications. Lactose used to be one of the cheapest fermentation carbohydrate on the market for some time. It is not inconceivable that lactose, in its crudest form as whey or UF permeate, could become an

economical source of biogas or bioethanol. Unfortunately, the literature of the last 65 years is replete with similar suggestions and pronouncements, while the dairy world is still waiting for the "final solution" of the whey (and thus lactose) problem.

Finally, addressing the widespread problem of lactose intolerance on a global level could bring major health benefits to many lactose intolerant populations around the world who presently have little access to milk and dairy products. This would also benefit the industrial dairy processors in opening up new attractive markets for dried whey and other dairy products containing lactose. Such a development would bring about a recognition of milk as a truly "mother nature's original nutraceutical product", as well as converting lactose from money-losing inconvenience of the past to money-making opportunity.

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