Current trends of β-galactosidase application in food technology

ZUZANA MLICHOVÁ - MICHAL ROSENBERG

Summary
β-Galactosidase, which hydrolyses lactose into glucose and galactose, is a widely used in food technology, mainly in the dairy industry. The enzyme is utilized in the development of new products with hydrolyzed lactose, which are suitable for lactose-intolerant people, for the improvement of technological properties of non-fermented milk products and for removing the lactose from a whey. Industrial application of β-galactosidase is also in the production of galacto-oligosaccharides. These are applied in a wide variety of foods because of their positive effect on the intestinal bacterial microflora.

Keywords
β-galactosidase; lactose hydrolysis; galacto-oligosaccharides; milk

Enzymatic hydrolysis of lactose is one of the most important biotechnological processes in the food industry. It is realised by enzyme β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23), trivial called lactase. The enzymatic hydrolysis of lactose proposes several benefits and advantages of industrial application, including:

- development of lactose hydrolysed products to present one of the possible approaches to diminish the lactose intolerance problem, prevalent in more than half of the world's population [1, 2],
- formation of galacto-oligosaccharides during lactose hydrolysis to favour the growth of intestinal bacterial microflora [3],
- improvement in the technological and sensorial characteristics of foods containing hydrolysed lactose [4],
- better biodegradability of whey after lactose hydrolysis [5].

The benefits of β-galactosidase in lactose hydrolysis and production of galacto-oligosaccharides are discussed in this work.

MICROBIAL SOURCES AND PRODUCTION OF β-GALACTOSIDASE

β-Galactosidase belongs to the group of saccharides converting enzymes in the family of hydrolases. They are widespread distributed in numerous biological systems, e.g. microorganisms, plants and animal tissues. Compared to animal and plant sources of enzyme, microorganisms produce enzyme at higher yields, resulted in decrease price of β-galactosidase [5]. Therefore the work is focused on microbial production of the enzyme. β-Galactosidase occurs in a variety of microorganisms, including yeasts, fungi, bacteria and actinomycetes [4].

Properties, specificity and structure of β-galactosidase significantly differ on the microbial source of the enzyme, e. g. different molecule weight, amino-acids chain length, position of the active site [6], pH- and thermal- optimum and stability (Tab. 1). The choice of suitable β-galactosidase source depends on reaction conditions of lactose hydrolysis. For example, dairy yeasts with a pH optimum 6.5–7 are habitually used for the hydrolysis of lactose in milk or sweet whey. On the other hand, the fungal β-galactosidases with optimum pH 3–5 are more suited for acidic whey hydrolysis [8, 15]. The activity of different β-galactosidases also depends on presence of ions. The fungal β-galactosidases are active without ions as cofactors, the yeast β-galactosidase isolated from *Kluyveromyces lactis* requires ions, such as Mn^{2+}, Na^{+}, and β-galactosidase from *Kluyveromyces fragilis* ions such as Mn^{2+}, Mg^{2+}, K^{+} [16]. On the contrary, Ca^{2+} and heavy metals inhibit the enzyme activity of all β-galactosidases [17].

Zuzana Mlichová, Michal Rosenberg, Institute of Biotechnology and Food Science, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovak Republic.

Correspondence:
Zuzana Mlichová, e-mail: zuzana.mlichova@stuba.sk

Tab. 1. Properties of microbial β-galactosidases.

Microorganism		Production of enzyme	pH-optimum	T-optimum [°C]	References
Fungi	*Aspergillus niger*	E	3,0–4,0	55–60	[7]
	Aspergillus oryzae		5,0	50–55	[8, 9, 10]
Yeasts	*Kluyveromyces lactis*	I	6,5–7,0	30–35	[8, 11, 12]
	Kluyveromyces fragilis		6,6	30–35	[8,12]
Bacteria	*Escherichia coli*	I	7,2	40	[7, 13]
	Lactobacillus thermophilus		6,2	55	[7]
	Leuconostoc citrovorum		6,5	66	[14]
	Bacillus circulans		6,0	65	[8]

I - intracellular, E - extracellular.

Although the most studied β-galactosidase is the one produced by *Escherichia coli*, possible toxic factors associated with coliforms make it unlikely that crude isolates of this enzyme will be permitted in food processes [5]. Therefore, β-galactosidases used in industrial scale for the production of milk and dairy products are isolated from microorganisms with GRAS status (generally recognized as safe). Yeast (mainly from *K. lactis* and *K. fragilis*) and fungal (mainly from *A. niger* and *A. oryzae*) enzymes have the greatest commercial significance [15].

Two types of β-galactosidases are of increasing significance in industrial processing: thermostable and cold-active [18]. Their use provides a number of advantages. Application of thermostable enzymes at high temperatures is connected with decreased viscosities of the substrate solution and with a reduction of undesired microbial contamination [19]. Cold-active enzymes provide treatment of milk and dairy foods under mild conditions so that taste and nutritional values remain unchanged [20].

LACTOSE HYDROLYSIS

Lactose (disaccharide consisted of glucose and galactose, linked by β-(1-4)-O-glycosidic bound) can be hydrolysed chemically (acid hydrolysis) [21] or by the use of enzymes. Compared to chemical hydrolysis, the enzymatic have several benefits: no by-products, no degradation of compounds in dairy products, no additional nasty flavours, odours and colours. Furthermore, milk treated by the enzyme retains its original nutrition value, especially since glucose and galactose are not removed [22]. Therefore, the use of enzyme for lactose hydrolysis is still very important in food and pharmaceutical applications.

The enzymatic hydrolysis of milk lactose has two alternatives, with free or immobilized β-galactosidase. Immobilization of β-galactosidase is a promising method to decrease the cost of the lactose hydrolysis process because of multiplied repetitive use of the enzyme for the bioprocesses. In addition, immobilized enzyme allows to hydrolyse lactose continuously. The successful verification of immobilized β-galactosidase by various methods, including entrapment, cross-linking, adsorption, or combination of these methods was described in several papers [22-24].

LACTOSE INTOLERANCE

Lactose is naturally found in highly concentrated only in milk and milk products. Cow's milk contains 4.5–5 % lactose, which is over one third of the solid phase in milk, approximately 20 % in ice cream and about 72 % in whey solids. The disaccharide performs important biological functions such as stimulating the growth of bifidobacteria and supplying galactose, an essential nutrient for the formation of galacto-oligosaccharides and cerebral galactolipids [25].

Lactose cannot be absorbed as such, but it must be hydrolysed into its component saccharides (which are easily absorbed from the intestine) by the action of intestinal β-galactosidase [26]. A significant number of the adult population (almost 75 %) is unable to digest lactose due to a genetic deficiency of this enzyme – lactase deficiency [1, 27].

Mammals are born with abundant β-galactosidase production, following weaning. All nonhuman mammals are genetically programmed to reduce β-galactosidase synthesis. β-galactosidase levels of adult animals are only about 1/10 of that infancy. The majority of human follow the animal pattern and are genetically programmed to lose the ability to synthesize β-galactosidase. However, a mutation

in some population groups allows the infantile level of β-galactosidase to persist throughout adulthood [25, 27]. There are three main types of lactase deficiency: congenital, primary and secondary lactase deficiency. Congenital lactase deficiency is a very rare genetic abnormality in which the enzyme β-galactosidase is very low or absent at birth. Primary lactase deficiency is the most common type and occurs as a normal physiological process in which β-galactosidase production in the brush border of the small intestine is reduced. Primary lactase deficiency occurs in people between 2 and 20 years old. Secondary lactase deficiency is usually a temporary condition in which low levels of the enzyme occur as a result of an underlying disease that affected the gastrointestinal tract [28].

The lactose reaching the large intestine can cause symptoms such bloating, flatulence, abdominal pain, cramps and diarrhoea (a clinical condition known as lactose intolerance). Temporary lactase deficiencies in individuals with normal β-galactosidase levels may also result from damage to the intestinal lining caused by viral or bacterial infections, cancer chemotherapy, allergic or autoimmune conditions, and from decreases in β-galactosidase associated with aging [28, 29].

Appearance of lactase deficiency depends on ethnic and racial group of population. Most of the Asians (more than 90%), most of Africans (80–100%), Native Americans (more than 90%), Southern Europeans (more than 80%) are reported to be lactose intolerant. On the other side, real lactose intolerance is confined mainly to people whose origins lie in Northern Europe (including Sweden, Great Britain, Holland, Germany, etc. less than 5 %) or the Indian subcontinent and is due to "lactase persistence" [30].

It is generally recommended for lactose intolerant people to avoid consumption of milk and dairy products. However, most lactose intolerant people are able to digest small amount of milk (approximately 200 ml). They can also consume fermented milk products (such as yoghurts), cheeses (hard and semi-hard) that contains no or only small amounts of lactose (present in only 10% of soft cheeses). These products are very important sources of calcium. Compared to milk, the lactose content of yoghurt is usually lower by about one third. In addition, lactose presented in yoghurt is very well tolerated by lactose intolerant people because of presence of living lactic acid bacteria with β-galactosidase activity [27].

The lactose intolerance in people can be solved in several ways. The pre-hydrolyzation of milk and milk-based products with the use of β-galactosidase offers an attractive way how to get nutritional rich milk with reduced levels of lactose. The fungal or yeast β-galactosidases serving for lactose-containing foods treatments are commercially available. The β-galactosidase may also be in a tablet form and ingested immediately before the consumption of milk products. These tablets contain β-galactosidase derived from the strains of *Aspergillus* (active at low pH), which allows the function in human stomach [31].

There is a relatively large group of lactose intolerant people (with different level of β-galactosidase deficiency) in Slovak Republic. However, the lactose hydrolysed milk products are absent at the market. That is one of the reasons resulting in drastically decrease of milk consumption (about 30% since 1990). The total consumption of milk and milk products is approximately 158.3 kg/person/year, which is significantly less than in most other parts of the European Union (with average consumption 240 kg/person/year). The consumption of liquid milk (65.1 kg/person/year) and fermented milk products decreased extensively, including the consumption of yoghurts (12,45 kg/person/year) [32]. This may lead to negative health state of Slovak population, because milk is a natural and important source of energy, proteins, fat, vitamins and minerals [33].

ENZYMATIC LACTOSE HYDROLYSIS IN MILK

The enzymatic hydrolysis of lactose is also desired in food technology because of low solubility of lactose. High lactose concentration in non-fermented milk products such as ice cream and condensed milk, can lead to excessive lactose crystallisation resulting in products with a mealy, sandy or gritty texture. Hydrolysis of lactose in ice cream and other dairy products improves scoopability and creaminess significantly, and these products are more digestible. Also for this purpose, the use of β-galactosidase enzyme prior to the condensing operation can reduce the lactose content to a point where lactose is no longer a problem [4].

Moreover, compared to lactose, monosaccharides formed by lactose hydrolysis are fermented more easily, and that results in reduction of the period from the addition of starter culture to obtaining the desired low pH in certain products such as cottage cheese and yoghurts. In addition, glucose and galactose considerably increase sweetness of the products (about 50%). Therefore, the amount of additional sweeteners in yoghurts is reduced resulting in fewer calories of final product [4, 34].

ENZYMATIC LACTOSE HYDROLYSIS IN WHEY

Lactose is a waste in the cheese industry, which causes several economical and environmental problems. Cheese production creates large quantities of whey as a by-product (150 million tons annually worldwide), whose main components are lactose (44–52 $g.l^{-1}$), proteins (6–8 $g.l^{-1}$) and minerals (4.3–9.5 $g.l^{-1}$) [35].

A substantial portion of cheese whey is treated by ultrafiltration to produce a protein rich ingredient - whey protein concentrate (WPC). However, the ultrafiltration process generates large quantities of permeate, which still contain 4–5% of lactose [36]. A part of whey and whey permeate lactose can be purified by crystallization and used together with its derivates as a supplement in foods and as an excipient for pharmaceutical products [37]. In spite of this, approximately 47% of whey is disposed in waters or loaded onto the land [38]. This leads to critical environmental problems of the dairy industry, because lactose is associated with the high biochemical and chemical oxygen demand (BOD/COD). More than 90% of whey BOD is determined by lactose [39]. Therefore, there is a need for investigation about further treating possibilities of lactose from whey. The process based on microbial cultivation of cheese whey permeate may offer a suitable alternative. The number of commercially interesting microorganisms that are able to directly utilize lactose, as a carbon source, is notably lower than the number of microorganisms that are able to metabolise glucose and galactose directly. Therefore, the preliminary hydrolysis of disaccharide will significantly increase the number of biological systems able to utilize lactose and the range of the bioproducts that can be obtained, i.e. biomolecules (lactates, acetates, ethanol, buthanediol, etc.), biopolymers, biomass [38, 40, 41]. Another perspective application of lactose from whey (after enzymatic hydrolysis) is manufacturing of syrup sweeteners and galacto-oligosaccharides [42, 43].

FORMATION OF GALACTO-OLIGOSACCHARIDES (GOS)

β-Galactosidases are also very useful for the human health because of the formation of galacto-oligosaccharides (GOS), used as prebiotic food ingredients. GOS are produced simultaneously during lactose hydrolysis due to transgalactosylation activity of β-galactosidase. The total amount of these oligosaccharides varies from 1–45% of the total saccharides present and depends on the source of enzyme [3, 44]. The ability of β-galactosidases to produce a series of oligosaccharides containing galactose was reported in the early 1950s [45, 46]. Later studies were focused on the optimization of conditions for their production [8, 47]. More recently, interest in the positive effect of oligosaccharides addition to the human health has been reported [48]. GOS belong to prebiotics – nondigestible food ingredients that are able to modify the intestinal microflora in favour of health promoting bacteria (*Bifidobacterium sp.* and *Lactobacillus sp.*) [49, 50]. Compared to other oligosaccharides, which supported the metabolic activity of all intestinal bacteria, the GOS are selective for probiotic acidic bacteria [51].

Commercially available GOS, in powder or liquid form, are mixtures of several types of GOS (more than 50%), lactose (20%), glucose (20%) and a small amount of galactose. GOS are quite stable during long-term storage at room temperature even in acidic conditions. Therefore, GOS

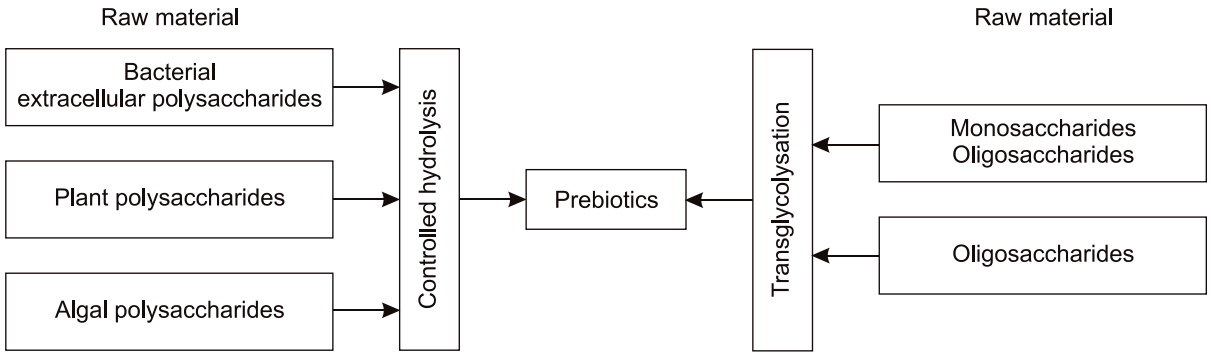

Fig. 1. Biotechnological production of prebiotics.

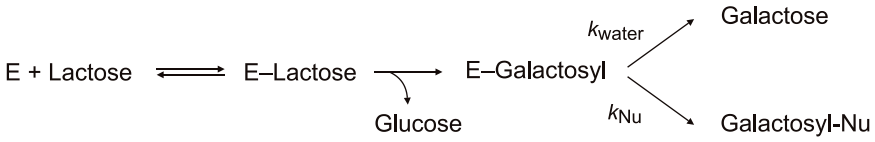

Fig. 2. Synthesis of galacto-oligosaccharides.
E - β-galactosidase, Nu - nucleophil-saccharide, *k* - reaction constant.

can be applied without decomposition in variety of foods. Major companies dealing with oligosaccharides production (including GOS) are in Japan [48]. Recently, there is also increasing trend of GOS production in Europe. Besides lactulose and soybean oligosaccharides, all oligosaccharides are prepared by transglycosylation from mono- and disaccharides or by controlled hydrolysis of polysaccharides (Fig. 1) [52].

PROPERTIES AND STRUCTURE OF GALACTO-OLIGOSACCHARIDES

The production of GOS involves three main steps. First, the glucose is released as a product and leaves an enzyme-galactosyl complex for further reaction (Fig. 2). Secondly, this complex is transferred to an acceptor containing a hydroxyl group (water or other saccharides). In the solution with low lactose concentration, the acceptor is water which results in galactose formation. In lactose concentrated solutions, lactose molecules act as the acceptor and bind the enzyme-galactose complex resulted in galactosyl-oligosaccharides formation, which is the last step [53, 54]. The transglycosylation activity of β-galactosidase increases with higher initial lactose concentration [55].

The GOS have the following molecular structure: α-D-Glu (1→4)-[β-D-Gal (1→6)]$_n$, where n = = 2–10. Their quantitative abundance decreases seriately: di-, tri-, tetra-, and higher oligosaccharides. The primary transferase product is allolactose (β-D-Gal (1→6)-D-Glc) formed along with galactobiose (β-D-Gal (1→6)-D-Gal) at all substrate concentrations (Fig. 3). They are formed directly from monosaccharides whose concentration rises as lactose is hydrolysed, or from degradation of trisaccharides. Other di- and higher saccharides are formed at higher initial lactose concentration. The composition of the GOS also changed during the reaction as indicated by the increase in the galactose/glucose ratio [3, 37].

Many β-galactosidases were isolated and purified to produce GOS, but yields are generally poor and only a few have been used for large-scale production. Concentrations and structures of the GOS depend on several factors such as character of enzyme (link with the source of β-galactosidase), concentration and character of the substrate, the conversion and the reaction conditions [37, 56]. For example, RABIU et al., [44] investigated several strains of *Bifidobacterium* to produce GOS from 30% initial lactose solution. They obtained the maximum of GOS production (43.8 %) using *B. angulatum*, whereas the strain *B. pseudolongum* produced no more than 26.8% of GOS. There are several types of bounds between two galactose units of GOS, which vary and depend on enzyme source, e.g. the β(1-4)-bounds are in β-galactosidase derived from *Bacillus circulans* [57], β(1-6) bounds when enzymes are isolated from *A. oryzae, Streptococcus thermophilus* [58]. *Bifidobacterium bifidum* is known to synthesize GOS from lactose with primal β(1-3) linkages [59]. Another factors influencing GOS synthesis are reaction conditions: pH and temperature. Although there are some differences of GOS yields, these influences are statistic unimportant compared to the influence of initial substrate concentration [3, 8].

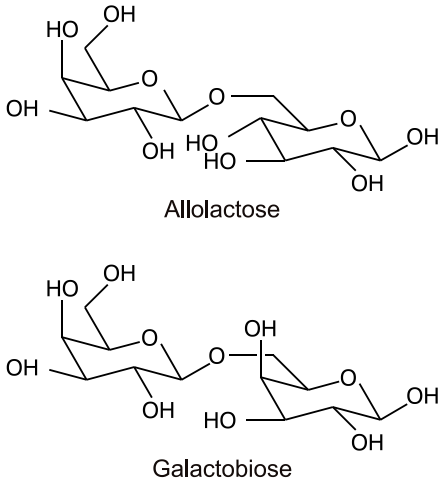

Fig. 3. Structure of principal disaccharides.

FUNCTION AND APPLICATION OF GALACTO-OLIGOSACCHARIDES

GOS are commonly present in human milk. It has been shown that human milk oligosaccharides induce an increase in the number of bifidobacteria of colonic flora in breast-feed infant, accompanied with a significant reduction in the number of potential pathogenic bacteria, due to their bifidogenic activity. Therefore some infant food companies have included GOS in the composition of their products in an attempt to emulate the beneficial action of complex carbohydrates in human milk. In others, they are used in cereal-based baby foods [48]. However, they are used not only in infant foods, but also in those aimed for adults.

The human body lacks the enzymes required to hydrolyse beta links, including the links formed among oligosaccharides. The GOS are considered to behave as soluble alimentary fibres. They arrive whole to the large intestine where they are fermented by the colonic flora. One of the results of this fermentation process is creating of notable short chain fatty acids linking with decrease in faecal pH [50]. The acidic medium is an unfavourable to the development of pathogenic microorganisms [49]. The GOS are also not metabolised by microorganisms present in the mouth (*Streptococcus mutants*), which do not produce cariogenic compounds [60].

System oligosaccharides/bifidobacteria provides a wide variety of health benefits, including anticarcinogenic effects, reduction in serum cholesterol, improved liver function, reduction of the colon cancer risk and improved intestinal health [50, 61]. Therefore, the public demand for their production is significantly increased together with the development of an effective and inexpensive GOS production. GOS are currently used as low calorie sweeteners, food ingredients, and cosmetic additives. They are included in a wide variety of foods such as soft drinks, cookies, cereals, chewing gums, candies, ice cream, yoghurts, powdered milk, clabbered milk, etc. [3, 37, 62].

The beneficial effects of consuming GOS for both nursing children and during other stages of life, have demonstrated their safety and efficacy. The proof of this is their more frequent inclusion in food products. In addition, the formation of GOS from lactose is also great interest for whey processing.

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