Pol. J. Food Nutr. Sci., 2017, Vol. 67, No. 1, pp. 33–39
DOI: 10.1515/pjfns-2016-0002
http://journal.pan.olsztyn.pl

Original article
Section: Food Technology

Synthesis of Galactosyl Mannitol Derivative Using β-Galactosidase from *Kluyveromyces lactis*

Robert Klewicki[1]*, *Iwona Belina*[1], *Aleksandra Wojciechowska*[1], *Elżbieta Klewicka*[2], *Michał Sójka*[1]

[1]*Lodz University of Technology, Institute of Food Technology and Analysis, Stefanowskiego 4/10, 90–924 Łódź, Poland*
[2]*Lodz University of Technology, Institute of Fermentation Technology and Microbiology,*
Wólczańska 171/173, 90–924 Łódź, Poland

Key words: mannitol, β-galactosidase, transglycosylation, lactose

The purpose of the study was to identify the influence of reactive mixture concentration (23–48 g/100 mL), pH (6.5–9.0), presence of NaCl (0.05–0.25 mol/L) and enzyme dose (2850–28,500 LAU/100 g of lactose) on the synthesis of galactosyl mannitol derivative using β-galactosidase from *Kluyveromyces lactis*. The use of the enzyme dose ranging from 2850 to 11,400 LAU/100 g lactose allowed obtaining gal-mannitol at the level of 21.8% total saccharides; higher doses intensified product decomposition. An increase in the concentration of the reactive mixture had a positive impact on the quantity of gal-mannitol obtained every single time, *i.e.* 4.39 g were obtained from 100 mL of a 23 g/100 mL solution and over 10 g were obtained from a 48 g/100 mL solution. A relatively low increase in product quantity (by *ca*. 5%) occurred after the pH was increased from 6.5 to 9.0. The use of NaCl rendered better results. An increase in the maximum content of gal-mannitol in the total sugar by 12.8% was observed at the concentration of 0.25 mol/L.

INTRODUCTION

Substances with a saccharide-polyol structure demonstrate interesting characteristics. Once used in the diet of rats, they have a positive influence on the parameters of blood and cecum [Juśkiewicz *et al*., 2006]. In their presence, lactic acid bacteria reveal antagonistic action against selected pathogens [Klewicki & Klewicka, 2004]. Lactitol (combination of galactose and sorbitol) has prebiotic properties, typical of transglycosylation products (oligosaccharides) [Saarela *et al*., 2003] with properties characteristic of alditols such as low hygroscopicity, reduced energy value, low susceptibility to non-enzymatic browning, resistance to hydrolysis and increased temperature [Linden & Lorient, 1999]. Lactitol is the only compound of that kind which is relatively cheap and commonly available on the market. It is obtained by catalytic hydrogenation of lactose. It is used as a sweetening agent [Seki & Saito, 2012]. Besides lactulose, lactitol is also used as an anti-hepatic encephalopathy agent [Sharma & Sharma, 2013].

The volume of data on enzymatic synthesis of galactosyl-polyols is relatively limited in the literature. Synthesis is based on the use of transglycosylation properties of β-galactosidase. Galactooligosaccharides are generated in lactose solutions by attaching a galactosyl residue to the saccharides present in the reaction medium [Guerrero *et al*., 2015]. The presence of polyol besides lactose in the reactive mixture makes transglycosylation activity of the enzyme greatly oriented towards the synthesis of gal-polyols. Glycerol seems to be most commonly used as the acceptor, according to the reports on the issue. Wei *et al.* [2013], Cardelle-Cobas *et al.* [2009] and Stevenson *et al.* [1993] used β-galactosidase from *Kluyveromyces lactis* for the synthesis of its galactosyl derivative. Irazoqui *et al.* [2009] used an enzyme from *Aspergillus oryzae*. Nakano *et al.* [2003] obtained a glucose derivative of glycerol carrying out the reaction in the mixture of soluble starch and glycerine using cyclodextrin glucanotransferases. Moreover, α-galactosyl derivative was obtained by using guar gum as a source of galactose and α-galactosidase from *Aspergillus* sp. MK14 [Kurakake *et al*., 2015]. Sorbitol was another polyhydroxyalcohol used as an acceptor. Klewicki [2007a] used β-galactosidase from *Kluyveromyces fragilis, Kluyveromyces lactis* and *Aspergillus oryzae*. Lu *et al.* [2009] used an enzyme from *Enterobacter cloacae* for gal-sorbitol synthesis. Moreover, the following compounds were also used as acceptors: erythritol [Irazoqui *et al*., 2009; Klewicki, 2007a], maltitol (using cyclodextrin glucanotransferase) [Kim *et al*., 1997] and lactitol [Klewicki, 2007a; Yanahira *et al*., 1997]. The possibility of attaching a galactosyl residue (as well as other saccharides) to an acceptor other than sorbitol, provides conditions for obtaining gal-polyols with properties potentially different than these of lactitol. It provides good prospects for the development of the market of prebiotics.

The paper presents conditions of gal-mannitol synthesis. Mannitol itself demonstrates interesting properties. Its physiological calorific value amounts to 1.6 kcal/g, which

---

* Corresponding Author: E-mail: robert.klewicki@p.lodz.pl

is 2.5 times lower than that of sucrose. It is about half less sweet, though, and is used as an agent improving products texture. It reduces sugar tendency to crystallize. Its hygroscopicity is low, hence products containing mannitol are more stable under increased humidity. It has a cooling effect and masks unpleasant tastes. In medicine, it is used as a diuretic or agent facilitating drugs transport to the brain [Saha & Racine, 2011].

In the available literature it is mentioned as an acceptor for galactosyl residues transmitted from *o*-nitrophenyl-β-D-galactopyranoside (*o*NPGal) by β-galactosidase from *Enterobacter cloacae* B5 [Lu *et al*., 2009; Lu *et al*., 2010].

The purpose of the study was to identify the impact of the reactive mixture concentration, pH, temperature and enzyme dose on the synthesis of galactosyl mannitol derivative using β-galactosidase from *Kluyveromyces lactis* and lactose as a donor.

MATERIALS AND METHODS

Materials

The following compounds and reagents were used in the study: Lactozym – an enzymatic preparation of β-galactosidase from *Kluyveromyces lactis* (Novozymes A/S, Bagsvaerd, Denmark); lactose (Bayerische Milchindustrie eG, Landshut, Germany); mannitol (Ubichem, Redditch, United Kingdom); acetonitrile (J.T. Baker from Witko, Łódź, Poland); KH_2PO_4 (POCh, Gliwice, Poland), and $Na_2HPO_4 \times 12H_2O$ (POCh, Gliwice, Poland).

Gal-mannitol synthesis in solutions with different contents of dry mass

β-Galactosidase in the amount of 11,400 LAU/100 g of lactose (1 LAU is defined as the amount of enzyme which releases 1 mmol glucose per minute under the following standard conditions: 4.75 % (w/w) lactose, pH 6.5, 37°C, reaction time 30 min) was added to 150 mL of a reactive mixture containing lactose and mannitol in a 1:1 ratio (w/w) with the following dry matter contents: 23, 28, 33, 38, 43 or 48 g/100 mL. The reaction was carried out at 37^{o}C for 3 to 8 hours. The pH value was 6.5±0.1 (adjusted with a solution: 3.471 g KH_2PO_4 + 8.954 g $Na_2HPO_4 \times 12H_2O$/ 100 mL). Samples (10 mL) were collected every 30 minutes. Experiments were performed in two replications.

Gal-mannitol synthesis using different enzyme doses

The following quantities of β-galactosidase were added to 150 mL of a 33 g/100 mL reactive mixture containing lactose and mannitol in a 1:1 ratio (w/w): 2850; 5700; 11,400; 17,100; 22,800 or 28,500 LAU/100 g of lactose. The reaction was conducted at 37^{o}C, for 3 to 8 hours. The pH level was 6.5±0.1 (adjusted with a solution: 3.471 g KH_2PO_4 + 8.954 g $Na_2HPO_4 \times 12H_2O$/100 mL). Samples (10 mL) were collected every 15 or 30 minutes. Experiments were performed in two replications.

Gal-mannitol synthesis in solutions with different pH

β-Galactosidase in the amount of 11,400 LAU/100 g of lactose was added to 150 mL of a 33 g/100 mL reactive mixture containing lactose and mannitol in a 1:1 ratio (w/w). The reaction was conducted at 37^{o}C, for 4 to 10 hours. The pH level was 6.5; 7.5; 8.5 or 9.0±0.1 (adjusted with 2 mol/L NaOH solution). Samples (10 mL) were collected every 30 or 60 minutes. Experiments were performed in two replications.

Gal-mannitol synthesis in the presence of NaCl of different concentrations

β-Galactosidase in the amount of 11,400 LAU/100 g of lactose was added to 150 mL of a 33 g/100 mL reactive mixture containing lactose and mannitol in a 1:1 ratio (w/w) and 0.05 mol/L, 0.1 mol/L or 0.25 mol/L NaCl. The reaction was conducted at 37^{o}C, for 4 to 10 hours. The pH level was 6.5±0.1 (adjusted with a solution: 3.471 g KH_2PO_4 + 8.954 g $Na_2HPO_4 \times 12H_2O$/ 100 mL). Samples (10 mL) were collected every 60 minutes. Experiments were performed in two replications.

Identifying gal-mannitol content using HPLC method

A sample (10 mL) of the reactive mixture was introduced into 20 mL of boiling distilled water; boiling was maintained for 3 minutes. After cooling down to room temperature, it was placed in a 50 mL volumetric flask and filled up with water. It was filtrated after mixing. A 5 mL sample was desalinated using a column containing a mixture of cationite and anionite (1:2). The first part of the filtrate (3 mL) was removed and another 2 mL were collected and sent for HPLC analysis (KNAUER). Separation conditions were as follows: column – Shodex Asahipak NH_2P, mobile phase – acetonitrile: water (80:20 ratio), flow rate – 0.8 mL/min, detector – RI.

MS analysis of gal-mannitol

The gal-mannitol peak was collected from five repeated HPLC separations. The obtained sample was then subjected to MS detector Q Exactive Orbitrap equipped with HESI probe (Thermo Scientific, Waltham, MA, USA). The sample was directly injected (10 μL/min) into MS detector. Analyses utilized the negative ion mode. The source parameters were as follows: ion spray voltage, 3.00 kV; capillary temperature, 300°C; and sheath gas and auxiliary gas, 10 and 0 units/min, respectively.

Statistical analysis

STATISTICA 10 (StatSoft. Inc., USA) was used to create figures. Mean values ± standard deviation were presented.

RESULTS AND DISCUSSION

The tendency to use transglycosylation products as substrates at a significant degree of using the basic substrate is the unfavorable property of glycosidases; and in the case of β-galactosidase this phenomenon is observed for far reached lactose hydrolysis [Rodriguez-Colinas *et al*., 2014; Manera *et al*., 2012]. The reduced content of gal-polyols in the reactive mixture is also attributed to this phenomenon. Therefore, identifying the right dose of the enzyme in relation to the reaction time is an essential factor for selecting optimum conditions for synthesis of transglycosylation products

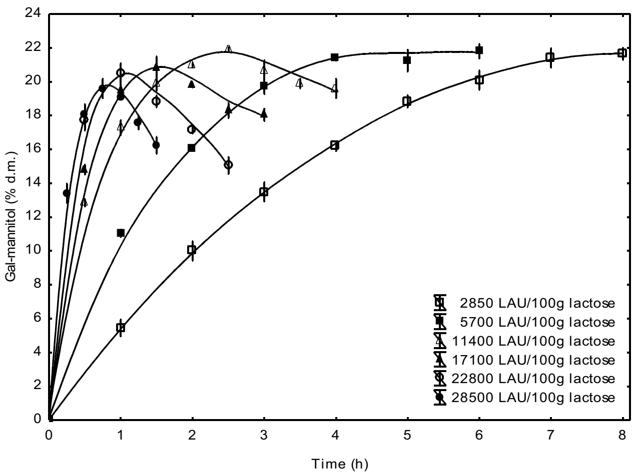

FIGURE 1. Changes in the content of gal-mannitol in the total sugar during synthesis in 33 g/100 mL solution of lactose and mannitol (1:1 ratio, w/w) depending on the enzyme dose. Reaction conditions: 37°C, pH 6.5, β-galactosidase from *Kluyveromyces lactis.*

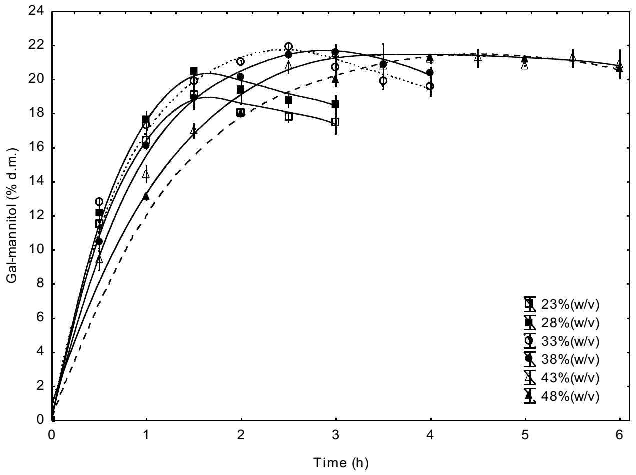

FIGURE 2. Changes in the content of gal-mannitol in the total sugar during synthesis in a solution of lactose and mannitol (1:1 ratio, w/w) depending on the dry mass content. Reaction conditions: 37°C, pH 6.5, β-galactosidase from *Kluyveromyces lactis* in the amount of 11400 LAU/100 g of lactose.

[Del-Val *et al*., 2001]. From the point of view of the obtained maximum content of gal-mannitol, using the enzyme dose from 2850 to 11,400 LAU/100 g of lactose provides similar results (product content in the total sugar of *ca.* 21.8%; Figure 1). The dose increase to 22,800 LAU/100 g of lactose resulted in an almost 7% decrease in the maximum content of gal-mannitol. A ten-fold increase in the quantity of β-galactosidase caused the value decrease by *ca*. 10%. However, increasing the enzyme dose significantly modified the reaction time to reach the product content close to the maximum. Although the time for the lowest dose is *ca*. 6 hours, the dose increase to 17,100 LAU/100 g of lactose reduces the time to 1.5 hours, while only an hour is sufficient for a ten-fold higher dose. It shall be emphasized that although for the two lowest doses no reduction in the content of gal-mannitol was observed, for the dose of 17,100 LAU/100 g of lactose extending the reaction time by an hour resulted in a 12.8% loss in the product content, and for the dose of 22800 LAU/100 g of lactose – in a 16.1% loss.

The content of lactose in the reactive mixture is an important factor influencing the efficiency of galactooligosaccharide synthesis [Iwasaki *et al*., 1996; Sen *et al*., 2014]. An increase in its concentration causes reduction in water activity in the mixture, which reduces the hydrolytic activity of β-galactosidase [Hansson *et al*., 2001]. Transglycosylation products are formed more intensively. An increase in the concentration is, however, limited by a limited lactose solubility; in solutions for obtaining galactooligosaccharides the concentration reaches 55 g/100 g [Manera *et al*., 2012; Shin & Yang, 1994; Tzortzis *et al*., 2005]. The use of higher concentrated solutions (33–48 g/100 mL) in the performed tests provided double benefits (Figure 2). Firstly, a certain increase in the maximum content of gal-mannitol in the total sugar was observed (from 19.1% in 23 g/100 mL solution to 21.9% in 33 g/100 mL solution). Simultaneously, the product quantity obtained individually from 100 mL of the solution increased from 4.39 g to 7.23 g (Table 1). Using a 48 g/100 mL solution over 10 g were obtained. A higher efficiency in more concentrated solutions is obtained at the expense of the reaction time needed to reach the maximum product content. At an increase from 23 g/100 mL to 48 g/100 mL, the time was 2.7 times longer. In solutions containing more water, faster hydrolysis of mannitol derivative was observed, which resulted in a lower final content of the product in the dry mass (Figure 2). As compared to the literature data on the synthesis of galactooligosaccharides the achieved efficiencies are in many cases higher, if applied to dimers synthesis and lower if we consider the total quantity of di- and trimers (the main fractions of galactooligosaccharides). Martínez-Villaluenga *et al.* [2008] using an enzyme from *Kluyveromyces lactis* obtained the maximum efficiency of disaccharides at the level of 15.2% in a 35% lactose solution (40°C, pH 7.5, 5 h), which reached 30% together with trisaccharides. Gobinath & Prapulla [2014] came to a similar fraction profile (13% of dimers, 17% of trimers) using β-galactosidase from *Lactobacillus plantarum* in a 40 g/100 mL solution of lactose (pH 7.0, 50°C, 12 h). A similar level of dimer content was observed after using an

TABLE. 1. Influence of lactose and mannitol solution concentration (1:1 ratio, w/w) on the reaction time and quantity of gal-mannitol obtained from 100 mL of mixture as a result of transgalactosylation using β-galactosidase from *Kluyveromyces lactis*. Reaction conditions: 37°C, pH 6.5, enzyme dose: 11,400 LAU/100 g of lactose.

Concentration of lactose and mannitol solution (1:1 w/w) (% (w/v))	Maximum quantity of gal-mannitol (g/100 mL solution)	Time to reach maximum content of gal-mannitol (h)
23	4.39	1.5
28	5.74	1.5
33	7.23	2.5
38	8.21	3.0
43	9.29	3.0
48	10.2	4.0

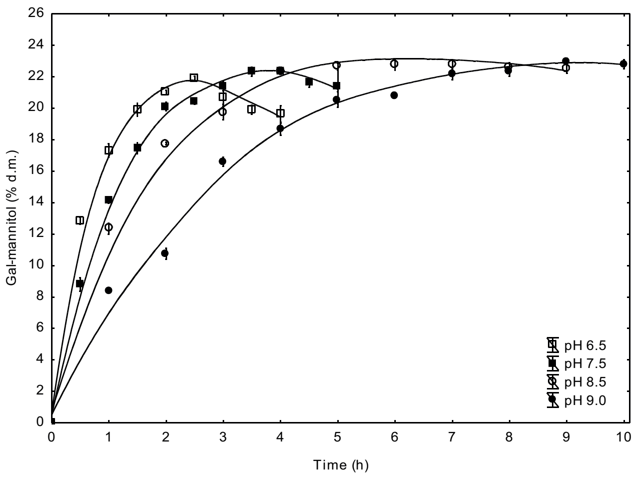

FIGURE 3. Changes in the content of gal-mannitol in the total sugar during synthesis in 33 g/100 mL solution of lactose and mannitol (1:1 ratio, w/w) depending on the pH value. Reaction conditions: 37°C, β-galactosidase from *Kluyveromyces lactis* in the amount of 11400 LAU/100 g of lactose.

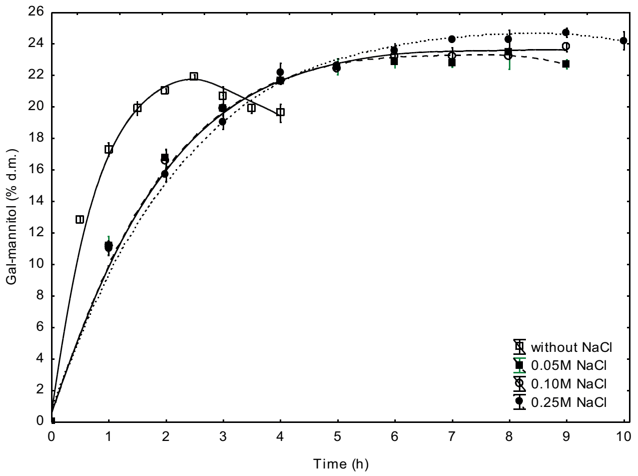

FIGURE 4. Changes in the content of gal-mannitol in the total sugar during synthesis in 33 g/100 mL solution of lactose and mannitol (1:1 ratio, w/w) in the presence of NaCl at a different concentration. Reaction conditions: 37°C, pH 6.5, enzyme dose: 11400 LAU/100 g of lactose.

enzyme from *Lactobacillus reuteri* [Splechtna *et al*., 2006]. Experiments carried out by Braga *et al*. [2003] using an enzyme from *Kluyveromyces marxianus* (35 g lactose/100 mL, pH 7.0, 40°C) resulted in the total GOS efficiency of *ca.* 16–21% (per initial lactose quantity) comparable with gal-mannitol content in the total sugars. Sen *et al*. [2014] using an immobilised enzyme from *Bacillus circulans* in 20 g/100 mL solution of lactose (35°C, pH 6.5; in packed bed bioreactor) reached the GOS content of over 8.5 g/100 mL, which indicates more than 42% content in dry mass. The content of gal-mannitol in the total sugar is in turn higher than in the case of galactosyl derivatives of polyols (sorbitol, xylitol, erythritol) synthesised using an enzyme originating from different microorganisms (14.7–18.8%) [Klewicki, 2007a]. The mannitol derivative reached a yield of 14.6% in the experiments performed by Lu *et al*. [2010] for β-galactosidase from *Enterobacter cloacae* B5 and *o*-nitrophenyl-β-D-galactopyranoside as a donor.

The pH value is a parameter which can be significant for the efficiency of transglycosylation products synthesis. An increase in its value is related to conformance changes within the active enzyme centre. They result from the changes in the ionisation condition of acids which occur therein [Zhou & Chen, 2001]. A greater enzyme capacity for using larger particles than water as an acceptor for galactosyl residues (shift of the reaction balance from hydrolysis towards transglycosylation) can be the effect of conformance changes (also caused by factors other than pH). It results in a greater quantity of the synthesised products, *e.g.* galactooligosaccharides [Giancomini *et al*., 2002; Bonnin & Thibault, 1996]. Martinez-Villaluenga *et al*. [2008] carried out tests using β-galactosidase from *Kluyveromyces lactis* in a lactose solution with different pH, reaching an almost 8% increase in the maximum content of trisaccharides at the pH increase from 6.5 to 7.5. In our tests, the quantity of the synthesised gal-mannitol was raised by *ca.* 5% by increasing pH from 6.5 to 9.0 (Figure 3). Gal-sorbitol synthesis conducted in previous tests under similar conditions resulted in an increase about twice as high [Klewicki, 2007b]. A better result can be obtained for mould enzymes from *Aspergillus* spp. Cardelle-Cobas *et al*. [2008] obtained an almost three-fold increase in the content of trisaccharides produced by the enzyme from *A. aculeatus,* increasing the pH from 4.5 to 6.5 (a significant drop in the produced dimers was observed simultaneously). The reaction was carried out in a 28 g/100 mL solution of lactose. Previously, del-Val & Otero [2003] achieved an almost 40% gain in the content of trisaccharides in 30 g/100 mL solution of lactose using an enzyme from the same source and increasing the pH from 5.0 to 6.5. Longer time to reach the maximum product concentration is the negative effect of an increase in the pH value. Gal-mannitol synthesis extended from 2.5 to 9 hours when pH was raised from 6.5 to 9.0.

The presence of salt in the reactive mixture was the last tested agent that could increase the quantity of transglycolysation products. It reduces water activity and shifts the enzyme activity from hydrolytic towards synthesis of oligosaccharides [Matsue & Miyawaki, 2000]. Sodium salt (NaCl) was used for the tests since sodium ions are mentioned as β-galactosidase activators [Fischer & Kleinschmidt, 2015; Zhang *et al*., 2013]. The use of salt at the concentration of 0.05 mol/L, 0.1 mol/L and 0.25 mol/L increased the maximum content of gal-mannitol (in the total sugar) by 7.1%, 8.7% and 12.8% respectively (Figure 4). Furthermore, the presence of salt also causes a favourable effect of impeding the product hydrolysis, which can be easily observed in the case of reactions without salt. The negative effect involves extension of the time necessary to reach the maximum share of mannitol derivative in the total sugar (from 2.5 h for the option without NaCl to 7–9 h for 0.25 mol/L NaCl). An almost 13% increase in the content of gal-mannitol is slightly higher than the one obtained for sorbitol derivative (*ca.* 11%) [Klewicki, 2007b]. Fortun & Colas [1991] obtained similar results during synthesis of galactosyl derivative of phenylethanol using an enzyme from *Aspergillus oryzae* in the presence of 0.5 mol/L lithium chloride. Del-Val & Otero [2003] reported an unfavourable influence of the presence of NaCl (concentration of up to 15 g/100 g) on GOS (trimers) synthesis carried out using β-galactosidase from A*spergillus aculeatus* in PEG 6000 environment.

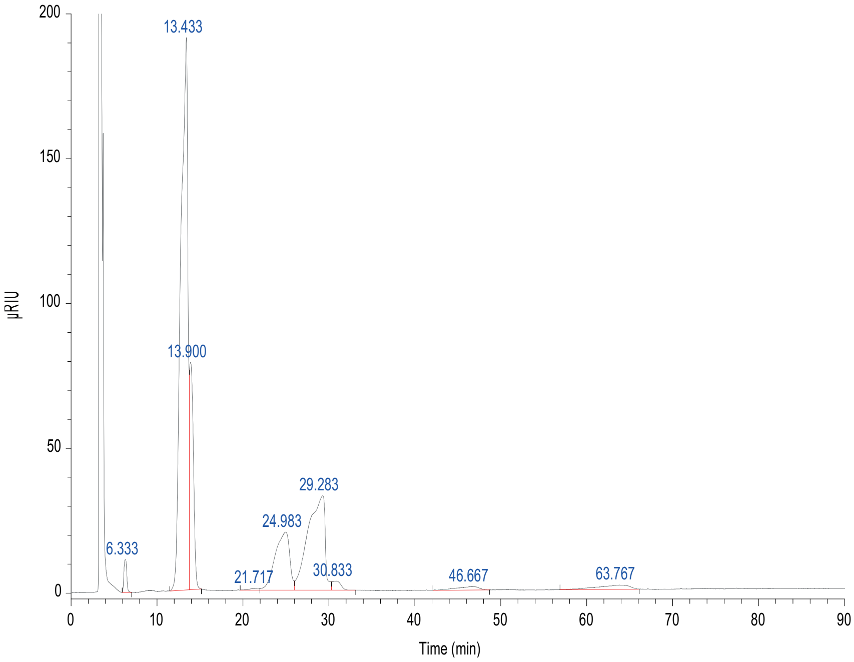

FIGURE 5. HPLC profile of the lactose hydrolysate containing gal-mannitol. Separation conditions: column – Shodex Asahipak NH_2P, mobile phase – acetonitrile: water (80:20 ratio), flow rate – 0.8 mL/min, detector – RI. Peaks: 6.333 min. – glycerol, 13.433 min. – glucose and galactose, 13.900 min. – mannitol, 21.717 min. – oligosaccharides, 24.983 min. – lactose, 29.283 min. – gal-mannitol, 30.833 min. – oligosaccharides, 46.667 min. – oligosaccharides, 63.767 min. – oligosaccharides.

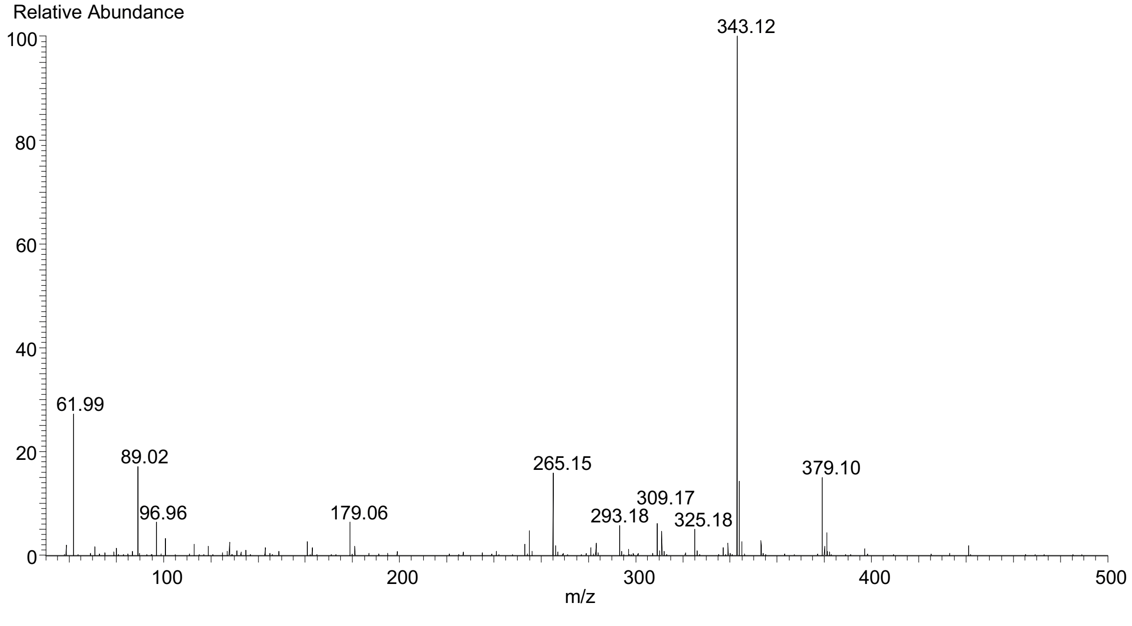

FIGURE 6. MS profile of the gal-mannitol fraction from the HPLC separation (Figure 1, peak – 29.283 min.).

Figure 5 shows a typical HPLC chromatogram. The retention time (RT) of galactosyl-mannitol is 29.283 min. The MS analysis confirmed the legitimacy of assigning the peak to gal-mannitol (molecular mass: 344.3; Figure 6 shows signal 343.12 m/z for the negative ion mode). It is known that galactose is mainly attached to the primary hydroxyl of acceptor during transgalactosylation [Shen *et al.*, 2012; Klewicki & Klewicka, 2004], thus *O*-β-D-galactosyl-(1,1)-mannitol was probably the predominant fraction in the product (it needs confirmation). Another oligosaccharides (derived from lactose; *e.g.*, RT: 21.717, 30.833, 46.667) were also present however they were not identified.

CONCLUSIONS

An increase in the enzyme dose over 11,400 LAU/100 g of lactose, by reducing the reaction time contributes to a simultaneous drop in the maximum content of gal-mannitol, which amounts to 21.9% of total sugar in a 33 g/100 mL solution, at 37°C and pH 6.5.

The use of solutions at a concentration over 33 g/100 mL ensures over 21% gal-mannitol content in the total sugars. The quantity of product obtained individually from 100 mL of the solution increases from 4.4 g to over 10 g at an increase in the concentration of the reactive mixture from 23 g/100 mL to 48 g/100 mL. A higher efficiency is achieved at the expense of the reaction time by reaching the maximum product content; the time is then 2.7 times longer. Faster hydrolysis of mannitol derivative is observed for solutions with a higher content of water, resulting in a lower final product content in the dry mass.

The pH increase from 6.5 to 9.0 causes an increase (by 5%) in the content of gal-mannitol in the total saccharides to a smaller extent than it is observed for galactooligosaccharides or derivatives of other polyols.

The use of salt concentration ranging from 0.05 mol/L to 0.25 mol/L contributes to an increase in the maximum content of gal-mannitol within the 7.1–12.8% range.

RESEARCH FUNDING

The work was financed from statutory funds of the Institute of Food Technology and Analysis, Faculty of Biotechnology and Food Sciences, Lodz University of Technology.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Bonnin E., Thibault J.-F., Galactooligosaccharide production by transfer reaction of an exogalactanase. Enzyme Microb. Technol., 1996, 19, 99–106.
2. Braga A.R.C., Manera A.P., da Costa Ores J., Sala L., Maugeri F., Kalil S.J., Kinetics and thermal properties of crude and purified β-galactosidase with potential for the production of galactooligosaccharides. Food Technol. Biotechnol., 2003, 51(1), 45–52.
3. Cardelle-Cobas A., Martínez-Villaluenga C., Sanz M.L., Montilla A., Gas chromatographic-mass spectrometric analysis of galactosyl derivatives obtained by the action of two different β-galactosidases. Food Chem., 2009, 114, 1099–1105.
4. Cardelle-Cobas A., Villamiel M., Olano A., Corzo N., Study of galacto-oligosaccharide formation from lactose using Pectinex Ultra SP-L. J. Sci. Food Agr., 2008, 88, 954–961.
5. Del-Val M.I., Hill Jr. C.G., Jiménez-Barbero J., Otero C., Selective enzymatic synthesis of 6'-galactosyl lactose by Pectinex Ultra SP in water. Biotechnol. Lett., 2001, 23, 1921–1924.
6. Del-Val M.I., Otero C., Biphasic aqueous media containing polyethylene glycol for the enzymatic synthesis of oligosaccharides from lactose. Enzyme Microb. Technol., 2003, 33, 118–126.
7. Fischer C., Kleinschmidt T., Synthesis of galactooligosaccharides using sweet and acid whey as a substrate. Int. Dairy J., 2015, 48, 15–22.
8. Fortun Y., Colas B., Lithium chloride effect on phenylethyl-β-D-galactoside synthesis by *Aspergillus oryzae* β-D-galactosidase in the presence of high lactose concentration. Biotechnol. Lett., 1991, 13, 863–866.
9. Giancomini C., Irazoqui G., Gonzalez P., Batista-Viera F., Brena B.M., Enzymatic synthesis of galactosyl–xylose *by Aspergillus oryzae* β-galactosidase*.* J. Mol. Catal. B-Enzym., 2002, 19–20, 159–165.
10. Gobinath D., Prapulla S.G., Permeabilized probiotic *Lactobacillus plantarum* as a source of β-galactosidase for the synthesis of prebiotic galactooligosaccharides. Biotechnol. Lett., 2014, 36, 153–157.
11. Guerrero C., Vera C., Conejeros R., Illanes A., Transgalactosylation and hydrolytic activities of commercial preparations of β-galactosidase for the synthesis of prebiotic carbohydrates. Enzyme Microb. Technol., 2015, 70, 9–17.
12. Hansson T., Andersson M., Wehtje E., Adlercreutz P., Influence of water activity on the competition between β-glycosidase-catalysed transglycosylation and hydrolysis in aqueous hexanol. Enzyme Microb. Technol., 2001, 29, 527–534.
13. Irazoqui G., Giacomini C., Batista-Viera F., Brena B.M., Cardelle-Cobas A., Corzo N., Jimeno M.L., Characterization of galactosyl derivatives obtained by transgalactosylation of lactose and different polyols using immobilized β-galactosidase from *Aspergillus oryzae*. J. Agric. Food. Chem., 2009, 57, 11302––11307.
14. Iwasaki K., Nakajima M., Nakao S., Galacto-oligosaccharide production from lactose by an enzymic batch reaction using β-galactosidase. Process Biochem., 1996, 31, 69–76.
15. Juśkiewicz J., Klewicki R., Zduńczyk Z., Consumption of galactosyl derivatives of polyols beneficially affects cecal fermentation and serum parameters in rats. Nutr. Res., 2006, 26, 531–536.
16. Kim T.-K., Park D.-C., Lee Y.-H., Synthesis of glucosyl-sugar alcohols using glycosyltransferases and structural identification of glucosyl-maltitol. J. Microbiol. Biotechnol., 1997, 7, 310–317.
17. Klewicki R., Effect of selected parameters of lactose hydrolysis in the presence of β-galactosidase from various sources on the synthesis of galactosyl-polyol derivatives. Eng. Life Sci., 2007a, 7, 268–274.
18. Klewicki R., Formation of gal-sorbitol during lactose hydrolysis with β-galactosidase. Food Chem., 2007b, 100, 1196–1201.
19. Klewicki R., Klewicka E., Antagonistic activity of lactic acid bacteria as probiotics against selected bacteria of the *Enterobaceriacae* family in the presence of polyols and their galactosyl derivatives. Biotechnol. Lett., 2004, 26, 317–320.
20. Kurakake M., Okumura T., Morimoto, Y., Synthesis of galactosyl glycerol from guar gum by transglycosylation of α-galactosidase from *Aspergillus* sp. MK14. Food Chem., 2015, 172, 150–154.
21. Linden G., Lorient D., New Ingredients in Food Processing. Biochemistry and Agriculture. 1999, Woodhead Publishing, Cambridge, England, pp. 226–231.
22. Lu L., Xu X., Gu G., Jin L., Xiao M., Wang F., Synthesis of novel galactose containing chemicals by β-galactosidase from *Enterobacter cloacae* B5. Bioresour. Technol., 2010, 101, 6868–6872.
23. Lu L.-l., Xiao M., Li Z., Li Y., Wang F., A novel transglycosylating β-galactosidase from *Enterobacter cloacae* B5. Process Biochem., 2009, 44, 232–236.
24. Manera A.P., Zabot, G.L., Oliveira J.V., de Oliveira D., Mazutti M.A., Kalil S.J., Treichel H., Maugeri F., Enzymatic synthesis of galactooligosaccharides using pressurised fluids as reaction medium. Food Chem., 2012, 133, 1408–1413.
25. Martínez-Villaluenga C., Cardelle-Cobas A., Corzo N., Olano A., Villamiel M., Optimization of conditions for galactooligosac-

charide synthesis during lactose hydrolysis by β-galactosidase from *Kluyveromyces lactis* (Lactozym 3000 L HP G). Food Chem., 2008, 107, 258–264.

26. Matsue S., Miyawaki O., Influence of water activity and aqueous solvent ordering on enzyme kinetics of alcohol dehydrogenase, lysozyme, and β-galactosidase. Enzyme Microb. Technol., 2000, 26, 342–347.
27. Nakano H., Kiso T., Okamoto K., Tomita T., Abdul Manan M., Kitahata S., Synthesis of glycosyl glycerol by cyclodextrin glucanotransferases*.* J. Biosci. Bioeng., 2003, 95, 583–588.
28. Rodriguez-Colinas B., Fernandez-Arrojo L., Ballesteros A.O., Plou F.J., Galactooligosaccharides formation during enzymatic hydrolysis of lactose: Towards a prebiotic-enriched milk. Food Chem., 2014, 145, 388–394.
29. Saarela M., Hallamaa K., Mattila-Sandholm T., Mättö J., The effect of lactose derivatives lactulose, lactitol and lactobionic acid on the functional and technological properties of potentially probiotic *Lactobacillus* strains. Int. Dairy J., 2003, 13, 291–302.
30. Saha B.C., Racine F.M., Biotechnological production of mannitol and its applications. Appl. Microbiol. Biotechnol., 2011, 89, 879–891.
31. Seki N., Saito H., Lactose as a source for lactulose and other functional lactose derivatives. Int. Dairy J., 2012, 22, 110–115.
32. Sen P., Nath A., Bhattacharjee C., Chowdhury R., Bhattacharya P., Process engineering studies of free and micro-encapsulated β-galactosidase in batch and packed bed bioreactors for production of galactooligosaccharides. Biochem. Eng. J., 2014, 90, 59–72.
33. Sharma P., Sharma B.C., Disaccharides in the treatment of hepatic encephalopathy. Metab. Brain Dis., 2013, 28, 313–320.
34. Shen Q., Yang R., Hua X., Ye F., Wang H., Zhao W., Wang K., Enzymatic synthesis and identification of oligosaccharides obtained by transgalactosylation of lactose in the presence of fructose using β-galactosidase from *Kluyveromyces lactis*. Food Chem., 2012, 135, 1547–1554.
35. Shin H.-J., Yang J.-W., Galacto-oligosaccharide production by β-galactosidase in hydrophobic organic media. Biotechnol. Lett., 1994, 16, 1157–1162.
36. Splechtna B., Nguyen T.-H., Steinböck M., Kulbe K.D., Lorenz W., Haltrich D., Production of prebiotic galacto-oligosaccharides from lactose using β-galactosidases from *Lactobacillus reuteri*. J. Agric. Food. Chem., 2006, 54, 4999–5006.
37. Stevenson D.E., Stanley R.A., Furneaux R.H., Optimization of alkyl β-D-galactopyranoside synthesis from lactose using commercially available β-galactosidases. Biotechnol. Bioeng., 1993, 42, 657–666.
38. Tzortzis G., Goulas A.K., Gibson G.R., Synthesis of prebiotic galactooligosaccharides using whole cells of a novel strain, *Bifidobacterium bifidum* NCIMB 41171. Appl. Microbiol. Biotechnol., 2005, 68, 412–416.
39. Wei W., Qi D., Zhao H., Lu Z., Lv F., Bie X., Synthesis and characterisation of galactosyl glycerol by β-galactosidase catalysed reverse hydrolysis of galactose and glycerol. Food Chem., 2013, 141, 3085–3092.
40. Yanahira S., Morita M., Aoe S., Suguri T., Takada Y., Miura S., Nakajima I., Effects of lactitol-oligosaccharides on calcium and magnesium absorption in rats. J. Nutr. Sci. Vitaminol., 1997, 43(1), 123–132.
41. Zhang H., Li W., Rui X., Sun X., Dong M., *Lactobacillus plantarum* 70810 from Chinese paocai as a potential source of β-galactosidase for prebiotic galactooligosaccharides synthesis. Eur. Food Res. Technol., 2013, 236, 817–826.
42. Zhou Q.Z.K., Chen X.D., Effects of temperature and pH on the catalytic activity of the immobilized β-galactosidase from *Kluyveromyces lactis*. Biochem. Eng. J., 2001, 9, 33–40.

Submitted: 17 July 2015. Revised: 27 November 2015 and 28 January 2016. Accepted: 1 February 2016. Published on-line: 5 September 2016.