Influence of Type of Substrate and Enzyme Concentration on Formation of Galacto-oligosaccharides

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Abstract: Different substrates and different concentrations of enzyme (Maxilact LX 5000) for galacto-oligosaccharides synthesis were tested. Lactose in phosphate buffer (138 mmol/l), ultrafiltration permeate (115 mmol/l), recombined whey (136 mmol/l) were used as substrates. Concentrations of used enzyme were from 0.15 to 15 U/ml for lactose in buffer, from 0.12 U/ml to 1.5 U/ml for ultrafiltration permeate and 1.5 U/ml for recombined whey. Reaction products were analysed by HPLC. There was obtained 6.4 ± 0.4 mmol/l of galacto-oligosaccharides (GOS) for lactose in buffer, it means that 0.0633 ± 0.0025 g/g of lactose was converted to GOS. The conversions of lactose to GOS for recombined whey and ultrafiltration permeate were 0.0669 ± 0.0079 and 0.0920 ± 0.0010 g/g. There was obtained 7.3 ± 0.1 mmol/l of GOS for ultrafiltration permeate and for recombined whey 5.9 ± 0.1 mmol/l of GOS.

Keywords: galacto-oligosaccharides; β-galactosidase; permeate; whey; lactose

INTRODUCTION

GOS can be prepared from lactose by the enzymatic reaction using β-galactosidase. The reaction produces mainly disaccharides and in lower amount tri- and higher oligosaccharides (Mahoney 1998). This reaction is influenced by many factors e.g. concentration of substrate, the higher is concentration of lactose the higher is amount of GOS (Boon *et al*. 2002), and origin and concentration of enzyme (Rustom *et al*. 1998). The enzyme from different origin synthesises various types and amounts of oligosaccharides, the highest amount of GOS is obtained from yeast origin (Boon *et al*. 2002), but Rustom *et al*. (1998) claimed that with yeast enzyme was obtained the lowest amount of GOS. The concentration of enzyme is influenced with following factors: the lower is concentrations of enzyme the higher is amount of oligosaccharides, but higher concentrations of enzyme produces mainly disaccharides (Martínez-Villaluenga *et al*. 2007).

The GOS have biological functions. Many positive physiological effects in humans are attributed to GOS, from selective improvement of beneficial intestinal bacteria grow to improved absorption of calcium and magnesium (Czermak *et al*. 2004). These GOS are components of many foods, from bisquit to milk products (Kakita *et al*. 2002). The aim of this work was the determination of influence of the enzyme concentration on GOS formation in different substrates.

MATERIALS AND METHODS

Materials. Lactose in phosphate buffer (138 mmol/l, pH = 6.75), ultrafiltration permeate (115 mmol/l) and recombined whey (136 mmol/l) were used as substrates. Enzyme Maxilact LX 5000 (DSM Food Specialties, Heerlen, the Netherlands) was used. Lactose (food grade) and whey powder (PML Nový Bydžov, Czech Republic), permeate was prepared from cheese whey supplied by local dairy (Moravia Lacto, Jihlava, Czech Republic) by ultrafiltration using ceramic membrane (50 kDa, Tami Industries, Hermsdorf, Germany). Phosphate buffer was prepared from 0.01 mol/l K_2HPO_4, 0.015 mol/l KCl, 0.012 mol/l $MgCl_2 \cdot 6\,H_2O$ and citric acid (30% w/w)) was used for pH adjustment. All

chemicals were of analytical grade (Penta, Praha, Czech Republic). O-nitrophenyl-β-D-galactoside (ONPG) (Chemos, Praha, Czech Republic) was used for β-galactosidase activity assay.

Methods. The amount of substrate for batch experiment was 100 g. The temperature during all experiments was kept at 37°C. Concentrations of enzyme (Maxilact LX 5000) were from 0.12 U/ml to 1.5 U/ml for ultrafiltration permeate, from 0.15 U/ml to 15 U/ml for lactose in buffer and 1.5 U/ml for recombined whey. Samples were taken in regular intervals and were analysed by HPLC (Agilent, series 1100, Waldbronn, Germany) according to method described previously (Hellerová & Čurda 2008). Activity of β-galactosidase was determined according to Lin *et al.* (1989) and Nakayama and Amachi (1999). One unit of β-galactosidase activity is defined as the amount of enzyme necessary to release 1 μmol ONP/min at 30°C.

RESULTS AND DISCUSSION

The concentrations of used enzyme were from 0.15 to 15 U/ml for lactose in buffer. The highest amount of GOS (6.4 ± 0.4 mmol/l) was obtained for the lowest concentration of enzyme (0.15 U/ml), which corresponds with the study of Martínez-Villaluenga *et al.* (2007). According to the results in Figure 1 it is evident that decreasing concentration of enzyme caused the extension of time needed for maximal amount of GOS formation. For preparation of GOS is thus more suitable to use the concentration of 1.5 U/ml.

The concentrations of used enzyme were from 0.12 to 1.5 U/ml for ultrafiltration permeate, because higher dose of enzyme lead to the undesirable shortening of reaction time. The highest amount of GOS (7.3 ± 0.1 mmol/l) was obtained for the highest concentration of enzyme (1.5 U/ml),

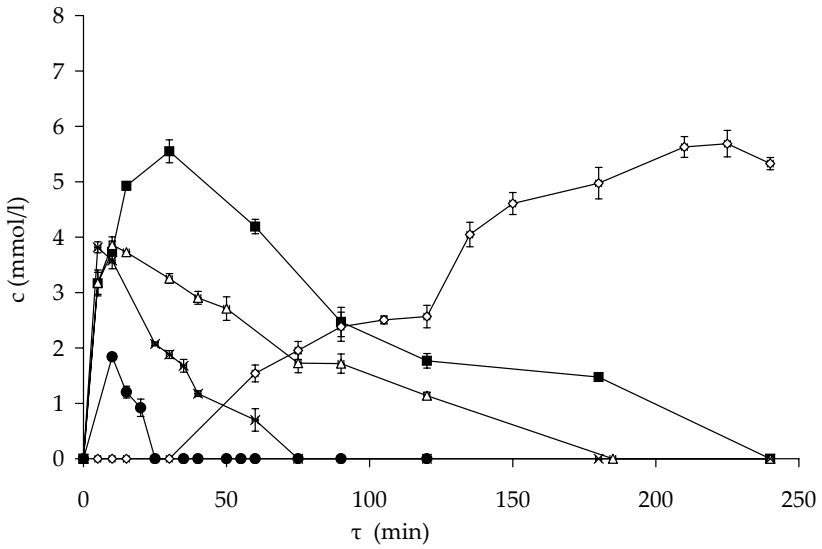

Figure 1. Changes of GOS concentration during experiment using lactose in buffer

◊ – c_{enzyme} = 0.15 U/ml, ■ – c_{enzyme} = 1.5 U/ml, Δ – c_{enzyme} = 3 U/ml, × – c_{enzyme} = 6 U/ml, ● – c_{enzyme} = 15 U/ml

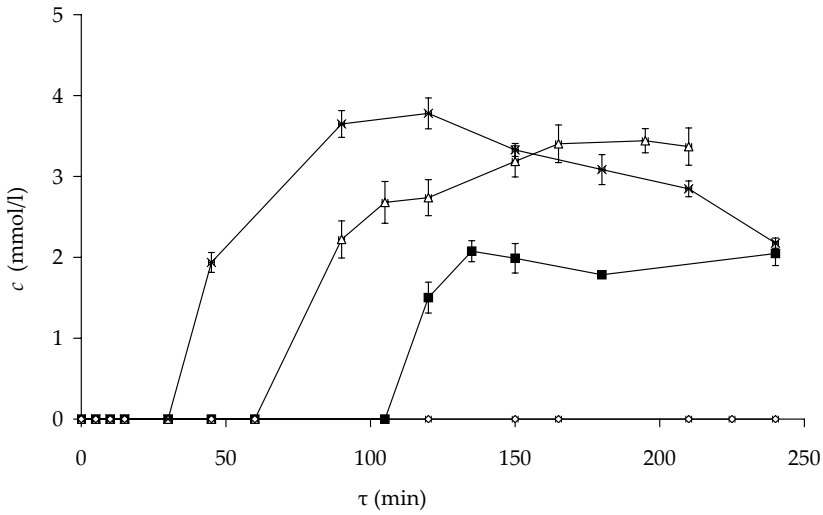

Figure 2. Changes of GOS concentration during experiment using ultrafiltration permeate

◊ – c_{enzyme} = 0.12 U/ml, ■ – c_{enzyme} = 0.24 U/ml, Δ – c_{enzyme} = 0.6 U/ml, × – c_{enzyme} = 1.2 U/ml

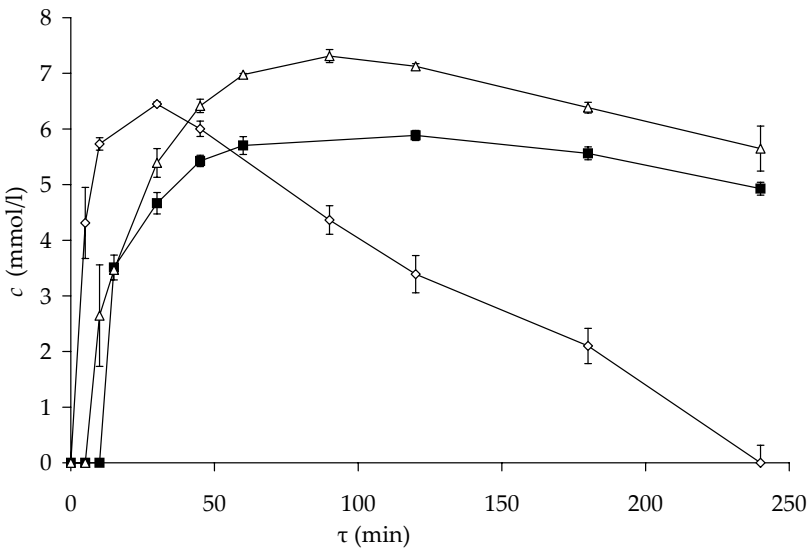

Figure 3. Comparison of substrates for synthesis of GOS

c_{enzyme} = 1.5 U/ml; ◊ – lactose in buffer, ■ – recombined whey, Δ – ultrafiltration permeate

(Figure 2). For the experiment with recombined whey was therefore used 1.5 U/ml concentration of enzyme. The highest amount of GOS reached during this experiment was 5.9 ± 0.1 mmol/l.

The comparison of substrates (lactose in buffer, ultrafiltration permeate and recombined whey) is shown on Figure 3. It is obvious from these results that ultrafiltration permeate is the best substrate for synthesis of GOS, because the highest amount of GOS was obtained. We can conclude that the optimal concentration of enzyme for all substrates is 1.5 U/ml.

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