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Optimization of the enzymic process for manufacturing low-lactose milk containing oligosaccharides

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Abstract

Methods and enzymic processing conditions for manufacturing low-lactose milk containing oligosaccharides were evaluated. Comparisons were made between the method of using β-galactosidase to transform the lactose in milk into oligosaccharides directly and the method of applying ultrafiltration techniques to separate lactose from milk proteins and then transform the lactose in the permeate into oligosaccharides. Since the β-galactosidase exhibited higher transgalactosylative activity in the permeate than in the milk and the higher the initial lactose concentration the higher the conversion to oligosaccharides, a useful procedure for manufacturing low-lactose high-oligosaccharides milk would be to separate milk proteins from lactose by ultrafiltration first, concentrate the UF permeate by evaporation, apply enzymic reaction in the concentrated permeate, and add the hydrolysate to the retentate to obtain the final milk product. The optimal conditions for the enzymic reaction in the permeate was 25.3% of initial lactose concentration, an *E*/*S* ratio of 6.7%, and carried out at 50 °C for 3.5 h.

Keywords: Oligosaccharide; β-Galactosidase; Ultrafiltration; Milk; Lactose

1. Introduction

Galactooligosaccharides can be produced from lactose in milk through the enzymic transgalactosylation reactions of β-galactosidase. It has been shown that oligosaccharides can promote the growth of bifidobacteria, healthy microbes, in the large intestine of humans [1–4]. The improvement of intestinal microflora and the suppression of putrefaction in the gut allow oligosaccharides to be considered a beneficial component in milk. Another benefit resulting from transforming lactose into oligosaccharides is the manufacture of low-lactose milk. It is well known that many people suffer from gastrointestinal problems because of the high lactose content in the milk products. Therefore, it is beneficial and desirable to produce low-lactose high-oligosaccharides milk.

There have been many researches on the hydrolysis of lactose in milk. However, only a few of these studies investigated the production of oligosaccharides through a transgalactosylation reaction during the hydrolysis of lactose in milk [5–9]. Although it was found that the maximum amount of oligosaccharides that could be formed in a 5% lactose solution was about 5% of the original lactose content, [6], other reports showed that oligosaccharides represented about 11.3% of the total lactose for 5% lactose solution and 16.0% for 20% lactose solution [10]. However, maximum oligosaccharide conversion of over 30% was obtained with an initial lactose concentration greater than 1.11 mol/l [11]. The amount of oligosaccharides formed in milk was considerably less than formed in a lactose solution. In fact, Kwak and Jeon [12] stated that the total concentration of the oligosaccharides formed in enzyme-treated milk was insignificant. The inhibition of milk or whey proteins on the activity of β-galactosidase appeared to be one of the major causes for the low production of oligosaccharides in milk [8,13]. In this study, two methods for manufacturing low-lactose and high-oligo-

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saccharides milk products were investigated. The first method used β-galactosidase to transform the lactose in evaporated milk into oligosaccharides directly. The second method applied ultrafiltration techniques to separate the lactose in milk from milk proteins, concentrated the permeate by evaporation, and then transformed the lactose in the permeate into oligosaccharides. Finally, the hydrolysate was added back to the retentate. The objectives of this study were to investigate the optimal conditions for the enzymic reactions in the milk and in the permeate using response surface methodology, and to compare the process performance of these two methods at their optimal conditions. A processing procedure and conditions for manufacturing low-lactose high-oligosaccharides milk product could then be proposed.

2. Materials and methods

2.1. Materials

Cow's milk was obtained from the dairy farm of National Taiwan University. After removing the fat by centrifuging, 0.1% sodium azide was added to prevent the growth of microbes. The β-galactosidase (9 unit/mg, one unit activity was defined as the amount of enzyme producing 1 μmol of glucose per min at 37 °C and pH 4.5 using lactose as substrate) from *Aspergillus oryzae*, lactose, glucose and galactose for preparing standard were purchased from Sigma Chemical Co. (St. Louis, USA).

2.2. Ultrafiltration

An ultrafiltration system equipped with a Romicon PM-50 hollow fibre cartridge (molecular weight cut off 50 000 Da, membrane area 5 ft^2, HFXS-5/10 Lab. System, Romicon Inc., Woburn, MA, USA) was used. The inlet and outlet pressures were controlled at 138 and 103 KPa, respectively, and the flow rate was maintained at 25.28 kg/min. Skim milk was concentrated three times by ultrafiltration at 50 °C. The resulting permeate was then treated with β-galactosidase.

2.3. Enzymic reaction

A rotary vacuum evaporator (Model N1, Rikakikai Inc., Tokyo, Japan) was used to concentrate the milk or the permeate at 45 °C. A 250 ml flask, containing 30 ml of concentrated milk or permeate sample and a suitable amount of β-galactosidase, was incubated in a shaker (230 rpm) at a predetermined temperature and enzyme/substrate ratio (*E*/*S* ratio). One millilitre of the samples of the mixture was removed periodically, heated in boiling water for 10 min to inactivate the enzyme and then analyzed for lactose and oligosaccharides contents.

2.4. Lactose and oligosaccharides determination

An equal volume of acetone solution (75%) was added to milk samples to precipitate proteins. After filtration through a 0.45 μm syringe filter (Gelman Sciences, Ann Arbor, MI), the filtrate was analyzed with a high-performance liquid chromatograph (HPLC) (ICI Victoria, Australia). For permeate samples, a ten times dilution was made by adding deionized water prior to filter through the syringe filter. The HPLC system consisted of a differential refractometer and a high pressure pump (Model LC1100, ICI Victoria, Australia) with a Rezex RAM Carbohydrate column (300 × 7.8 mm i.d., Phenomenex Co., Torrance, CA). Aliquots of 20 μl were injected and eluted at a flow rate of 0.5 ml/min using deionized water as mobile phase. The temperatures of the differential refractometer and the column were controlled at 25 and 80 °C, respectively.

A typical HPLC chromatogram of lactose solution after the enzymic reaction by β-galactosidase is shown in Fig. 1. Standard solutions of lactose, galactose, and glucose were used to identify these monosaccharides and disaccharide in the chromatograms. However, pure compounds of galactooligosaccharides were difficult to obtained, therefore, peaks 1–3 in the chromatogram

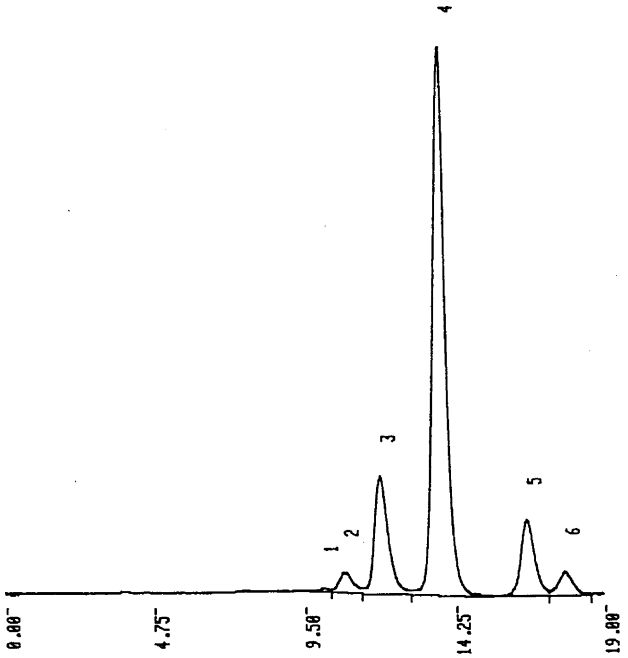

Fig. 1. The chromatogram for the oligosaccharides, glucose, galactose formations under the enzymic transgalactosylation reaction of lactose by β-galactosidase.

were tentatively identified as pentasaccharides, tetrasaccharide and trisaccharide, respectively, based on the retention time and the properties of the separation column used in this analysis. The quantity of each carbohydrate was calculated from the HPLC chromatogram and reported as the ratio of its peak area to the total peak area.

2.5. Experimental design and model building

To develop the mathematical models giving oligosaccharide concentrations as functions of the substrate concentration, the E/S ratio and the reaction temperature, an appropriate experimental design was needed to limit the number of experimental points. A face-centred central composite design [14] for three variables with three different levels each was adopted. The experimental levels for the enzymic reaction in the milk were: lactose concentration 7.5; 12.1; and 16.7%, temperature 35; 50; and 65 °C, E/S ratio 0.3; 3.5; and 6.7%. The experimental levels for the enzymic reaction in the permeate were: lactose concentration 6.4; 15.9; and 25.3%, temperature 30; 45; and 60 °C, E/S ratio 0.2; 3.4; and 6.7%. The time course for measuring the enzymic reaction in the milk was 10 min, 0.5, 1, and 2 h, while the time course for the permeate was 0.5, 1, 2, and 4 h.

A multiple regression procedure in the SAS package (SAS Institute, Inc., Cary, NC) was used to fit the data for the oligosaccharide concentration into second order polynomial equations with interaction terms:

$$Y = B_o + B_i \Sigma X_i + B_{ii} \Sigma X_i^2 + B_{ij} \Sigma X_i X_j (i \neq j)$$

where Y is the dependent variable (in percentage), B_o, B_i, B_{ii}, B_{ij} are regression coefficients of the model, and X_i, X_j, are the magnitudes of the enzymic variables. Once the regression models were developed, one can search the optimal level of each variable that resulted in a maximum yield of oligosaccharides using various numerical techniques.

3. Results and discussion

3.1. Oligosaccharides production in the milk

It had been reported that the production of galactooligosaccharides from lactose increased with the concentration of lactose [2,15]. Thus, it was necessary to concentrate the milk samples in order to manufacture low-lactose milk containing high amounts of oligosaccharides. Table 1 shows the time course of the enzymic reaction of β-galactosidase in the concentrated milk. The lactose content in the milk decreased and the amount of glucose and galactose increased with time due to the hydrolysis of lactose. However, the content of galactose was significantly lower than that of glucose because galactose was used to form galactooligosaccharides. The yield of pentasaccharides increased with time while the yields of trisaccharides, tetrasaccharides, and total oligosaccharides (the sum of tri-, tetra-, and penta-saccharides) only increased with the reaction time up to 0.5 h, and then a slightly decrease in the yields of trisaccharides and tetrasaccharides were observed as the reaction time further increased. As expected, the yield of oligosaccharides followed the order: trisaccharides > tetrasaccharides > pentasaccharides, because tetra- and penta-saccharides were converted from trisaccharides due to the transgalactosylation reaction of β-galactosidase. In order to search the optimum conditions for producing low-lactose milk containing oligosaccharides, the effects of lactose concentration, E/S ratio, reaction time and temperature on the contents of di- and oligosaccharides were investigated using a face-centered central composite design. Table 2 shows the regression models for the yields of disaccharides, trisaccharides, tetrasaccharides, and total oligosaccharides resulted from the enzymic reaction in the concentrated milk by β-galactosidase. Many regression coefficients for the interaction and quadratic terms of the equations were significant ($P < 0.05$), indicating that second order equations were needed to describe the processing performance. All the determination of the regression models, R^2, for the equations were reasonably high, indicating, in general, good fit of the equations to the data. Based on the equations in Table 2, the optimal conditions for the production of total oligosaccharides, trisaccharides, and tetrasaccharides were searched using numerical techniques and the results are shown in Table 3. The optimal conditions for the direct enzymic reaction in the milk was to use concentrated milk containing 16.7% lactose, and the reaction was carried out at 5.1% E/S ratio, 47 °C for 1.4 h. The process could yield a product containing 22.8% oligosaccharides, and 34.7% of the original disaccharides (mainly lactose) remained in the milk. The optimal lactose

Table 1
The enzymic reaction of β-galactosidase in the concentrated milk for the center point in the face-centered central composite design

Content (%)	Time (h)			
	0.167	0.5	1	2
Pentasaccharides	0.00 ± 0.00^{a}	0.86 ± 0.08^{b}	1.00 ± 0.08^{bc}	1.13 ± 0.07^{c}
Tetrasaccharides	2.40 ± 0.44^{a}	3.88 ± 0.08^{b}	3.84 ± 0.11^{b}	3.69 ± 0.09^{b}
Trisaccharide	12.73 ± 0.68^{a}	15.72 ± 0.03^{b}	14.16 ± 0.15^{c}	12.64 ± 0.03^{a}
Total oligosaccharides	15.13 ± 0.24^{a}	20.47 ± 0.10^{b}	19.01 ± 0.18^{c}	17.45 ± 0.02^{d}
Lactose	68.25 ± 0.70^{a}	55.68 ± 0.11^{b}	47.58 ± 0.23^{c}	40.10 ± 0.02^{d}
Glucose	12.16 ± 0.65^{a}	16.64 ± 0.09^{b}	22.25 ± 0.30^{c}	26.97 ± 0.11^{d}
Galactose	4.46 ± 0.33^{a}	7.21 ± 0.06^{b}	11.17 ± 0.19^{c}	15.48 ± 0.11^{d}

The experimental conditions for the center point were: lactose: 12.1%; temperature: 50 °C; E/S: 3.5%.

Table 2
The regression models for the yields of trisaccharides, tetrasaccharides, total oligosaccharides (the sum of tri-, tetra-, and penta-saccharides) and disaccharides after the enzymic reaction of β-galactosidase in the milk

Parameter	Total oligo	Trisaccharide	Tetrasaccharides	Disaccharides
INTERCEPT	−16.3149	−6.8053	−6.9097*	197.7411***
X1	0.5145	0.9628	−0.2391	4.4102**
X2	0.7355	0.2558	0.3259**	−5.3177***
X3	3.0893**	2.3778***	0.7091*	−6.9242***
T	10.5378**	7.4344**	2.7716*	−35.2398***
X1 × X1	−0.0335	−0.0446	0.0057	−0.1089
X2 × X1	0.00376	0.0016	0.0010	−0.0313**
X2 × X2	−0.0086*	−0.0035	−0.0034**	0.0572***
X3 × X1	0.0640	0.0286	0.0274**	−0.0756
X3 × X2	0.01881	0.0129	0.0037	−0.0353*
X3 × X3	−0.4192***	−0.2761***	−0.1177***	0.7797***
T × X1	0.2818*	0.1736	0.0708	−0.1837
T × X2	−0.0619	−0.0394	−0.0194	0.2486***
T × X3	−0.5563**	−0.5258***	−0.0496	−0.9446**
T × *T*	−3.5059***	−2.3223**	−0.9469**	7.9662***
R^2	0.82	0.79	0.79	0.96

*, Significant level at 0.05; **, significant level at 0.01; ***, significant level at 0.001. X1: lactose (%); X2: temperature (°C); X3: *E*/*S* (%); *T*: time (h).

concentrations for both total oligosaccharides and tetrasaccharides were at the highest level, 16.7%, suggesting that further increase the lactose concentration of milk might obtain even higher total oligosaccharides and tetrasaccharides. However, it was found that 16.7% was nearly the highest lactose content that could be reached practically by evaporation. Further concentration would cause the milk to form a paste, which was too sticky to carry out enzymic reaction efficiently.

Compared with the optimal conditions for trisaccharides, more severe conditions for the enzymic reaction were required to maximize the yield of tetrasaccharides. Results also showed that the yield of tetrasaccharides was significantly lower than that of trisaccharides. This was expected because tetrasaccharides were converted from trisaccharides through the transgalactosylation reaction of β-galactosidase [2,16].

Fig. 2 shows the effects of lactose concentration and the *E*/*S* ratio on the yields of total oligosaccharides, trisaccharides, and tetrasaccharides in the milk when the temperature and reaction time were at the optimal conditions for total oligosaccharides. The production of total oligosaccharides increased linearly with the lactose content in the milk. This result agreed with the reports from Prehosil et al. [15], Matsumoto et al. [2], Lopez-Leiva and Guzman [17], and Iwasaki et al. [11]. Raising the *E*/*S* ratio in the range of 0.3–5.1% increased the yield of total oligosaccharides, but the yield decreased slightly as the *E*/*S* ratio rose over 5.1%. As the hydrolysis of lactose and the synthesis and the hydrolysis of oligosaccharides occurred simultaneously, the decline in total oligosaccharides implied that the formed oligosaccharides were immediately hydrolyzed to disaccharide or monosaccharide at high *E*/*S* ratio. The effects of lactose concentration and *E*/*S* ratio on the yields of trisaccharides and tetrasaccharides were similar to that for total oligosaccharides (Fig. 2), except at high lactose concentration. Unlike the approximately linear increase with lactose concentration found in total oligosaccharides, the yield of trisaccharides decreased slightly when the lactose concentration was over 14%. Lopez-Leiva and Guzman [17] and Iwasaki et al. [11] stated that the hydrolysis of lactose occurred predominantly at low lactose concentrations, while oligosaccharides production through the transgalactosylation reaction increased with increasing concentrations of lactose. Therefore, a reasonable explanation for the decrease in trisaccharides at high lactose concentrations is that some fraction of the trisaccharides was converted into tetrasaccharides.

Table 3
The optimal conditions and yields of trisaccharides, tetrasaccharides, and total oligosaccharides for the enzymic reaction in the milk

	Trisaccharides	Tetrasaccharides	Total oligo
Lactose (%)	15.6	16.7	16.7
Temperature (°C)	42	49	47
E/*S* (%)	4.9	5.4	5.1
Time (h)	1.3	1.4	1.4
Yield (%)	16.6	5.1	22.8
Disaccharides (%)	40.5	32.7	34.7

Fig. 3 shows the effects of temperature and reaction time on the production of total oligosaccharides, trisaccharides, and tetrasaccharides in the milk when the lactose concentration and the *E*/*S* ratio were at the optimal conditions for total oligosaccharides production. With the increase in temperature, the yield of total oligosaccharides production increased gradually in the temperature range of 35–47 °C, but decreased when the temperature exceeded 47 °C due to the inactivation of the enzyme. The yield of total oligosaccharides reached a maximum at 1.4 h and then declined as the reaction time increased. This was because the rate of synthesis of oligosaccharides was faster than the rate of hydrolysis for oligosaccharides in the initial stages of enzymic reaction. The rate of synthesis became slower as the reaction time increased. The effects of temperature and reaction time on the yields of trisaccharides and tetrasaccharides were similar to that of total oligosaccharides production.

3.2. Oligosaccharides production in the UF permeate

After removing milk proteins by ultrafiltration, the lactose concentration in the permeate could be increased to 25.3% by evaporation, which was significantly higher than that in the milk. Table 4 shows the regression models for total oligosaccharides, trisaccharides, tetra-

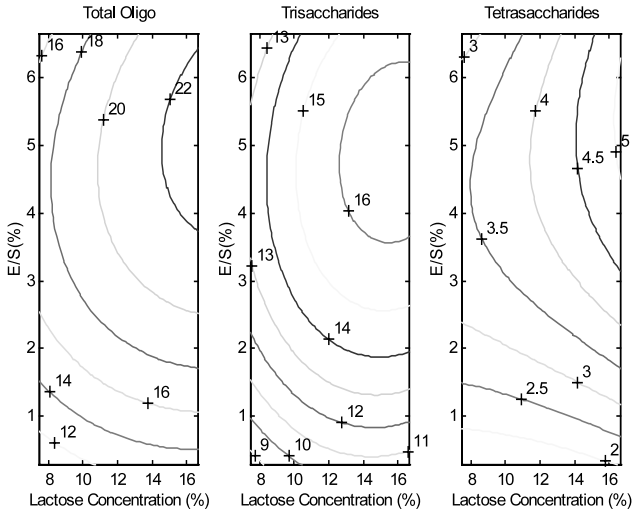

Fig. 2. Effects of lactose concentration and the *E*/*S* ratio on the yields of total oligosaccharides, trisaccharides, and tetrasaccharides in the milk when the temperature and reaction time were at the optimal conditions for total oligosaccharides.

saccharides, pentasaccharides, and disaccharides production during the enzymic reaction of β-galactosidase in the permeate. Second order equations were also required with reasonably high R^2 values. The optimal conditions for the yields of total oligosaccharides, trisaccharides, tetrasaccharides, and pentasaccharides are shown in Table 5. To obtain the maximum amount of oligosaccharides, the optimal conditions for the enzymic reaction were 25.3% lactose concentration, 6.7% *E*/*S* ratio, and 50 °C for 3.5 h. The process produced a product containing 31.1% oligosaccharides, and 35.3% of the original disaccharides remained in the permeate after the enzymic process. The optimal conditions for all oligosaccharides production were quite

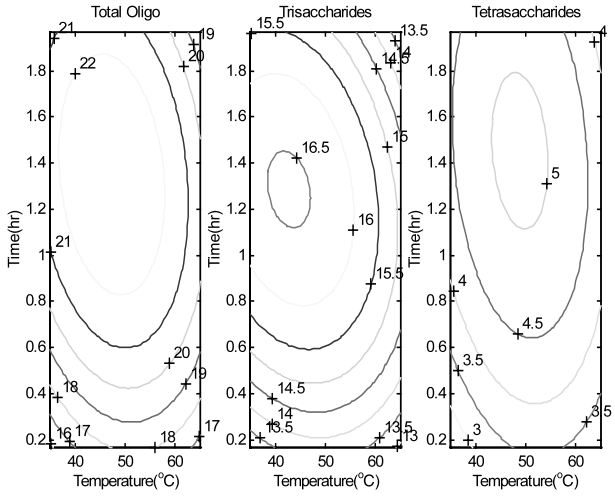

Fig. 3. Effects of temperature and reaction time on the production of total oligosaccharides, trisaccharides, and tetrasaccharides in the milk when lactose concentration and the *E*/*S* ratio were at the optimal conditions for total oligosaccharides.

similar. Again, the optimal lactose concentration for the production of all oligosaccharides from the permeate was at its highest level, 25.3%. Also the optimal *E*/*S* ratio for total oligosaccharides, tetrasaccharides, and pentasaccharides production was at the highest level, 6.7%.

Effects of lactose concentration and *E*/*S* ratio on the yields of oligosaccharides trisaccharides, tetrasaccharides, and pentasaccharides in the permeate are shown in Fig. 4. The yields of total oligosaccharides, trisaccharides, tetrasaccharides, and pentasaccharides all increased with the *E*/*S* ratio and lactose concentration. Fig. 5 shows the effects of temperature and reaction time on the yields of oligosaccharides, trisaccharides, tetrasaccharides, and pentasaccharides in the permeate. The yield of total oligosaccharides increased gradually in the range of temperature from 30–50 °C but decreased over 50 °C due to the inactivation of the enzyme. The yield of total oligosaccharides production reaches a maximum at 3.5 h and then declined as the reaction time increased. The effects of temperature and reaction time on the yields of trisaccharides, tetrasaccharides, and pentasaccharides were similar to that for total oligosaccharides production.

3.3. Effect of ultrafiltration

A comparison for the maximum yields of total oligosaccharides in the milk and permeate is shown in Table 6. As mentioned before, the maximum yield of total oligosaccharides in the permeate was 31.1%, while it was only 22.8% in the milk. However, in this case the lactose concentration in the permeate (25.3%) was significantly higher than that in the milk (16.7%). When the lactose concentration in the permeate was fixed at 16.7%, the maximum yield of total oligosaccharides estimated from the regression models was 27.9%, which was still significantly higher than the maximum yield of total oligosaccharides in the milk (22.8%). If the enzymic reaction was carried out in the permeate under the same conditions that resulted in a maximum yield of oligosaccharides for the milk (i.e. 16.7% lactose; *E*/*S* ratio of 5.1; reacted at 47 °C for 1.4 h), 25.5% oligosaccharides yield could be obtained in the permeate, but would have 53.6% of the original disaccharide remaining after the enzymic reaction (Table 6). It appeared that β-galactosidase had a higher activity in the permeate than that in the milk. Since milk proteins were removed by the ultrafiltration membrane for obtaining permeate, it could be inferred that milk proteins might inhibit the activity of β-galactosidase. Jakubowski et al. [13] reported that whey proteins decreased the activity of β-galactosidase from *A. niger*. Mozaffar et al. [8] also found that milk proteins decreased the activity of β-galactosidase from *E. coli* and *K. lactis*. However, the mechanism of the inhibition

Table 4
The regression models for the yields of total oligosaccharides, trisaccharides, tetrasaccharides, pentasaccharides, and disaccharides after the enzymic reaction of β-galactosidase in the permeate

Parameter	Total oligo	Tri-saccharide	Tetra-saccharides	Penta-saccharides	Di-saccharides
INTERCEPT	−31.0168***	−20.2082***	−8.3008***	−2.4853**	184.1667***
X1	−1.0834*	−0.6759**	−0.3070*	−0.0869	3.2750**
X2	1.9237***	1.3004***	0.4725***	0.1450***	−5.1414***
X3	3.8821***	3.3455***	0.6256*	−0.1088	−5.4598*
T	5.2406*	3.5837*	1.3618*	0.3481	−10.3565*
X1 × X1	−0.0081	−0.0076	−0.0005	−0.0006	−0.0400
X2 × X1	0.0224***	0.0158***	0.0051***	0.0014***	−0.0366***
X2 × X2	−0.0238***	−0.0163***	−0.0057***	−0.0017***	0.0605***
X3 × X1	0.0645***	0.0357**	0.0214***	0.0073***	−0.0129
X3 × X2	0.0102	0.0055	0.0029	0.0018	−0.0415
X3 × X3	−0.4013***	−0.3185***	−0.0794*	−0.0004	0.5201*
T × X1	0.0856*	0.0458	0.0266*	0.0141***	−0.0334
T × X2	−0.0460*	−0.0302	−0.0126	−0.0034	0.0709
T × X3	−0.1355	−0.1977*	0.0312	0.0309*	−0.4949
T × *T*	−0.6000	−0.3542	−0.1840	−0.0729*	1.1094
R^2	0.87	0.87	0.83	0.76	0.84

*, Significant level at 0.05; **, significant level at 0.01; ***, significant level at 0.001. X1: lactose (%); X2: temperature (°C); X3: *E*/*S* (%); *T*: time (h).

caused by milk proteins is still unknown. Another possible cause for the higher activitiy of β-galactosidase in the permeate than in the milk was the difference in the metal ions composition between these two solutions. There has been much research focused on the effects of Mg^{+2}, Ca^{+2}, M^{+2}, or Na^{+} on the activity of β-galactosidase from various sources and the results have been quite contradictory [8,18,19]. Agbebavi et al. [19] reported that Mg^{+2} and Ca^{+2} activated β-galactosidase, while Itoh et al. [20] found that Mg^{+2}, Ca^{+2}, and Zn^{+2} inhibited the activity of β-galactosidase. These contradictory findings regarding the effects of metal ions may be due to differences in the enzyme sources or controlled conditions. For example, Mozaffar et al. [8] stated that Ca^{+2} and Na^{+} decreased the activity of β-galactosidase from *E. coli* and *K. lactis*, but not from *B. circulans*. It is known that the milk and the permeate had a different composition of metal ions, [21,22] and these differences may have caused significant differences in the activity of β-galactosidase.

The total yield of 31.1% oligosaccharides in the permeate did not include the lactose which remained in the retentate. The UF membrane used in this study could not retain lactose, and approximately 30% of the lactose remained in the retentate when the ultrafiltration produces a three-fold concentrate. After adding the enzyme-treated permeate to the retentate, the total yield of oligosaccharides in the reconstituted milk will become 22.0%, which is similar to that in whole milk. However, this does not mean that the use of ultrafiltration cannot improve the total yield of oligosaccharides. Since the β-galactosidase showed a higher activity in permeate than in milk, the total yield for oligosaccharides is dependent upon the extent that the UF system separates the lactose. For example, if the milk is ultrafiltered to 5-fold concentrate (ca. 80% lactose in milk goes to the permeate with 20% lactose remains in the retentate), it is estimated that 25% total yield could be obtained. Also, as mentioned before, if it is necessary, the total yield of oligosaccharides in the permeate could be further increased by raising the *E*/*S* ratio.

The use of ultrafiltration to manufacture low-lactose milk containing oligosaccharides may also prevent damage to the protein and/or flavor qualities due to

Table 5
The optimal conditions and yields for trisaccharides, tetrasaccharides, pentasaccharides and total oligosaccharides for the enzymic reaction in the permeate

	Trisaccharide	Tetrasaccharide	Pentasaccharide	Total oilgo
Lactose (%)	25.3	25.3	25.3	25.3
Temperature (°C)	51	50	52	50
E/*S* (%)	6.2	6.7	6.7	6.7
Time (h)	2.8	4.0	4.0	3.5
Yield (%)	20.9	8.2	2.6	31.1
Disaccharides (%)	39.1	33.9	34.1	35.3

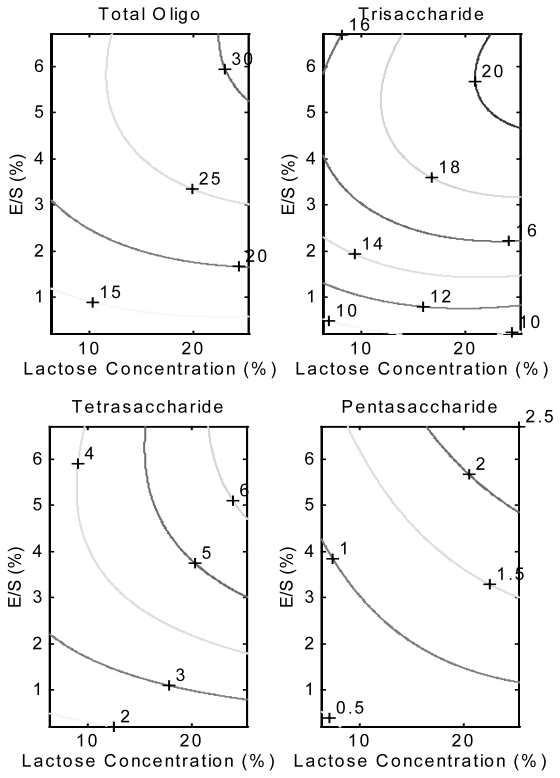

Fig. 4. Effects of lactose concentration and the E/S ratio on the yields of oligosaccharides trisaccharides, tetrasaccharides, and pentasaccharides in the permeate when the temperature and reaction time were at the optimal conditions for total oligosaccharides.

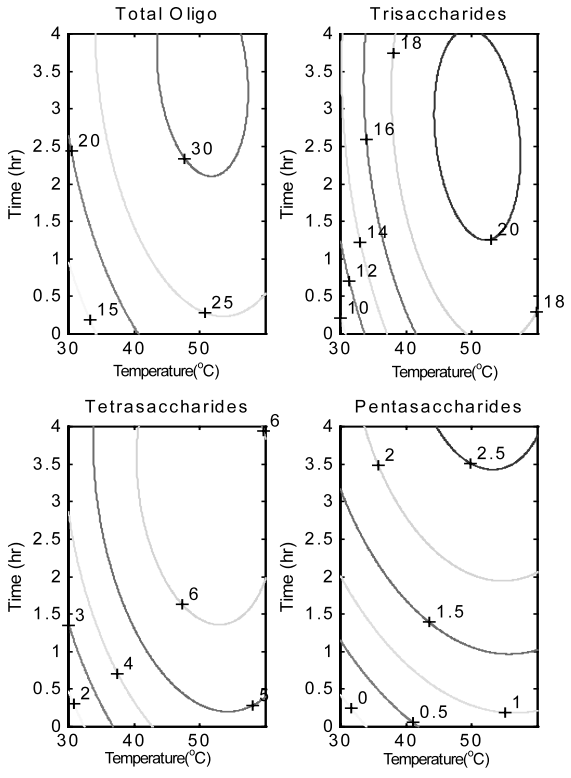

Fig. 5. Effects of temperature and reaction time on the yields of oligosaccharides, trisaccharides, tetrasaccharides, and pentasaccharides in the permeate when lactose concentration and the E/S ratio were at the optimal conditions for total oligosaccharides.

the lack of heating for processing concentrated milk and avoid direct contact between the milk proteins and the β-galactosidase. The effect of industrial processing on the biological value of milk proteins was investigated by Parlapanova [23] who reported that β-galactosidase treatment caused a decrease in milk albumins and some globulins and an increase in γ-globulin. Therefore, when high quality milk products containing oligosaccharides are required, the use of ultrafiltration is a better way to manufacture these products.

4. Conclusion

β-Galactosidase exhibited higher transgalactosylation activity in the permeate than in milk. The milk should be processed by ultrafiltration first, the resulting permeate is then treated with β-galactosidase to convert lactose to oligosaccharides. Finally, the enzyme-treated permeate is added back to the UF retentate to obtain a complete milk containing oligosaccharides. The extent of ultrafiltration would affect the total oligosaccharides content in the final product. When the milk is ultrafiltered to a concentration ratio higher than three, the enzyme-treated UF milk will have a higher oligosaccharides content than the enzyme-treated evaporated milk. Since the β-galactosidase may also react with the milk protein, and the ultrafiltration process could separate the milk protein from lactose before enzyme treatment, it appears that the ultrafiltration process can also aid the manufacture of a better quality milk product containing oligosaccharides.

Table 6
A comparison of the yields of total oligosaccharides in the milk and the permeate

	The milk	The permeate		
Lactose (%)	16.7	25.3	16.7	16.7
Temperature (°C)	47	50	47	47
E/S (%)	5.1	6.7	6.3	5.1
Time (h)	1.4	3.5	3.1	1.4
Yield (%)	22.8	31.1	27.9	25.5
Disaccharides (%)	34.7	35.3	41.1	53.6

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