J. Dairy Sci. 99:1–7
http://dx.doi.org/10.3168/jds.2016-11065

Modeling lactose hydrolysis for efficiency and selectivity: Toward the preservation of sialyloligosaccharides in bovine colostrum whey permeate

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ABSTRACT

Enzymatic hydrolysis of lactose has been shown to improve the efficiency and selectivity of membrane-based separations toward the recovery of bioactive oligosaccharides. Achieving maximum lactose hydrolysis requires intrinsic process optimization for each specific substrate, but the effects of those processing conditions on the target oligosaccharides are not well understood. Response surface methodology was used to investigate the effects of pH (3.25–8.25), temperature (35–55°C), reaction time (6 to 58 min), and amount of enzyme (0.05–0.25%) on the efficiency of lactose hydrolysis by β-galactosidase and on the preservation of biologically important sialyloligosaccharides (3′-siallylactose, 6′-siallylactose, and 6′-sialyl-*N*-acetyllactosamine) naturally present in bovine colostrum whey permeate. A central composite rotatable design was used. In general, β-galactosidase activity was favored at pH values ranging from 3.25 to 5.75, with other operational parameters having a less pronounced effect. A pH of 4.5 allowed for the use of a shorter reaction time (19 min), lower temperature (40°C), and reduced amount of enzyme (0.1%), but complete hydrolysis at a higher pH (5.75) required greater values for these operational parameters. The total amount of sialyloligosaccharides was not significantly altered by the reaction parameters evaluated, suggesting specificity of β-galactosidase from *Aspergillus oryzae* toward lactose as well as the stability of the oligosaccharides at pH, temperature, and reaction time evaluated.

Key words: lactose hydrolysis, sialyloligosaccharides, colostrum

INTRODUCTION

Bovine milk and dairy streams such as whey permeate have been identified as sources of bioactive oligosaccharides that are structurally identical/similar to the oligosaccharides present in human milk. Recently identified milk oligosaccharides in bovine whey permeate contain branched oligosaccharides decorated with sialic acid and fucose (Barile et al., 2009, 2010), exhibiting greater similarity to human milk oligosaccharides than currently available prebiotics such as inulin and galacto-oligosaccharides (Barile and Rastall, 2013). The recovery of highly bioactive oligosaccharides from whey permeate, a co-product from the removal of whey proteins during ultrafiltration, has the added potential to mitigate environmental and economic problems associated with the disposal of this underutilized dairy stream.

The major challenge in isolating oligosaccharides from dairy streams is to enrich oligosaccharides while simultaneously reducing the content of lactose and other simple sugars that do not possess the desired prebiotic or protective functions and that would confound the biological activity of oligosaccharides for in vitro testing. The high lactose content (44–52 g/L; Jelen, 1979) and the low concentration of the most abundant sialyloligosaccharides in bovine milk (0.033 g/L; Martín-Sosa et al., 2003), in addition to their molecular weight proximity, demonstrates the processing challenge associated with the recovery of oligosaccharide fractions with a high degree of purity when using membrane techniques.

Upstream lactose hydrolysis by β-galactosidases has been shown to improve the efficiency and selectivity of membrane-based separations toward the recovery of biologically bioactive oligosaccharides (Sarney et al.,

Received February 21, 2016.
Accepted April 5, 2016.
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2000). Nearly 100% lactose hydrolysis was achieved when β-galactosidase from *Aspergillus oryzae* was incubated in human milk at pH 5.2 for 4–5 h. The combination of selective protein precipitation, lactose hydrolysis, and nanofiltration (followed by diafiltration) enabled the recovery of more than 50% of the human milk oligosaccharides (Sarney et al., 2000). However, residual lactose and monosaccharides remained in the retentates produced by this approach, which when concentrated by membrane filtration often became more concentrated than the target oligosaccharides, thus markedly reducing the purity of the final product.

The objective of the present work was to maximize the efficiency of lactose hydrolysis by β-galactosidase while achieving maximum preservation of biologically important sialyloligosaccharides (3′-siallylactose [**3′-SL**], 6′-siallylactose [**6′-SL**], and 6′-sialyl-*N*-acetyllactosamine [**6′-SLN**]) naturally present in bovine colostrum whey permeate. We chose to use bovine colostrum to facilitate the analytical quantification of oligosaccharides, but this approach is easily translatable to more typical dairy streams derived from cheese-making. This investigation is of great importance to maximizing the purity and yield of acidic oligosaccharides when considering downstream isolation by membrane filtration. In the present work, the effects of pH, temperature, reaction time, and amount of enzyme were simultaneously evaluated. To identify the ideal combination of pH, reaction time, temperature, and amount of enzyme, a central composite rotatable design totaling 28 experimental conditions was used.

MATERIALS AND METHODS

Bovine Colostrum Whey Permeate

Bovine colostrum whey permeate was kindly provided by La Belle Colostrum (Bellingham, WA). Liquid colostrum was initially defatted via cream separators and decaseinated by rennet addition. The whey obtained after the removal of caseins was pasteurized at 63°C for 30 min. Whey proteins were removed by ultrafiltration (10-kDa membrane) under continuous diafiltration to produce whey permeate. Lactose and solid contents of the colostrum whey permeate (starting material) were 17.4 ± 0.62 g/L and 3.15 ± 0.03%, respectively.

β-Galactosidase Treatment of Bovine Colostrum Whey Permeate

A food-grade fungal lactase (Bio-Cat Inc., Troy, VA) derived from the fungus *A. oryzae* was used to hydrolyze lactose into β-D-galactose and α-D-glucose.

Table 1. Variables and levels evaluated in the experimental design to optimize lactose hydrolysis efficiency by β-galactosidase and preservation of acid oligosaccharides[1]

	Level				
Variable	$-\alpha$	-1	0	1	$+\alpha$
pH, X_1	3.25	4.5	5.75	7.0	8.25
Time (min), X_2	6	19	32	45	58
Temperature (°C), X_3	35	40	45	50	55
Enzyme (%), X_4	0.05	0.10	0.15	0.20	0.25

[1]Complete 2^4 factorial design parameters, with 4 independent variables in 2 levels, 4 repetitions in the central point, and 8 axial points.

Thirteen milliliters of whey permeate was adjusted to 3.25 to 8.25 pH with 0.1 *N* HCl or 0.1 *N* NaOH, and β-galactosidase was added to achieve 0.05 to 0.25% (wt/wt) (Table 1). Whey permeate samples were incubated at 35 to 55°C for 6 to 58 min (Table 1) at 50 rpm in 250-mL Erlenmeyer flasks. After lactose hydrolysis, samples were immediately centrifuged at 3,900 × *g* for 30 min using a 30-kDa molecular weight cutoff centrifugal filter device (Amicon Ultra-15 Centrifugal Filter, Millipore, Billerica, MA) to separate the enzyme from the hydrolyzed fraction. Unequal permeate volumes due to the addition of different amounts of HCl or NaOH used during the pH adjustment step were equalized by the addition of distilled water to achieve a uniform final volume. Samples from all experiments were analyzed for glucose, galactose, lactose, 3′-SL, 6′-SL, and 6′-SLN.

Experimental Design and Statistical Analysis

Response surface methodology was used to investigate the optimal reaction parameters affecting the efficiency of lactose hydrolysis by β-galactosidase and the preservation of the major oligosaccharides present in bovine colostrum whey permeate. The use of a factorial design methodology, associated with response surface analysis, enables simultaneous analysis of multiple variables, thus reducing processing time and costs. The individual and combined effects of the most important parameters that affect β-galactosidase activity (pH, reaction time, temperature, and amount of enzyme) were evaluated by a central composite rotatable design, with 4 central points and 8 axial points (Hatzinikolaou et al., 2005; Rodrigues and Iemma, 2014). The total number of experiments followed the equation $2^k + 2k + n_c$, where k is the number of independent variables and n_c is the number of repetitions in the central point.

The effects of pH (3.25–8.25), temperature (35–55°C), reaction time (6–58 min), and amount of enzyme (0.05–0.25% wt/wt) on the efficiency of lactose hydrolysis by β-galactosidase and preservation of sialyloligosac-