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Enzymatic formation of galactooligosaccharides in goat milk

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ABSTRACT

Goat milk has become more popular due to its attractive nutritional profile. Galactooligosaccharides (GOS) are prebiotics with health benefits. The enzymatic formation of GOS in reconstituted goat milk using two commercial β-galactosidases (L6500 from *Kluyveromyces lactis* and L10 from *Aspergillus oryzae*) was studied. The carbohydrate composition of the milk system was quantified using high-performance anion-exchange chromatography. Major GOS produced by β-galactosidases under the experimental conditions (45 °C, pH 6.7) were 6-galactobiose, allolactose, and 6-galactosyllactose. The maximum GOS yields in goat milk with 10% total solids were obtained when the lactose conversion rates were 59% for L10 and 88% for L6500. Further increasing lactose conversion decreased GOS formation. Increasing milk concentration from 10% to 30% total solids (lactose concentration from 47 to 143 g/L) increased GOS yields up to 22% of the total sugars using L6500. It may be concluded that suitable experimental conditions can be used to produce GOS in goat milk with prebiotic function.

1. Introduction

Dairy goat farming is a significant industry in many countries such as South Asia, Mediterranean regions, Middle East, and Africa (FAO, 2018). Goat milk, like other milks, is a good source of various nutrients such as protein, lipids, and certain minerals and vitamins. Compared to cow or human milk, goat milk tends to have higher digestibility, buffering capacity, and alkalinity with certain therapeutic values for medicine and human nutrition (Park, Juárez, Ramos, & Haenlein, 2007). There has been increasing interest in developing goat milk based products for human consumption. For example, goat cheese and yogurt are popular products with uniquely-bodied flavour and texture.

Lactose is the major carbohydrate in goat milk, in which it makes up ~4.1% of the total solid content. The lactose content of goat milk tends to be lower than that of sheep (4.9%), cow (4.7%), and human (6.9%) (Park et al., 2007). There is a significant population with lactose intolerance (Vesa, Marteau, & Korpela, 2000). Enzymatic hydrolysis by β-galactosidases has been commonly used to reduce the lactose concentration in milk products to aid people with lactose intolerance and mal-digestion (Fischer & Kleinschmidt, 2018; Rodriguez-Colinas, Fernandez-Arrojo, Ballesteros, & Plou, 2014). Lactose is enzymatically hydrolysed into glucose and galactose which are sweeter in taste. Galactooligosaccharides (GOS) may also be produced from this enzymatic hydrolysis through transgalactosylation (Martínez-Villaluenga et al., 2008). GOS, as prebiotics for human nutrition, have shown a range of health benefits (Azcarate-Peril et al., 2017; Rodriguez-Colinas et al., 2014). GOS can promote the proliferation of *Bifidobacterium* spp. and *Lactobacillus* spp. in the colon. This not only provides protection from infections by inhibiting the growth of pathogens and modulating the immune system, but also facilitates the normal functions of the human gut by improving the absorption of minerals (Sako, Matsumoto, & Tanaka, 1999). Therefore, it is nutritionally desirable to use β-galactosidases to increase the GOS concentration in milk while decreasing the lactose content.

Factors affecting the enzymatic formation of GOS from lactose in solution include pH, temperature, the enzyme source, substrate and enzyme concentration, the presence of other components, and so on (Rodriguez-Colinas et al., 2014). A previous study reported the enzymatic formation of GOS in skim cow milk using commercial β-galactosidases from different sources (Rodriguez-Colinas et al., 2014). In this short communication, two commercial β-galactosidases were used to produce GOS in reconstituted goat milk systems. The changes in the carbohydrate composition in goat milk were followed using high-performance anion-exchange chromatography. The results of this study may provide a basis to support the further development of the goat milk industry.

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2. Materials and methods

2.1. Materials

Lactozym® Pure 6500 L (L6500) (Novozymes®, ≥ 6500 Acid Lactase Unit (ALU)/g) and Enzidase® Lactase 10 (L10) (Zymus®, ≥ 10,000 ALU/g) were obtained from Novozymes (Bagsværd, Denmark) and Zymus International (Auckland, New Zealand), respectively. The L6500 and L10 were from *Kluyveromyces lactis* and *Aspergillus oryzae*, respectively. The enzymatic activities were according to the suppliers. All the other chemicals were of analytical grade from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Goat skim milk powder (batch number: TD0600M) was provided by Dairy Goat Co-operative (Hamilton, New Zealand). The composition of the skim milk was 50.6% lactose, 35.6% protein, 9.3% ash, 3.5% moisture and 1% total fat according to the manufacturer.

2.2. Formation of galactooligosaccharides in reconstituted goat milk

Goat milk was reconstituted the day before the experiment. Goat milk powder (10 − 30%, w/w) was stirred in Milli-Q water (Millipore Corporation, Burlington, MA, USA) at room temperature (~23 °C) for ~2 h and left in the refrigerator overnight for full hydration. Before measurements, the samples were equilibrated at room temperature for 2 h. The milk with 10% total solids was closest to normal milk. The enzymatic processing used the method of Rodriguez-Colinas et al. (2014) with the exception that the reactions were done at 45 °C for optimal enzyme activities from a preliminary test. The amount of enzymes used in this study (12.2 mg of L6500 and 7.9 mg of Lactase 10 in 1 mL of reconstituted milk solution) was targeted to rapidly and completely (within an h) hydrolyze lactose so as to be potentially workable in industrial applications. The hydrolysed samples were mixed with Carrez solutions before centrifugation at 4000 × *g* (DW-41, Qualitron, Seoul, South Korea). The supernatant was filtered through a 0.45 μm filter syringe (Microscience, Auckland, New Zealand) before high-performance anion-exchange chromatography (HPAEC) analysis. The lactose conversion rate (%) was calculated as the difference in the lactose concentration between the final and initial milks.

2.3. Formation of galactooligosaccharides in lactose solution

Lactose solutions (45–135 g/L) in 10 mM potassium phosphate buffer (pH = 6.7) were prepared. The reaction conditions were the same as in the above section and the reaction was carried-out for 30 min. An aliquot (200 μL) was taken and boiled in a water bath for 5 min to inactivate the enzymes before HPAEC analysis.

2.4. HPAEC

The carbohydrate composition of goat milk or the lactose model system were analyzed on a Dionex ICS5000$^+$ HPAEC-PAD (pulsed amperometric detection) system (Thermo Scientific™, Sunnyvale, CA, USA). The analytical column was a CarboPac PA-1 anion-exchange column with a CarboPac PA-1 guard column (Thermo Scientific™). The flow rate was 1.0 mL/min and column compartment temperature was 30.0 °C. The injection volume was 25.0 μL. A gradient elution consisted of eluent A (125 mM NaOH) and eluent B (12.5 mM NaOH): 0–15 min, 100% B; 15–20 min, 100–50% B; 20–50 min, 50–10% B; 50–52 min, 10–0% B; 52–59 min, 0% B; 59–60 min, 100% B. The identification and quantification of sugars including lactose, galactose, glucose, (Sigma-Aldrich Chemical Co., USA, purity > 99%), 6-galactobiose, allolactose, and 6-galactosyllactose were based on external standards kindly donated by Yakult (Tokyo, Japan), as well as results of previous reports (Rodriguez-Colinas et al., 2014) (Supplementary Fig. 1). The areas under the curve was intregrated using the Chromeleon™ Chromatography Data System (CDS) 7.0 software (Thermo Scientific™).

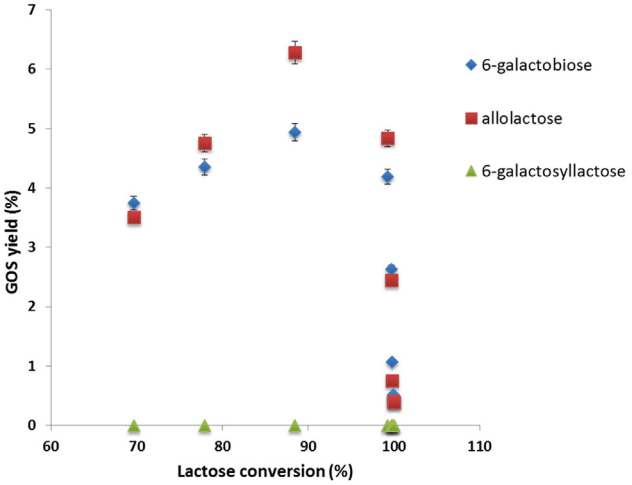

Fig. 1. Relationships between GOS yield and lactose conversion rate (%) in reconstituted goat milk with 10% total solids during hydrolysis using L6500.

2.5. Data analysis

All the experiments were done in triplicate. Results were expressed as mean ± SD (error bars in the figures). ANOVA with Duncan's test was used to determine the difference of means among different treatment ($p < 0.05$) using SPSS software (Version 22.0, IBM Corporation, Armonk, New York, USA). Only significant differences are discussed in this paper.

3. Results and discussion

3.1. GOS formation by enzymes in goat milk

Initially, a lactose solution as a model system was used to obtain the optimal conditions (pH, temperature, and time of hydrolysis) (45 °C and 30 min for L6500 and 45 °C and 5 h for L10, pH 6.7 as in goat milk) (the experimental conditions were used based on preliminary trials) for the enzyme hydrolysis (data not shown). These experimental conditions were extended to catalyze lactose transformation in the goat milk with 10% total solids using the enzymes L6500 and L10. The relationships between the lactose conversion and GOS yields were obtained (Figs. 1 and 2). The initial lactose concentration in the goat milk with 10% totals solids was 47.2 g/L. It was observed that the rate of lactose conversion in the 10% total solid goat milk was close to that of lactose solution (45 g/L). The minimum rate of lactose conversion was 69.7% after 15 s of catalysis with the enzyme L6500 (Fig. 1). The fast

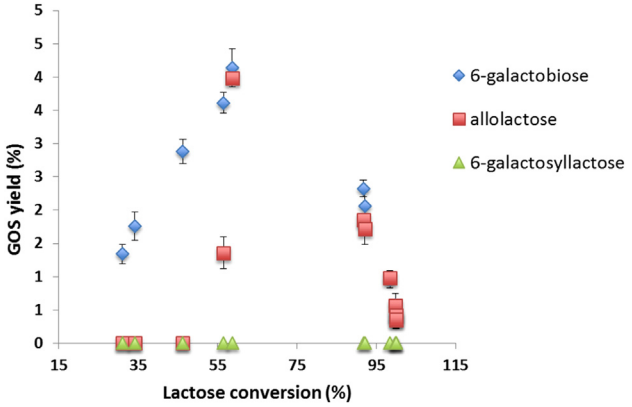

Fig. 2. Relationships between GOS yield and lactose conversion rate (%) in reconstituted goat milk with 10% total solids during hydrolysis using L10.