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ARTICLE

Galactooligosaccharide and Sialyllactose Content in Commercial Lactose Powders from Goat and Cow Milk

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Abstract

The most commonly used infant formulas contain lactose originating from cow milk. Goat milk has recently been claimed to be nutritionally more effective for infants than other milks. In baby foods, much emphasis is placed on the concentrations of intestinal microflora-promoting oligosaccharides, which are generally transferred into lactose from milk during crystallization process. Here we show that higher level of free sialic acid is present in goat lactose powder compared to cow lactose powder. Without proteinase K treatment, the amount of 3-sialyllactose and 6-sialyllactose were similar in goat and cow lactose powders. However, after proteolysis, 6-sialyllactose was present at higher levels in goat than in cow lactose powder. Galactooligosaccharides, a group of prebiotics, are present in milk in the form of glycoproteins. Galactooligosaccharide content was also higher in goat lactose powder than in cow lactose powder.

Keywords: galactooligosaccharide, sialyllactose, goat milk, cow milk

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Introduction

Infant formulas are manufactured to feed babies and infants. Recently, mammalian milk with a composition similar to that of human milk has received much attention. In Korea, dairy goat breeding began with the introduction of the Saanen breed in 1903, and sales of goat milk are gradually increasing as its advantages and health benefits are advertised (Coveney and Darnton-Hill, 1985; Park 2006). Dairy goats are known for their excellent immunity, ease of breeding, and environmental friendliness; they produce more than twice the amount of milk per kilogram of weight than cows, making them an industrially valuable livestock (Jenness, 1980).

Goat milk has many similarities with human milk. In comparison with cow milk, protein and fatty acid composition of goat milk is more similar to that of human milk (Prosser *et al*., 2007; Urbiene *et al*., 1997). In particular, the primary structure of goat casein is closer to that of human casein, which ensures greater digestive absorption (Tomotake *et al*., 2006). Thus, goat milk consumption is associated with fewer digestive disorders, such as upset stomach and diarrhea (Haenlein *et al*., 1984). In comparison with cow milk, goat milk has a lower content of α_{S1}-casein, which plays a critical role in many digestive disorders (Yangilar, 2013). Furthermore, goat milk contains approximately twice as much medium-chain fatty acids as cow milk, which contributes to its better digestibility (Jenness, 1980).

In addition to similarities in protein and fatty acid composition, the oligosaccharide profile of goat milk is more similar to that of human milk than that of cow milk. Although the lactose contents of cow milk and goat milk are similar (about 70% that of human milk), goat milk rarely causes lactose intolerance (Jandal, 1996). Furthermore, lactose present in milk increases acidity in the intestinal tract, which not only facilitates the growth of health-beneficial bacteria, but also enhances the absorption of minerals, such as calcium, phosphate, and magnesium (Daddaoua *et al*., 2006). Moreover, oligosaccharides act as prebiotics and support intestinal function in infants and toddlers (Chung, 2010; Hernandez *et al*., 2009). Goat milk contains five to eight times higher levels of oligosaccha-

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rides than cow milk, the major ones being 3-sialyllactose, 6-sialyllactose, disialyllactose, N-glycolylneuraminyllactose, 3-galactosyllactose, N-acetylglucosaminyllactose, and lacto-N-hexose (Martinez-Ferez *et al*., 2006).

Commercially available lactose powders used as ingredients for powdered infant formulas are produced through concentration and crystallization of cheese whey, and trace amounts of oligosaccharides remain in the final product after during the manufacturing process (Stephen, 2013). In the present study, we analyzed the oligosaccharide content in commercially available cow lactose powder and goat lactose powder. The levels of 3-sialyllactose, 6-sialyllactose, and galactooligosaccharides (GOS), the major oligosaccharides of both goat and cow milks, were assessed by HPLC and LC-MS/MS analysis.

Materials and Methods

Materials

Commercial cow and goat lactose powders within 6 mon after manufacture were purchased from a local store in Seoul, Korea. The reference standards (N-acetylneuraminic acid [Neu-5Ac] and N-glycolylneuraminic acid [Neu-5Gc]) for sialic acid analysis were obtained from Sigma-Aldrich (USA). 3-Sialyllactose, 6-sialyllactose (USA), and Sunoligo L500 (Samyang Genex, Korea) containing >50% (w/v) of GOS were used as reference standards for oligosaccharide analysis. Proteinase K and activated charcoal powder (Samchun Chemicals, Korea) were used for sample pretreatment. Other chemicals and reagents were obtained from Sigma-Aldrich.

Sialic acids analysis

Samples were pretreated as described by van der Ham *et al*. (2007) with modifications. Lactose powder (2 g) was reconstituted in distilled water (5 mL) at 50°C for 2 h and activated charcoal was added to a final concentration of 0.1% (w/v), and the samples were boiled and filtered through filter paper (Whatman No. 2; GE Healthcare Life Sciences, UK), followed by filtration through a 0.45 μm syringe filter (Advantec, USA) to remove possible contaminating microorganisms. Sialic acid was determined with the technical assistance of the Cawthron Institute (New Zealand) according to the HPLC-MS/MS condition of van der Ham *et al*. (2007). The reference standards were prepared at the concentration of 20 g/mL.

Oligosaccharide Analysis

Lactose powder (2 g) was reconstituted in 5 mL of deionized distilled water to a final concentration of 40% (w/v). The samples were treated as follows: 1) activated charcoal was added to a final concentration of 0.1% (w/v), and the samples were boiled and filtered through filter paper (Whatman No. 2; GE Healthcare Life Sciences, UK), followed by filtration through a 0.45 μm syringe filter (Advantec, USA) to remove possible contaminating microorganisms; 2) additionally treated with proteinase K. The reference standard for GOS was prepared at a concentration of 1000 mg/mL. The GOS content was analyzed under two different experimental conditions using the following HPLC systems: 1) Waters e2695 separation module (Waters, USA) equipped with a YMC-Pack Polyamine II column (4.6×250 mm., YMC, Japan); 2) Waters 515 (Waters, USA) pump with an Aminex HPX-87C column. A Waters 2414 refractive index detector (Waters, USA) was used for the analysis. Acetonitrile in water (64%) was used as a mobile phase at a flow rate of 1.0 mL/min. The total GOS content was calculated according to the reference to the Food for Specified Health Use (FOSHU) status manual by Ministry of Health and Welfare of Japan. The peak area (from two independent experiments) with fructose, glucose, galactose and lactose peak areas subtracted was considered as the GOS content. In addition, total GOS content was calculated using the ratio of analyzed values between the Aminex HPX-87C and YMC-Pack Polyamine II columns.

For the analysis of sialyllactose, 3-sialyllactose and 6-sialyllactose were used as reference standards. Pretreated samples were diluted to a concentration of 0.1% prior to use. Sialyllactose was analyzed using a BioLC system (model ICS-2500; Dionex, USA). A CarboPac PA-100 column (Dionex) and 100 mM sodium acetate (pH 4.0) containing 100 mM sodium hydroxide were used for the analysis. The flow rate was 1.0 mL/min. The contents of 3-sialyllactose and 6-sialyllactose were calculated based on the peak area.

SDS-PAGE for glycoprotein determination

Lactose powder samples (3 g) were dissolved in 5 mL of deionized distilled water or Tris buffer (100 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.5) to a final concentration of 60% (w/v). Proteinase K (200 μL; 4.0 mg/mL) was added to both samples every 8 h, and the mixtures were incubated for up to 24 h at 55°C as described previously (de Castro *et al*., 2013). An 8% (w/v) stacking gel and 15% (w/v) separation gel were used. The samples (16 μL) were combined with 4 μL of sample buffer (12.5 mM Tris, 2% β-mercaptoethanol, 2% SDS, 20% glycerol,

0.02% bromophenol blue, pH 6.8), and heated for 5 min at 95°C prior to loading. Samples were electrophoresed for 2 h at 80 V. The gels (100 mm in length, 80 mm in width and 1 mm in thickness) were stained using Bio-Safe Coomassie G-250 stain (Bio-Rad, USA) for 1 h and destained in distilled water. The molecular weights of glycoproteins were estimated using SolGent Protein Marker (Solgent, Korea).

Growth of lactic acid bacteria in cow and goat milk

Lactic acid bacteria were obtained from Chr. Hansen (Denmark). ABT-5 (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus acidophilus*) was cultured in lactobacilli MRS broth (Difco, BD Biosciences, USA) for 1 d, and then the seed culture was inoculated (5% v/v) into cow and goat milk. Cultures were incubated for 8 h at 37°C. At the end of the incubation, living lactic acid bacteria numbers were counted using BCP added-MRS agar and expressed as Log CFU/mL.

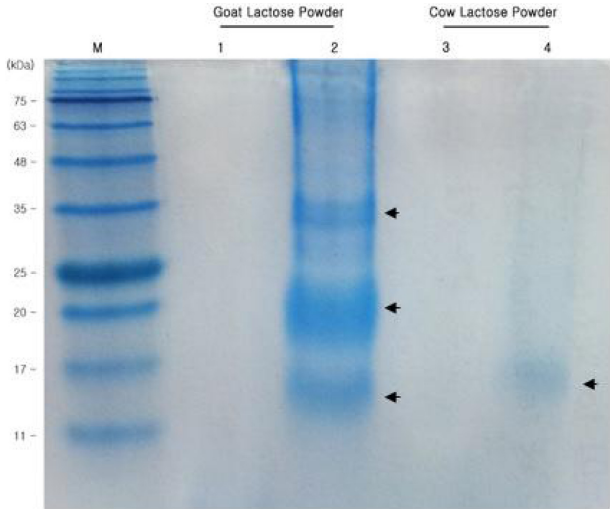

Fig. 1. SDS-PAGE of goat and cow lactose powders. Lane 1: Goat lactose powder treated with proteinase K (4.0 mg/mL); Lane 2: Untreated goat lactose powder; Lane 3: Cow lactose powder treated with proteinase K (4.0 mg/mL); Lane 4: Untreated cow lactose powder. Arrows indicate glycoproteins.

Results

Sialic acids content

As shown in Table 1, the content of Neu-5Ac in goat lactose powder was 273 mg/kg, while it was undetectable in cow lactose powder. Similarly, the content of Neu-5Gc in goat lactose powder was 50 mg/kg, while it was not detected in cow lactose powder. Thus, the total level of sialic acid in goat lactose powder was 323 mg/kg, but it was undetectable in cow lactose powder. A free form of sialic acid given that the content of sialic acid (Neu-5Ac, Neu-5Gc) in goat milk powder was higher than those of cow milk powder, it may have been detached from the acidic fraction of animal milk oligosaccharides. As stated by Schauer (1982), sialic acid was bound to lactose, more sialic acid may exist as a bound form in the lactose of goat milk powders.

Determined protein of lactose powder

To examine whether sialyllactose and oligosaccharides are bound to glycoproteins, lactose powder samples were analyzed by SDS-PAGE. As shown in Fig. 1, three protein bands around 15 kDa, 22 kDa, and 34 kDa were present in goat lactose powder (lane 2). In contrast, there was only a faint protein band around 17 kDa in cow lactose powder (lane 4). Both goat and cow lactose powder samples treated with proteinase K did not show any protein bands (lanes 1 and 3, respectively). This demonstrates that protein content is higher in goat lactose powder than in cow lactose powder; however, no protein bands were found when concentration of lactose powder samples was below 60% (w/v), indicating that only trace amounts of proteins are present in goat lactose powder (data not shown).

Sialyllactose contents

The content of 3'-N-acetylneuraminyl-D-lactose (3'-sialyllactose, 3'-SL) and 6'-N-acetylneuraminyl-D-lactose (6'-sialyllactose, 6'-SL) and glycoprotein forms in goat lactose powder and cow lactose powder was analyzed using Bio-LC. As shown in Table 2, no significant differences

Table 1. Sialic acid contents in goat and cow lactose powders

Sialic acid species	Goat[1)]		Cow[1)]	
	Mean	RSD (%)	Mean	RSD (%)
N-acetylneuraminic acid (Neu-5Ac)	273	7	ND[2)]	0
N-glycolylneuraminic acid (New-5Gc)	50	9	ND	0
Total sialic acid (New-5Ac + Neu-5Gc)	323		ND	

[1)]Each measurement (mg/kg) was made in triplicate.
[2)]ND : not detected.

in the levels of 3'-sialyllactose and 6'-sialyllactose were found between goat and cow lactose powders filtered through 0.45 μm syringe filters. The 3'-sialyllactose content was 0.02% for goat and 0.03% for cow. The 6'-sialyllactose content was 0.01% for both samples. However, when samples were treated with proteinase K followed by syringe filtration, the content of 6'-sialyllactose in goat lactose powder was higher than that in cow lactose powder (0.05% vs. 0.01%), while no significant difference was found in 3'-sialyllactose. These results show that more 6'-sialyllactose is present in the form of glycoproteins in goat lactose powder than in cow lactose powder.

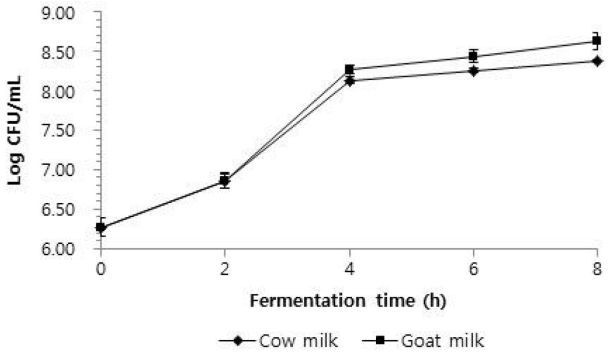

Fig. 2. Viable cell counts of lactic acid bacteria grown in cow and goat milk.

Galactooligosaccharide content

The GOS content determined by using an Aminex HPX-87C column and an YMC-Pack Polyamine II column is summarized in Table 3. The GOS content in goat lactose powder was approximately four times that in cow lactose powder when samples were filtered without proteinase K treatment. Similarly, the GOS content in goat lactose powder was approximately two times that in cow lactose powder when samples were treated with proteinase K followed by filtration. These results indicate that the level of GOS in goat lactose powder is higher than in cow lactose powder. The GOS levels in goat and cow lactose powders increased approximately twofold and fourfold, respectively, followed by proteinase K treatment, indicating that considerable amount of GOS exist in the form of glycoproteins in lactose powders.

Growth behavior of lactic acid bacteria in cow and goat milk

To evaluate the effect of higher levels of oligosaccharides in goat milk on the growth of probiotics, we inoculated commercially available cow milk and goat milk with ABT-5 (*S. thermophilus, L. bulgaricus, L. acidophilus*), which is widely used to produce fermented milk. As depicted in Fig. 2, the number of living cells increased remarkably for up to 8 h in both cow and goat milk (Log 8.37 and Log 8.63, respectively). The number of lactic acid bacteria in goat milk was higher than in cow milk, suggesting that the higher content of oligosaccharides enhances the growth of probiotics more effectively. This is consistent with previous observations that oligosaccharides promote the growth of probiotics in the intestinal tract (Martinez-Ferez *et al*., 2006).

Discussion

It was found that oligosaccharides in commercially available lactose powders are present in a free form or are bound to proteins. The existence of the protein-bound forms suggests that various oligosaccharide forms are probably transferred into lactose concentrates in the pro-

Table 2. 3'-Sialyllactose and 6'-sialyllactose content in goat and cow lactose powders

Sample preparation	Goat[1)]				Cow[1)]			
	3'-sialyllactose		6'-sialyllactose		3'-sialyllactose		6'-sialyllactose	
	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
Filtration	0.02	8.21	0.01	6.18	0.03	8.33	0.01	10.21
Proteinase K + filtration	0.03	7.45	0.05	9.92	0.04	7.82	0.01	9.01

[1)] Each measurement (%) was made in triplicate.

Table 3. Galactooligosaccharides (GOS) contents in goat and cow lactose powders

Sample preparation	GOS (%)[1)]			
	Goat		Cow	
	Mean	RSD (%)	Mean	RSD (%)
Filtration	4.8	6.11	1.1	9.14
Proteinase K + filtration	9.7	7.28	4.6	6.84

Each measurement was made in triplicate.

cess of lactose crystallization. On the basis of the results, the proteinase K pre-treatment is recommended as a critical step for the micro-determination of milk originated oligosaccharides (using HPLC). By using the developed protocol, it was found that the amounts of oligosaccharides in goat lactose powder are higher than those in cow lactose powder.

Conclusions

In the present study, we quantified the amounts of oligosaccharides present in commercially available goat and cow milk powders. We found that GOS and sialyllactose were present in lactose powder mainly as glycoproteins. Upon treatment with proteinase K, 3'-sialyllactose, 6'-sialyllactose, and GOS were analyzed; the levels of 6'-sialyllactose and GOS were higher in goat lactose powder than in cow lactose powder. These results imply that goat lactose powder may provide benefits as prebiotics, and warrant its wide use in processed infant foods (e.g., prepared powdered milk). This protocol could be applicable to routine analyses of oligosaccharides as a measure of quality control of infant formulas or in the food industry.

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