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Goats' milk as a natural source of lactose-derived oligosaccharides: Isolation by membrane technology

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Abstract

Human milk oligosaccharides are thought to be beneficial for the infant with regard to their prebiotic and anti-infective properties. However, so far no milk from farm animals has been considered to be a good natural source of lactose-derived oligosaccharides for human nutrition. In this study, the characterization and quantitation of neutral and sialylated lactose-derived oligosaccharides in mature caprine milk was performed and compared to ovine, bovine and human milk. The quantification was carried out using high-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), and the characterization was performed by fast atom bombardment mass spectrometry (FAB-MS). A large amount and variety of acidic and neutral oligosaccharides were found in goats' milk when compared with cow and sheep milk. In addition, 15 new oligosaccharide structures were identified in caprine milk. In order to isolate the goats' milk oligosaccharide fraction, a two-stage tangential ultrafiltration–nanofiltration process was selected. Tubular ceramic membranes with molecular mass cut-offs of 50 and 1 kDa, respectively, were employed. A virtually lactose and salts-free product containing more than 80% of the original oligosaccharide content was obtained.

Keywords: Lactose-derived oligosaccharides; Ultrafiltration; Nanofiltration; Goats' milk; Human nutrition

1. Introduction

Interest in milk oligosaccharides started about a hundred years ago after observing that the carbohydrate fraction is most likely responsible for the development of a bifidogenic flora in breastfed children (Kunz, Rudloff, Baier, Klein, & Strobel, 2000). Nowadays, milk oligosaccharides are thought to be beneficial for the human milk fed infant with regard to their prebiotic and anti-infective properties (Kunz et al., 2000) as most oligosaccharides (>95%) from human milk are not digested in the gastrointestinal tract, which suggest they may also play a role in the local intestinal immune system of the breast-fed infants (Gnoth, Kunz, Kinne-Saffran, & Rudloff, 2000).

In addition, there is currently great interest in the role of these oligosaccharides as pathogen receptors. Oligosaccharides may indeed mimic epithelial receptors for pathogenic microorganisms, thus acting potentially as intestinal mucosa cell protectors. They may constitute an additional defense mechanism for newborn infants, whose gastric pH is less acidic than in adults, and whose immune system is not yet mature (Adlerberth, 1997; Lundquist, Nord, & Winberg, 1985). In fact, among breastfed newborn infants there is a lower rate of children suffering from diarrhoea, respiratory diseases, otitis and, in general, infectious diseases (Newburg,

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1999). Many in vitro studies have demonstrated the potential of human milk oligosaccharides to influence infectious events such as inhibition of the toxical effect of the *Escherichia coli* heat-stable enterotoxin (Cravioto et al., 1991; Schwertmann, Schroten, Hacker, & Kunz, 1999), inhibition of the infection caused by Campylobacter jejune (Cervantes, Newburg, & Ruiz-Palacios, 1995) and blocking of the union of *Streptococcus pneumoniae* and *Haemophilus influenzae* with their respective recipients (Zopf & Roth, 1995).

Another important research topic is currently the influence of oligosaccharides on leukocyte–endothelial cell interactions (Lasky, 1995; McEver, 1994; Schwertmann, Rudloff, & Kunz, 1996). Many sialylated and fucosylated oligosaccharides from human milk may block these interactions having dramatic effects on the progression of inflammatory responses (Kunz, Rodriguez-Palmero, Koletzko, & Jensen, 1999a).

Currently, infant formulas enriched with fructo- and/or galacto-oligosaccharides are commercially available. Those are simple structures that possess a prebiotic effect stimulating Bifidobacteria and Lactobacilli in the gut (Moro et al., 2002), but where the other important functions described for human milk oligosaccharides have not been tested.

Unfortunately, no company supplements their infant formulas with oligosaccharides similar to those found in human milk, because of the extreme difficulty of synthesis or, in the case of finding a natural source enriched in these biomolecules, isolation. Potential processes for the of bioactive compounds include chromatography, gel and capillary electrophoresis, selective precipitation and membrane technology (De-Frees, 2002; Sarney, Hale, Frankel, & Vulfson, 2000). Except the latter, none of them has been efficiently implemented at an industrial scale so far due to high capital cost, operational complexity and low productivity (Zydney, 1998). Among the membrane modules commercially available, ceramic membranes are extremely versatile. They are inert to common cleaning chemicals and solvents and offer wide temperature, pH and pressure limits, allowing extended operating lifetimes (Cheryan, 1998).

In this sense, besides human milk only few natural sources for such components are known. Milk from very few species (e.g., elephants or primates) contains rather high concentration and unique pattern of oligosaccharides (Kunz, Rudloff, Schad, & Braun, 1999b; Warren et al., 2001). Until now, among farm animals, there is not one type of milk which has been found to contain the proportion of oligosaccharides resembling that found in human milk, both in quantity and composition.

The aim of the present study was twofold. First, to find a natural source of lactose-derived oligosaccharides among the main farm mammals for the development of functional foods, for clinical and infant nutrition. To this end, the oligosaccharide composition of fresh mature milk from Spanish goats, cows and sheep were determined and compared to those found in human milk. Second, to select a feasible process (which can be easily implemented at an industrial scale) for the isolation of the oligosaccharide fraction.

2. Materials and methods

2.1. Comparison of caprine, bovine, ovine and human milk oligosaccharides

2.1.1. Chemicals and reagents

NaOH solution (50% wt/wt; low in carbonate) was purchased from J.T. Baker (Philadelphia, USA). Sodium acetate of analytical grade and thin-layer plates (Silica-gel 60, 100×100 mm) were purchased from Merck (Darmstadt, Germany). Sephadex G25 and LH-20 was obtained from Pharmacia (Uppsala, Sweden). All other reagents were of analytical grade.

2.1.2. Milk and sample preparation

Milk samples from Spanish goats ($n = 10$), sheep ($n = 5$) and cows ($n = 5$) were donated by Puleva Biotech S.A. Samples of 500 mL were taken by hand-milking and distributed into 10 mL collection tubes. Mature human milk ($n = 3$) was kindly donated by volunteers. The samples were stored at $-80\,^{\circ}C$ until further analysis. Two millilitres of whole milk were thawed and defatted by centrifugation at $6500 \times g$ for 25 min at $4\,^{\circ}C$. The viscous, upper cream layer, consisting primarily of fats and other lipids, was discarded by careful pipetting from the lower aqueous layer, which was completely transferred to sterile tubes. Four millilitres of precooled ($4\,^{\circ}C$) ethanol were added to the sterile tubes to precipitate the protein fraction, and the sample was kept on ice for 2 h with constant stirring. The ethanol was extracted in a refrigerated vapour trap with vacuum to yield a solution (OS-1 solution) containing the carbohydrate fraction.

2.1.3. Purification of oligosaccharides by FPLC

The OS-1 solution was diluted to 1 mL with Milli-Q^{TM} water before purification. Lactose and salts were partially removed by applying the diluted OS-1 solution onto a Sephadex G-25 column (900×25 mm i.d.) connected to a fast protein liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) and eluted with Milli-Q^{TM} water at a flow rate of $1.0\,mL\,min^{-1}$ (Kunz, Rudloff, Hintelmann, Pohlentz, & Egge, 1996). The resulting fractions were then pooled and further concentrated by freeze-drying. The final powder containing milk oligosaccharides was dissolved in 0.5 mL of Milli-Q^{TM} water (OS-2 solution) and stored at $-20\,^{\circ}C$ for further analysis.