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Comprehensive peptidomic and glycomic evaluation reveals that sweet whey permeate from colostrum is a source of milk protein-derived peptides and oligosaccharides

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ABSTRACT

Whey permeate is a co-product obtained when cheese whey is passed through an ultrafiltration membrane to concentrate whey proteins. Whey proteins are retained by the membrane, whereas the low-molecular weight compounds such as lactose, salts, oligosaccharides and peptides pass through the membrane yielding whey permeate. Research shows that bovine milk from healthy cows contains hundreds of naturally occurring peptides – many of which are homologous with known antimicrobial and immunomodulatory peptides – and nearly 50 oligosaccharide compositions (not including structural isomers). As these endogenous peptides and oligosaccharides have low-molecular weight and whey permeate is currently an under-utilized product stream of the dairy industry, we hypothesized that whey permeate may serve as an inexpensive source of naturally occurring functional peptides and oligosaccharides. Laboratory fractionation of endogenous peptides and oligosaccharides from bovine colostrum sweet whey was expanded to pilot-scale. The membrane fractionation methodology used was similar to the methods commonly used industrially to produce whey protein concentrate and whey permeate. Pilot-scale fractionation was compared to laboratory-scale fractionation with regard to the identified peptides and oligosaccharide compositions. Results were interpreted on the basis of whether industrial whey permeate could eventually serve as a source of functional peptides and oligosaccharides. The majority (96%) of peptide sequences and the majority (96%) of oligosaccharide compositions found in the laboratory-scale process were mirrored in the pilot-scale process. Moreover, the pilot-scale process recovered an additional 33 peptides and 1 oligosaccharide not identified from the laboratory-scale extraction. Both laboratory- and pilot-scale processes yielded peptides deriving primarily from the protein β-casein. The similarity of the laboratory- and pilot-scale's resulting peptide and oligosaccharide profiles demonstrates that whey permeate can serve as an industrial-scale source of bovine milk peptides and oligosaccharides.

1. Introduction

Peptidomics and glycomics are recent technologies that are allowing food scientists to develop a detailed molecular-level understanding of functional compounds in food and food byproducts. Bovine milk contains fragments of intact milk proteins that are visible by gel electrophoresis and referred to as the proteose peptone fraction (Andrews, 1979; Andrews & Alichanidis, 1983; Eigel, 1981; Paquet, Alais, & Aubert, 1989). Mass spectrometry-based analysis has revealed that among this peptide fraction are hundreds of endogenous peptide sequences (Dallas, Guerrero, Khaldi, et al., 2013; Dallas, Guerrero, Parker, et al., 2013). These peptides are released by a select group of proteases that occur naturally in bovine milk, including plasmin (Andrews, 1983; Politis, 1996) and cathepsins B and D (Hinz, Larsen, Wellnitz, Bruckmaier, & Kelly, 2012; Larsen, Boisen, & Petersen, 1993) and elastase (Dallas, Underwood, Zivkovic, & German, 2012). Bovine milk also contains at least 49 oligosaccharides (polymers of several monosaccharides) by composition (shown in Supplementary Table 1), not including isomers; and 62 including isomers (Barile et al., 2010; Barile, Meyrand, Lebrilla, & German, 2011; Barile et al., 2009; Mariño et al., 2011; Parkkinen & Finne, 1987; Saito, Itoh, & Adachi, 1984, 1987; Schneir & Rafelson, 1966; Sundekilde et al., 2012; Tao et al.,

Abbreviations: ACN, acetonitrile; TFA, trifluoroacetic acid; FA, formic acid; Q-TOF, quadrupole time-of-flight.

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2008; Urashima, Saito, Ohmisya, & Shimazaki, 1991; Urashima, Taufik, Fukuda, & Asakuma, 2013; Veh et al., 1981).

In cheese making, the liquid remaining after rennet-based casein precipitation is called "sweet whey." In the past, whey was treated as a waste product. Now, whey is recognized as a source of potentially valuable compounds. As a result of scientific and technological innovations, whey now serves as an economically important source of functional ingredients for value-added foods. The major products derived from whey are whey protein concentrate and whey protein isolate (Ramchandran & Vasiljevic, 2013). Membrane technology is increasingly deployed as a non-destructive technique for isolation of whey proteins (Marcelo & Rizvi, 2008; Tunick, 2008).

Membrane filtration of whey produces a protein-rich retentate (concentrated whey proteins) and a permeate. Whey permeate is still considered a waste product by the dairy industry and the economic value of this stream remains low (Barile et al., 2009). As the production of whey protein concentrate typically employs 10 kDa membranes (Marcelo & Rizvi, 2008), it was hypothesized that oligosaccharides and protein fragments (peptides) that are smaller than 10 kDa pass through the membrane into the whey permeate phase.

In this research, sweet whey from bovine colostrum was processed by membrane filtration on a pilot-scale as a model for industrial whey permeate products. The permeate was analyzed for peptides and oligosaccharides by nano-liquid chromatography tandem mass spectrometry and database searching. Peptides and oligosaccharides present in this permeate were compared to those present from the laboratory-scale filtration of the same bovine colostrum whey. Furthermore, peptides were assessed for which enzymes were most active in the bovine colostrum sample. This work was conducted to determine whether milk's naturally occurring peptides and oligosaccharides exist within whey permeate, and, thus, whether this whey permeate can serve as an industrial-scale source for these compounds.

2. Materials and methods

2.1. Whey from bovine colostrum

Bovine colostrum whey was a gift from Sterling Technology (Brookings, South Dakota, USA). Whey was produced by lipid removal via cream separators and followed by High Temperature Short Time pasteurization and rennet-based casein precipitation. To eliminate lactose contamination of the oligosaccharide fraction, lactose was hydrolyzed by the addition of 0.1% (wt/wt) fungal lactase and incubated for 30 min at 45 °C with constant stirring.

2.2. Sample preparation

An overview of the methodology employed to produce whey permeate from colostrum whey at the laboratory- and pilot-scale is presented in Fig. 1.

2.2.1. Laboratory-scale production of peptides from whey permeate

For comparison to the pilot-scale whey permeate sample, the colostrum whey (after lactose hydrolysis) was processed in the laboratory with 30 kDa molecular weight cut-off centrifugal filter devices (Amicon Ultra-15 Centrifugal Devices, Millipore, Cork, Ireland) to create a laboratory-scale whey permeate. The membranes in each centrifugal device were made of Ultracel low-binding regenerated cellulose and had an active membrane area of 7.6 cm^2. One milliliter of the original whey was split into 4 aliquots of 0.25 mL each. Each aliquot was mixed with 8.75 mL water (18-fold dilution) and centrifuged in a swinging bucket rotor at 4000 ×g, 20 min at 4 °C. After centrifugation, the permeate was collected, 9 mL of nanopure water was added to the retentate, and the sample was mixed using a vortex mixer and centrifuged again with the same conditions. This procedure was repeated

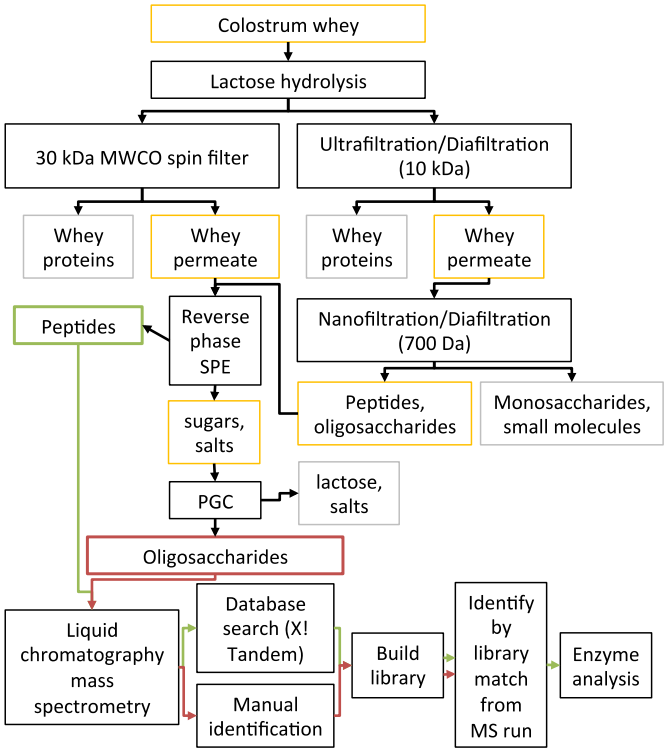

Fig. 1. Schematic representation of laboratory- (left) and pilot-scale (right) production of whey permeate, isolation of peptides and oligosaccharides and compound identification and analysis.

for a total of 5 wash steps. The combined permeate solution was dried to 10 mL by centrifugal evaporation at 37 °C.

2.2.2. Pilot-scale production of peptides from whey permeate

The whey was subjected to a two-stage filtration using a tangential-flow membrane system at pilot-plant scale (Model L, GEA Filtration, Hudson, WI, USA). The system was composed of a 2.5-in. diameter spiral membrane housing, a 95 L jacketed stainless steel reactor, a flow-meter, a heat exchanger and a feed pump (7.5 Hp). Ninety five liters of bovine colostrum whey were ultrafiltered as a single batch with a 10 kDa molecular weight cut-off polyethersulfone spiral-wound membrane with an effective area of 1.86 m^2. The whey was concentrated to a concentration factor of 5.3 (concentration factor = volume of feed/volume of retentate). This ultrafiltration and whey concentration produced a protein-rich retentate and a sugar- and peptide-rich permeate. Ultrafiltration was performed at 40–43 °C, with a transmembrane pressure of 3.0 bar and a recirculation flow rate of 10 L/min. The resulting permeate was nanofiltered using a 500–700 Da molecular weight cut-off sulfonated polyethersulfone spiral-wound membrane with an effective area of 1.86 m^2. The nanofiltration retentate was concentrated to a concentration factor of 8.5 to produce a peptide- and oligosaccharide-rich retentate and a monosaccharide- and salt-rich permeate. Nanofiltration was performed at 45–50 °C, with a transmembrane pressure of 20 bar and a recirculation flow rate of 9.5 L/min. Four diafiltration steps were performed to improve monosaccharide removal from the nanofiltration retentate. All membranes were manufactured by Hydranautics (Oceanside, CA, USA). The peptide- and oligosaccharide-rich retentate from the nanofiltration step was used. After this membrane filtration fractionation, the final whey permeate samples were immediately iced, transferred to the laboratory and stored at −30 °C. Prior to peptide extraction, 100 mL of whey permeate was centrifuged at 4000 ×g for 10 min at 4 °C. Precipitates from remaining salts were removed and the supernatant was collected. The supernatants were centrifuged again with the same parameters and the second