Pilot scale isolation of bioactive oligosaccharides from whey permeate

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Background

The intestinal microbiota is a recent target for research as it is an important health factor. A dysbiotic gut and chronic diarrhea are leading causes of infant mortality worldwide[1]. **Milk oligosaccharides are bioactive carbohydrates with prebiotic, anti-pathogen adhesion, immunomodulatory, and cognitive development roles in infants**. Human milk is an excellent source of these protective carbohydrates but is limited in availability for commercial use; therefore, our group is investigating dairy streams such as whey permeate (see figure 1 below) as a source of milk oligosaccharides[2]. **There is a high degree of similarity between oligosaccharides found in whey permeate and human milk**. The primary oligosaccharides in whey permeate are 6′ sialyllactosamine (6′-SLN), 6′-sialyllactose (6′-SL), and 3′-sialyllactose (3′-SL). Major challenges in isolating bovine milk oligosaccharides (BMO) from whey permeate include the low BMO concentration (<0.5 g/L), the very dilute nature of whey permeate (>95% water) and the high concentration of lactose (>85% on a solids basis), which yields a high biochemical oxygen demand (BOD, ~50,000 ppm), and is therefore a potentially strong, albeit non-toxic environmental pollutant[3,4]. Carbohydrates other than oligosaccharides in whey permeate such as lactose and monosaccharides will confound the *in vitro* bioactivity of BMO. Efforts to **isolate lactose- and monosaccharide-free BMO using membrane filtration** have previously been unsuccessful[5,6].

Objective

To isolate pure BMO from whey permeate, three techniques will be utilized: an enzymatic treatment, membrane filtration, and combined fermentation and membrane filtration, all completed at pilot scale. The commercial enzyme β-galactosidase (lactase, used to make lactose-free milk) will be used to examine if hydrolyzing lactose into its constituent monosaccharides glucose and galactose facilitates BMO isolation from whey permeate. Next, efforts to change the selectivity of the membrane to retain oligosaccharides and eliminate monosaccharides using pH and process pressure will be investigated, while an alternative strategy will use the yeast *Saccharomyces cerevisiae* to deplete the monosaccharide content through fermentation. By using sustainable and scalable technologies in the UC Davis Milk Processing Laboratory, such as membrane filtration at the pilot scale, we hope to provide a framework for industrial commercialization while simultaneously isolating sufficient quantities of BMO for *in vitro*, *in vivo*, and clinical trials to determine bioactivity.

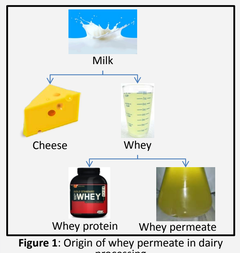


Figure 1: Origin of whey permeate in dairy processing

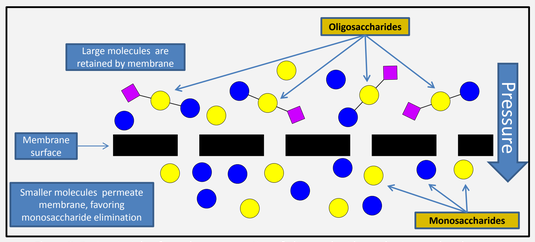


Figure 2: Basic principle of membrane separation of oligosaccharides and monosaccharides

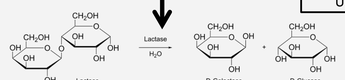
Methods

Whey permeate is a production stream from the dairy industry, derived from whey protein isolation using membrane filtration

Whey permeate 150-250 L

UC Davis Milk Processing Laboratory

Hydrolyzing lactose into glucose and galactose using the commercial enzyme β-galactosidase lowers the molecular weight (from 342 Da to 180 Da) and makes the sugars easily fermentable



Lactose hydrolysis

Nanofiltration and diafiltration

pH adjustment with NaOH or citric acid

Nanofiltration: concentrate oligosaccharides and eliminate monosaccharides to improve purity

Diafiltration: improves purity of oligosaccharides by "rinsing" out monosaccharides with water for a buffer exchange

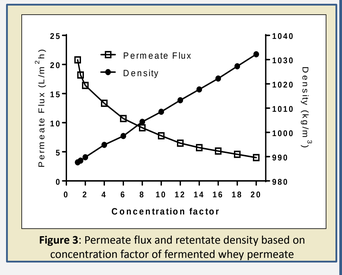
Membrane filtration: Molecular size-based separation

Fermentation and nanofiltration

Fermentation with *Saccharomyces cerevisiae*

Microfiltration: remove yeast cells and colloidal particles

Nanofiltration: concentrate oligosaccharides while permeating metabolites and minerals



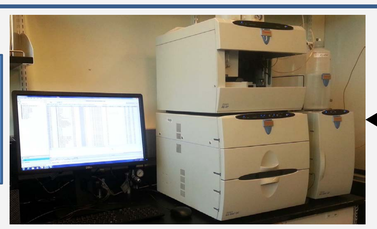
Freeze dry BMO to form usable powder

Purified bovine milk oligosaccharides (BMO)

Figure 3: Permeate flux and retentate density based on concentration factor of fermented whey permeate

High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

Allows for accurate quantification of compounds for which pure standards are available



Instrumental analysis: characterize process material and effect of parameters

Nano Liquid Chromatography Chip Quadrupole Time of Flight Mass Spectrometry (Nano LC Chip Q-ToF)

Extremely sensitive detection and identification of all oligosaccharides to understand entire carbohydrate profile

Results

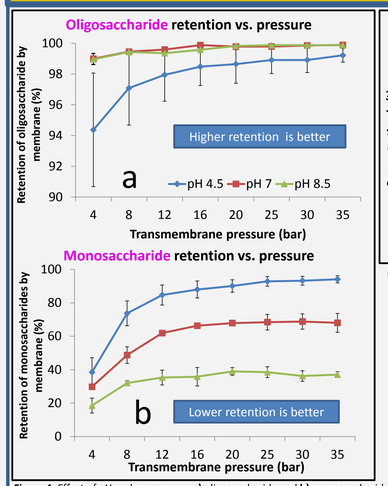


Figure 4: Effect of pH and pressure on **a)** oligosaccharide and **b)** monosaccharide retention during pilot scale nanofiltration

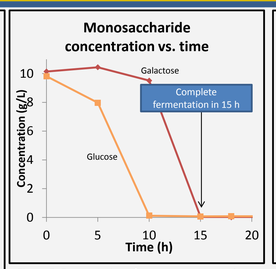


Figure 5: Fermentation of whey permeate at pilot scale (120L)

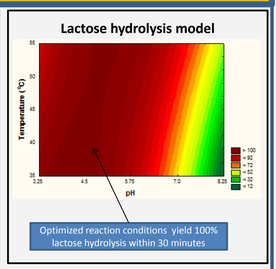


Figure 6: Response surface model of lactose hydrolysis as a function of reaction temperature and pH

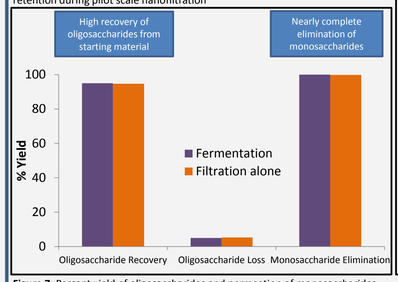


Figure 7: Percent yield of oligosaccharides and permeation of monosaccharides using two strategies to isolate oligosaccharides; fermentation and nanofiltration with diafiltration

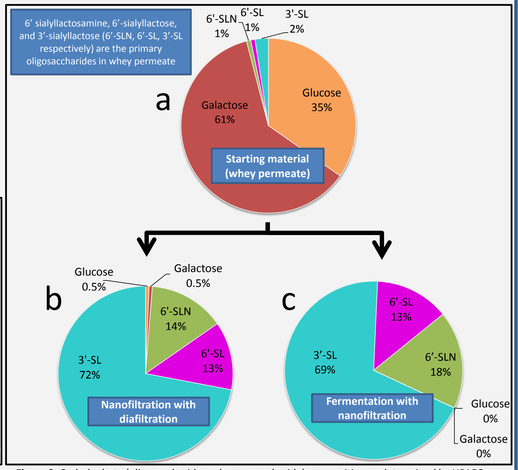


Figure 8: Carbohydrate (oligosaccharide and monosaccharide) composition as determined by HPAEC-PAD of **a)** starting whey permeate, **b)** nanofiltration with diafiltration final material, and **c)** fermentation with nanofiltration final material

Conclusions

- pH and operating pressure during filtration have profound effects on the selectivity of the nanofiltration membrane examined
- Both fermentation and nanofiltration, as well as nanofiltration combined with diafiltration were successful for isolation of bioactive oligosaccharides at pilot scale
- **High recovery yields (>95%) and high purity (<1% lactose/monosaccharides)** using sustainable technologies represent a promising approach for the dairy industry
- A pure BMO product will allow for functional testing in a variety of models, including *in vitro*, *in vivo*, and clinical trials

Implications

- Isolating oligosaccharides from a currently under-utilized food processing co-product creates a value added product for the dairy industry
- The yeast biomass could be used elsewhere in the food industry, and the monosaccharides recovered from filtration could be used as feedstock for alternative fermentations
- Reducing the amount of organic waste and pollutants generated from food processing is an important step towards a more sustainable global food industry
- **These functional oligosaccharides will improve infant nutrition worldwide**
- This research is building a wide network of collaborators across campus, the U.S., and the world

References

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