2001 MAFMA Final Report

Project Title **Development of an Enzymatic Process for Production of GOS from Whey Lactose**

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1. Objective Summary

The objectives of this project included: 1) optimizing enzyme immobilization on inexpensive cotton cloth as the support carrier, 2) to scale up the immobilized enzyme reactor for continuous GOS production from whey lactose, and 3) to evaluate nanofiltration and adsorption chromatography for GOS separation and purification.

2. Objective Accomplishments

We have developed a novel enzymatic process to produce galacto-oligosaccharides (GOS) from whey lactose and study the feasibility of using nanofiltration to separate and purify GOS. In this process, the enzyme β-galactosidase (lactase), commercially used to hydrolyze lactose in milk and whey, is used to produce oligosaccharides containing 2 to 5 galactose units and one glucose unit from lactose. These GOS have been found to efficiently and selectively accelerate the growth of *Bifidobacteria* in the lower intestine. These bacteria and GOS are known to have many beneficial effects on human health. In this project, we have optimized the immobilized enzyme reactor for GOS production from lactose. We have also evaluated the feasibility of several novel approaches to enhance and to control GOS formation from lactose. The reactor has also been evaluated for continuous production of GOS from lactose solution. The results to date show that the process has a very high productivity (100-fold higher than those reported in the literature) and is stable for long-term production. Scale up work on whey lactose as the feedstock has been delayed due to problems in shipping and storing large amounts of whey permeate on site. A pilot-scale process has been designed and will be evaluated at a cheese whey processing plant once a strategic partner has been identified in the near future. A nanofiltration process has also been studied for separating the GOS product from lactose and monosaccharides (glucose and galalctose). The results obtained from commercial nanofiltration membranes show good selectivity for GOS over monosaccharides and lactose. Further process evaluation and scale up study is underway. We have also evaluated chromatographic adsorption method for purifying GOS, and plan to develop a simulated moving bed process for this purpose.

3. Unexpected findings, if any

Methods to increase the galactosyl transferase activity and inhibit hydrolytic activity of the enzyme have also been studied with the goals of increasing GOS yield from current less than 40% (w/w) to more than 70% and reducing the product cost by ~50%. An integrated reaction and separation process that has a potential to further improve GOS production and reduce product costs is currently being evaluated. The product can be economically produced from the surplus whey permeate and lactose currently produced in the dairy industry, and used as a health-promoting food ingredient and dietary supplement.

4. Practical impacts of research efforts.

a. Short Term Impacts

Together with BIC, our industry partner, we have complete a market study and concluded that our technology has advantages over current technologies commercialized in Japan. One US patent application will be submitted in the near future.

b. Long Term Impacts

The product can be economically produced from the surplus whey permeate and lactose currently produced in the dairy industry, and used as a health-promoting food ingredient and dietary supplement. GOS are non-caloric and non-cariogenic sugars, and are classified as dietary fiber by FDA. They are currently used as a "health food" in Japan with an estimated annual sale of $200 million. In 1995, approximately 15,000 tons of GOS were produced worldwide, mainly in Western Europe and Japan. OS are already used in over 450 foods in Japan. The use of GOS in the US and North America is expected to grow rapidly with the growing US neutraceutical market as Americans are increasingly demanding “natural” foods with beneficial health effects. The initial applications for GOS in US would be as a health-promoting food ingredient and/or dietary supplement for adults. GOS can be included in infant formula to make the product more closely resemble mother’s milk, which should greatly benefit the health of bottle-fed infants. GOS also give similar benefits to animal health (cow, pigs, etc.) when they are included in animal feeds. Thus, the potential US market for GOS produced from whey lactose is estimated at hundred million to billion dollars per year. GOS at current market price of more than $10 per pound would be a high-value product for the dairy industry. However, the present use of GOS in foods is largely limited by the high production costs; these costs are attributed mainly to the high enzyme cost and low oligosaccharide yields (less than 30% w/w) associated with the present production technology. The new immobilized enzyme technology to be developed in this project would cut down the production costs by at least 50% due to increased reactor productivity and enzyme life. Coupled with a separation process, it would be possible to produce GOS from lactose with a high product yield (>50%) and high purity that is also appropriate for biomedical applications.

5. Publications resulting from this research

N. Albayrak and S.T. Yang. 2002. Immobilization of *Aspergillus oryzae* β–galacosidase on cotton cloth by tosyl chloride activation, *Enz. Micorb. Technol.*, 31:371-383.

N. Albayrak and S.T. Yang. 2002. Production of galacto-oligosaccharides from lactose by *Aspergillus oryzae* β-galacosidase immobilized on cotton cloth, *Biotechnol. Bioeng*., 77:8-19.

N. Albayrak and S.T. Yang. 2002. Immobilization of β-Galactosidase on Fibrous Matrix by Polyethylenimine for Production of Galacto-oligosaccharides from Lactose. *Biotechnol. Progress*, 18:240-251.

J.I. Sanz-Valero, I.C. Tang and S.T. Yang. 2002. Production of Galacto-oligosaccharides from Whey Lactose by Immobilized Enzyme Reaction and Nanofiltration, AIChE Annual meeting, Indianapolis, IN, November 3 – 8.

J. I. Sanz Valero and S.T. Yang. 2003. Production of galacto-oligosaccharides (GOS) from lactose by *Kluveromyces lactis* β–galacosidase, in preparation.

J.I. Sanz-Valero, I.C. Tang and S.T. Yang. 2003. Production of Galacto-oligosaccharides from Whey Lactose by Immobilized Enzyme Reaction and Chromatographic Separation, AIChE Annual meeting, San Francisco, CA, Nov. 16-21.

S. Pruksasri and S.T. Yang. 2003. Simultaneous Production and Separation of Galacto-Oligosaccharides from Lactose by -galactosidase Immobilized on Nanofiltration Membrane, AIChE Annual meeting, San Francisco, CA, Nov. 16-21.