Investigate polymerization of lactose by twin screw extrusion

September 2012

By:
Tonya Schoenfuss
University of Minnesota

Andrea Tremaine, graduate student

Funded in part by:
Midwest Dairy Association

Abstract

Current methods of oligosaccharide production include extraction, chemical synthesis and enzymatic synthesis which are often inefficient and result in poor yields. This project proposed using twin-screw extrusion of lactose to produce oligosaccharides. The effect of formula and processing conditions of a lactose and citric acid blend on the obtained sugar profiles and soluble fiber content were evaluated. The citric acid concentration and added glucose were varied. Conversion of up to 55% of the starting lactose to soluble fiber was achieved. It is envisioned that this research will lead to future funding to further characterize the extrudates and evaluate product purification processes.

Background Information

Research has revealed the prebiotic nature of many oligosaccharides (Barreteau *et al*, 2006), along with the functional properties and health benefits associated with oligosaccharide use and consumption. The most well-known health benefits include an improvement in immune system functioning (Mussatto and Mancilha, 2007), and creation of a bifidogenic effect (Playne and Crittenden, 2004; Taniguchi, 2005). These health effects have most notably been observed when milk formula has been supplemented with oligosaccharides in an attempt to mimic the composition of breast milk (Fanaro *et al*, 2005). The addition of oligosaccharides in foods is becoming more prevalent due to the GRAS status of many oligosaccharides. The leading oligosaccharides on the world market include fructo-oligosaccharides and galacto-oligosaccharides (Barreteau *et al*, 2006). In 2009, an estimated 6,255 metric tons of prebiotics were used in the United States alone (Frost and Sullivan, 2009). By 2014, the United States prebiotic market is expected to grow to 14,050.1 metric tons, with galacto-oligosaccharides (GOS) and other prebiotics expected to account for a larger share of the market. In addition to the supplementation of GOS and oligosaccharides in infant formulas, functional beverages, supplements (Frost and Sullivan, 2009), confectionary products, bakery products and yogurt can also serve as vehicles for oligosaccharides (Rudolfová and Curda, 2005). Future forecasts for the use of prebiotics such as GOS and other oligosaccharides illustrate the need to ensure that an economical oligosaccharide production process is in place in order to meet the future demand.

Currently, oligosaccharides can be produced by extraction, as well as by chemical and enzymatic methods. The current means of production, however, are associated with many problems. Problems associated with chemical syntheses include the corrosion of the reactor, the requirement to remove chemicals after syntheses, and the small output due to the batch process (Hwang *et al*, 1997). Problems with the enzymatic method can be illustrated by considering GOS synthesis. GOS are produced after the reaction between a concentrated solution of lactose and β-galactosidase via a transgalactosylation reaction (Barreteau *et al*, 2006; Gosling *et al*, 2010; Hellerová and Curda, 2009; Rastall, 2010; Rudolfová and Curda, 2005; Playne and Crittenden, 2004; Pandya and Haenlein, 2009; Gibson *et al*, 2004). Problems include long reaction times, enzyme inactivation, specific bioengineering control, difficulties in enzyme reuse and low efficiency (Hwang *et al*, 1998). Yields often do not exceed 50 percent, and more commonly are between 30 and 40 percent (Gosling *et al*, 2010). The low yield is a result of

multiple factors, which include the reaction time, initial lactose concentration, water activity, temperature and competitive and uncompetitive inhibition (Gosling *et al*, 2010). Only with further processing is it feasible to increase the percentage of oligosaccharides in the product. Enzyme characteristics including the source of the enzyme, the concentration, and pH and temperature optimums also affect GOS yield (Hellerová and Curda, 2009; Martínez-Villaluenga *et al*, 2008; Gosling *et al*, 2010). These problems are common to many enzymatic oligosaccharide syntheses.

Due to the difficulty of producing oligosaccharides by existing methods, new methods are being developed such as the use of new enzyme variants by site-directed and random mutagenesis (Monsan and Auriol, 2004), metabolic engineering (Rastall and Gibson, 2002), reverse enzyme technology, and by controlled polysaccharide hydrolysis and equilibrium synthesis of oligosaccharides. One promising method is the production of oligosaccharides by extrusion. It has already been established that sugars can be polymerized in the presence of heat and acid (Manley-Harris and Richards, 1993). Korean researchers, Hwang *et al*, have published two scientific papers on the extrusion of glucose as well as lactose to produce oligosaccharides. In their first paper, glucose syrup and lactose along with a catalytic amount of citric acid was extruded to produce gluco- and lacto-oligosaccharides (1997). The glucose and lactose were processed at 160, 180 and 200°C, which resulted in polymerization yields for the glucose syrup of 36.90%, 55.44% and 77.10%, respectively, and polymerization of lactose at 26.45%, 38.16% and 45.86%. The lactose may have had a lower polymerization yield due to the disaccharide structure of lactose, resulting in a less effective polymerization mechanism. Increasing the temperature led to an increase in viscosity and polymerization, and exposure to α-amylase and amyloglucosidase indicated the presence of random linkages rather than α-1,4 linkages. In their second paper, Hwang *et al*. extruded glucose with a catalytic amount of citric acid to produce gluco-oligosaccharides and polydextrose (1998). The temperature was varied similar to the first study, and resulted in similar results. The polymerization yields at 160, 180 and 200°C were 43.9%, 75.0% and 93.7%, respectively. A total of 63% GOS and 11.2% polydextrose were obtained at 180°C, while 30.7% GOS and 63.0% polydextrose was obtained at 200°C. These results outline the potential to control oligosaccharide yield during extrusion processing and offer a rapid, continuous and flexible manipulation of the composition during the production of oligosaccharides. Basic research into the effects of citric acid and temperature on the degree of polymerization and soluble fiber content needs to be conducted in order to reduce the cost and increase the efficiency of oligosaccharide production and improve the utilization of lactose.

The proposed study will be a 12-month project to evaluate the extruder conditions necessary to produce GOS and other oligosaccharides. Lactose will be extruded on a Buhler twin-screw extruder. A common screw configuration and rpm will be used, and the design parameters will include two levels of citric acid (1% and 2%) and three temperatures as determined by pre-trial experimentation. The resulting extruded product will be separated using chromatography according to the degree of polymerization (DP), and the DP fractions will be confirmed and further characterized by mass spectrometry as well as by determining the ratio of glucose and galactose. Other responses that will be examined include the color, soluble

fiber content as assessed by enzymatic stability, and the intrinsic viscosity. It is envisioned that this research will lead to future funding to further characterize the extrudates and evaluate product purification processes.

Objectives

Objective 1: **Determine the effect glucose concentration on the yield of soluble fiber of the extrudates**

Hypothesis 1: Seeding lactose with glucose will result in higher yield of soluble fiber.

This objective will be to investigate the effects of added glucose on yield, and the polymerization profile.

Objective 2: **Determine the effect of citric acid concentration on the sugar profile of the extrudates**

Hypothesis 2: Different concentrations of citric acid will affect the sugar profile of the extrudates

Because citric acid plays a catalytic role in the polymerization of sugars and production of soluble fiber. The hypothesis will be tested by varying the amount of citric acid added to the formula to change the pH during extrusion..

Materials and Methods

Materials

Refined edible fine grind lactose (Davisco Foods International, Inc., Eden Prairie, MN), Cerelose dextrose (Corn Products International, Newark, DE) and citric acid (Gadot Biochemical Industries Ltd., Haifa Bay, Israel) were used in all extrusion trials.

Pretrial Experiments to Determine Extrusion Conditions

Three pretrial extrusion runs took place in order to determine the extruder processing conditions (barrel temperatures, screw design, feed rate, screw speed), and to determine the rheological properties of the extruded product. Thermal energy alone could not melt the lactose during the residence time in our extruder, so mechanical energy also had to be utilized. Mechanical energy was added to the process by optimizing the screw design. Reverse elements were added to the screw shaft to increase the retention time, and allow for additional reaction time. Forward elements were added prior to many of the reverse elements in order to push the product forward, and to prevent the extruder from reaching maximum torque. Additionally, more mechanical energy was added towards the front end of the barrel, and this was accomplished by using larger elements, using kneading blocks (polypacks) and reverse elements in order to melt the dry feed as soon as possible upon introduction into the

extruder. Reverse elements were placed after the polypacks so that the lactose could be held in the melting region longer.

Experimental Design and Preparation of Raw Materials

The experimental design consisted of three glucose concentrations (0%, 10%, 20%) and two citric acid concentrations (1%, 2%), with the remainder of the formula consisting of lactose. All six formula combinations were processed in a single run, and the experiment was repeated on separate processing days. The order in which samples were collected was randomized for each set of product replicates.

The lactose, glucose and citric acid were mixed in 30 lb batches with a ribbon blender model IMS-1 (Bepex International LLC, Minneapolis, MN) in forward and reverse directions for 2 minutes each. The mix was processed on a Buhler 44mm co-rotating twin-screw extruder DNDL 44 (Bühler AG, Uzwil, Switzerland) with a L/D ratio of 28, which consisted of 4 barrel zones set at 230°C, 238°C, 238°C, and off from inlet to outlet. No die plate was used in the extrusion. The temperatures of the barrels were maintained by a heat transfer control system model H47212DT (Mokon, Buffalo, NY). The same screw configuration (Figure 1) was used throughout the experimental design. Dry feed was conveyed at 15 kg/h into the extruder with a K-Tron Soder K-ML-KT20 loss-in-weight feeder (K-Tron Ltd., Niederlenz, Switzerland). The screw speed was maintained at 250 rpm. The extruded product was collected in stainless steel trays after the die temperature and extruder shaft torque reached steady state, and then allowed to cool and solidify at room temperature. Process responses (die temperature, product temperature (measured with a probe after temperature zone 3) motor torque and specific mechanical energy) were collected at the beginning and end of sample collection for each treatment. The product was broken into small pieces and then placed in 1 L glass jars, and stored at room temperature until analyzed.

Chemical and Physical Analysis

Moisture content of the non-extruded and extruded samples was determined by vacuum oven. The samples were prepared for analysis by grinding with a spice grinder to a powder. 1-1.5 g of sample was weighed out in duplicate and placed in dried aluminum dishes and dried to constant weight at 100°C and 0.683 m mercury for 5 hours.

The pH of the raw mix formulations and the extrudates was measured on an Accumet AB15 pH meter (Thermo Fisher Scientific, Inc., Waltham, MA). The pH was measured after combining 10 mL reverse osmosis (RO) treated water with 15 g raw mix or crushed extrudate.

The color of crushed extrudates was determined using the method described by Wu et al. (2007). Extrudates were ground to a fine powder, the powder was placed in a glass Petri dish and the color was analyzed with a HunterLab D25 A Optical Sensor Colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). A total of 5 measurements were recorded, with rotating the Petri dish 90° after each reading.

To determine the degree of polymerization (DP) of the extrudates, test portions were prepared by grinding the extrudates to a fine powder with a spice grinder, and then dissolving 30 mg into 10 mL double distilled (DD) water. Prior to HPLC analysis, the sample was purified by solid phase extraction (SPE) using Strata-X SPE cartridges, 500 mg (Phenomenex, Torrance, CA). The cartridges were conditioned with 10 mL methanol followed by 10 mL DD water. 5 mL of sample was loaded into each cartridge, and then washed with 10 mL DD water. Samples were analyzed in duplicate. DP analysis was

performed on a HPLC (Beckman Coulter Inc., Fullerton, CA) consisting of a System Gold 508 auto sampler, System Gold 125 solvent module pump, a programmable C020 column heater (Torrey Pines Scientific, Carlsbad, CA), and an evaporative light scattering detector (ELSD-LTS, Shimadzu Corporation, Kyoto, Japan). All analyses were performed with a Phenomenex HPLC 0.5 μm porosity x 3.0 mm column in-line filter (Torrance, CA) coupled to a Rezex RSO-Oligosaccharide Ag^+ 4% column, 200 x 10 mm internal diameter (Phenomenex, Torrance, CA). A constant flow rate of 0.3 mL/min with isocratic flow consisting of DD water was used. 10 μL of sample and 5 μL of standard were injected each run with a 100 μL sample loop. The column temperature was maintained at 80°C, and the ELSD nebulizer was maintained at 40°C, and 250 kPa with nitrogen. The total run time was 60 minutes, and the chromatograms were monitored for 55 minutes by Clarity software version 3.0.06.589 (Data Apex Ltd., Czech Republic). Sample chromatograms were analyzed qualitatively, and compared to a column performance check standard oligosaccharide ladder (ALO-3038, Phenomenex, Phenomenex, Torrance, CA).

Total soluble dietary fiber, both high molecular weight and low molecular weight soluble dietary fiber, HMWSDF and LMWSDF, respectively, were determined using the integrated total dietary fiber assay procedure (Megazyme, Wicklow, Ireland). There were several modifications to the kit procedure. D-ribose (Sigma, St. Louis, MO) at a concentration of 10 mg/mL was used as the internal standard instead of D-sorbitol to enable greater resolution from other monosaccharides. After evaporating the deionized sample to dryness, it was reconstituted in 2.5 mL water instead of 2.0 mL. To prepare for HPLC analysis, the sample was then diluted by a factor of 10 with DD water. An oligosaccharide DP ladder (ALO-3038, Phenomenex, Phenomenex, Torrance, CA) was used to determine the demarcation point between DP 2 and DP 3 sugars. The same HPLC conditions were used in LMWSDF determination as in DP analysis, with the following exceptions. A full 100 μL loop of sample and standard was injected and the sugars were separated with a Transgenomics CHO-411 column (Omaha, NE). The response factor was determined as specified in the kit procedure; however, D-ribose (10 mg/mL) was used as an internal standard in 1000, 2000 and 3000 ppm glucose solutions. Standards were diluted 10 fold prior to injection, and a full 100 μL loop of sample was injected. LMWSDF was quantified by peak integration using Clarity software version 3.0.06.589 (Data Apex Ltd., Czech Republic). To correct for protein in HMWSDF, the Kjeldahl procedure was used to measure protein using 5 mL 95.0-98.0% sulfuric acid (Sigma-Aldrich, St. Louis, MO) and 1 Kjeldahl tablet (Merck KGaA, Darmstadt, Germany) added to sample residue. Digestion occurred in a block heater (Büchi Digest System K-437 and Büchi Distillation Unit K-350, Flawil, Switzerland) at 420°C for 90 minutes. After dilution with 50 mL of reverse osmosis (RO) water, samples were neutralized with sodium hydroxide and distilled into 4% boric acid (Mallinckrodt, Paris, KY). Distillate was titrated with 0.1 N hydrochloric acid (Alfa Aesar, Ward Hill, MA) to pH 5.2 (pH of a faint pink color with methyl red) with a DL22 food and beverage analyzer auto titrator (Mettler Toledo, Columbus, OH). The nitrogen conversion factor used in calculating the percent protein was 6.25.

The amount of lactose in the lactose powder and extruded samples was determined by the lactose and D-galactose assay procedure (Megazyme, Wicklow, Ireland), which hydrolyzes lactose with the enzyme β-galactosidase, followed by the mutarotation of α-D-galactose to β-D-galactose enzymatically. The β-D-galactose is oxidized to D-galactonic acid by NAD^+ and β-galactose dehydrogenase forming NADH, which can be measured spectrophotometrically. Samples were prepared for analysis by adding 0.19 g extruded sample, or 0.13 g of lactose to 250 mL volumetric flasks, and adjusting to volume with RO water. The pH was then adjusted to 8.6 with 1.0 M sodium hydroxide (Sigma-Aldrich, St. Louis, MO), and the absorbance was measured at 340 nm with a Beckman DU® 650 Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). The percentage of lactose remaining after extrusion was

determined by calculating the ratio of the % lactose in the extrudate divided by the % lactose in the raw mix, taking into account the purity of lactose, and multiplying by 100.

Statistical Analysis

Process and product responses were analyzed to assess the effects of the independent variables by conducting univariate analyses of variance (ANOVA) using SPSS version 17.0.2 (IBM SPSS, Chicago, IL). Based on the fitted ANOVAs, we presented pairwise comparisons using R version 2.12.0 adjusting for multiple testing using Tukey honestly significant difference (R Development Core Team, 2008). Pearson's correlation was calculated to estimate the correlation between responses (IBM SPSS, Chicago, IL).

Results and Discussion

Formula Effects on Process Measurements and Product Parameters

Glucose concentration had an effect on the specific mechanical energy (SME) and motor torque, but had no effect on the die temperature and product temperature (Table 1). The concentration of citric acid did not affect any of the process responses. The average values for SME and motor torque are shown in Table 2.

Heat transfer during extrusion processing is due to thermal energy and mechanical energy (Lei et al., 2008). SME represents a source of mechanical energy and is defined as the energy input transmitted to the material being extruded, and is produced as a result of the friction generated between the screw elements and the product (Ortiz et al., 2010; Schaer, 2010). A negative correlation existed between SME and the *L* value, with a correlation coefficient of -0.817 ($p= 0.001$), meaning that an increase in SME resulted in a decrease in the brightness of the extrudates. There was also a positive correlation between SME and the *a* value with a correlation coefficient of 0.783 ($p= 0.003$), so an increase in SME was associated with an increase in the redness of the extrudates. These color changes arose due to caramelization (discussed in detail later), and the correlations exist because the rate and extent of caramelization is dependent on heat transfer, so is therefore sensitive to changes in the SME.

The product responses observed in relation to motor torque were similar to SME. Torque is described as the effectiveness of a force to produce rotation (Ghebre-Sellassie and Martin, 2003). The torque required to rotate the screw is related to its speed, fill, as well as to the viscosity of the food material in the barrel (Mercier et al., 1989). The motor torque and SME were greatest during the processing of the 0% glucose formulas. This is most likely due to differences in viscosity upon exiting the extruder. Formulas with 0% glucose were described qualitatively as having a caramel-like consistency versus a honey consistency when glucose was added, upon exiting the extruder. Differences in the melting points of the individual sugars, and most importantly the effect of mixing different sugars likely accounted for the differences in the viscosity, and ultimately differences observed in motor torque. Krüger (2009) reported that the melting points of lactose monohydrate and dextrose monohydrate were greater than 200°C and 83°C, respectively. Regardless of the individual melting points, mixing two different sugars together may have had the greatest effect. Gillot (1904) examined the effect of mixing sugars on the melting point of several sugars. Overall, he found that the melting point of the two mixed sugars was never exactly the mean of the individual sugar's melting points. Small additions of one sugar into another resulted in a lower melting point, regardless of if the added sugar had a higher melting point. Ultimately, the differences in motor torque correlated with differences in extrudate color. A negative correlation existed between motor torque and the *L* value, with a correlation coefficient of -

0.813 (p= 0.001), or a decrease in brightness with increase in motor torque. A positive correlation also existed between motor torque and the *a* value, with a correlation coefficient of 0.810 (p= 0.001), or an increase in the redness with increase in motor torque. The increased levels of browning could have been due to the differences in product viscosity, and thus the formulations with the highest melting point (and viscosity), 0% glucose formulas, took a longer time to move from the inlet to the exit of the extruder, thereby having an extended heating time.

As shown in Table 1, glucose and citric acid concentration did not affect the die temperature or product temperature (as measured after the third temperature zone). There was, however, a positive correlation between product temperature and motor torque with a correlation coefficient of 0.611 (p= 0.035). The product temperature is the result of the mechanical energy and thermal energy inputs, and the amount of torque is indicative of the amount of energy absorbed by the material being extruded due to the shear generated from the screws (Fichtali and van de Voort, 1989; Lei et al., 2005). This higher energy affected the product color. The product temperature and the *L* and *a* values were correlated with correlation coefficients of -0.624 (p= 0.030) and 0.772 (p= 0.003), respectively, so an increase in the product temperature was associated with a decrease in extrudate brightness and increase in redness. There was also a correlation between the die temperature and the *b* value, which were positively correlated with a correlation coefficient of 0.709 (p= 0.010), so an increase in die temperature was associated with increase in yellowness.

Chemical and Physical Analysis

The moisture of the raw and extruded samples is shown in Table 2. The moisture content of the raw mix could affect the extent of polymerization, since polymerization and hydrolysis are two reactions that exist in equilibrium, and water that is present or produced during polymerization could ultimately result in the hydrolysis of polymers (Leuck, 1945, 1948; Fetzer et al., 1953). The moisture content of the raw mixes did affect the color of the extrudates, and was positively correlated with the *L* value with a correlation coefficient of 0.864 (p= 0.000), or increase in lightness with increase in moisture. Additionally, the moisture of the raw mixes and the *a* value were negatively correlated with a correlation coefficient of -0.770 (p=0.003), or decrease in sample redness with increase in moisture. Although the raw mix moisture was shown to affect the color properties of the extrudates, other factors such as the glucose itself could have also influenced color properties. The changes in the *L* and *a* values could be related to the effects of glucose addition, and how it can decrease the melting point of the sugars, thereby decreasing the viscosity of the material flowing through the extruder, and decreasing the amount of time exposed to heat in the extruder.

The extruded samples were lower in moisture than the raw mixes, suggesting that water was lost during processing and could have been lost as steam, or through decomposition, dehydration and/or polymerization reactions. The generation of black smoke as well as the melting of the sugar suggests that decomposition occurred. Lee et al. (2011) attributed the loss in crystalline structure (melting) in glucose, fructose and galactose to the kinetic process of thermal decomposition. The authors also mentioned that dehydration contributes to loss of crystallinity. Dehydration can occur in the heating period following sugar melting and is associated with caramelization (Shallenberger, 1993). The lower moisture of the extruded mix could also indicate that the process of condensation polymerization of the sugars occurred.

The pH of the extrudates was higher than that of the raw mixes, with a pH increase between 5.9 and 12.7%, independent of glucose concentration and citric acid concentration, suggesting that volatilization or decomposition of the citric acid occurred. It has been demonstrated that citric acid can

begin decomposition at 175°C, and the first decomposition results in the formation of aconitic acid, followed by the generation of carbonic anhyhdride, itaconic acid, acetone and carbonic acid with further heating (J.B. Lippincott & Co., 1877). As expected, extrudates with 2% citric acid had a lower pH than extrudates with 1% citric acid.

To indicate whether polymerization of the sugars occurred, color, the DP profile of the treatments by HPLC, analysis of dietary fiber, as well as the loss of lactose were evaluated.

Color and flavor are created through caramelization of sugars, and polymerization also occurs during the process. Jiang et al. (2008) studied the process of caramelization of fructose, glucose and sucrose and found that sugar degradation occurred in the early stages of caramelization followed by polymerization in the later stages. Glucose concentration affected the *L* value and the *a* value, while citric acid had no effect on the *L*, *a*, or *b* values (Table 3). Although there was a strong interaction between glucose concentration and the *a* value, there were no significant main effects of the predictors. Treatments did vary in the *L* values, and these average values are shown in Table 2. In general, there was a trend that lower citric acid concentrations were associated with darker extrudates (lower *L* values), while there was no clear trend with glucose concentration. Buera et al. (1987) studied the kinetics of caramelization in many sugar solutions that were adjusted to a water activity of 0.90, including glucose and lactose solutions. Browning rates were greater at pH 6 versus pH 5. In terms of the differences in reactivity rate of glucose versus lactose, both were assigned to the same kinetic model for caramelization, but the rate of color development of lactose was greater than the rate for glucose. Since the glucose concentration and citric acid had variable effects on the *L* value, it is likely that color could have been influenced by other factors, such as the extrusion residence time.

The DP as determined by HPLC revealed that profiles were similar between treatments and ranged from DP 1 to DP 15 or higher. A sample chromatogram is shown in Figure 2. Both polymerization products and a small quantity of lactose hydrolysis products were present in the extruded samples.

Similar to DP analysis, soluble dietary fiber analysis provided an indication of the extent of polymerization. Soluble dietary fiber was the combination of low molecular weight and high molecular weight fiber that remained in the filtrate after precipitation of the indigestible dietary fiber with ethanol. Any of the sugars in the filtrate with a DP greater than 2 were defined as soluble dietary fiber. Quantification of fiber revealed that 26-58% by weight of sample was low and high molecular weight soluble dietary fiber. Because of the high range of variability observed in the results, even amongst sample replicates, there were no differences in the amount of fiber between treatments. As mentioned previously, Hwang et al. (1997, 1998) polymerized glucose and lactose by extrusion and obtained polymerization up to 93.7% and 45.9%, respectively. Their polymerization yield for glucose was high though because anything larger than glucose was defined as polymerized (including disaccharides).

The assay for determining the percent of the original lactose remaining in the extrudates had less variability than the dietary fiber assay, and was thus able to indicate (indirectly) the effects of citric acid and glucose concentration on polymerization. As shown in Table 3, both glucose concentration and citric acid concentration affected the amount of the original lactose remaining after extrusion. Lactose was more efficiently converted in formulations containing 2% citric acid, with less of the original lactose remaining after extrusion (Table 2). Glucose concentration, on the other hand, did not exhibit a clear trend. Both 0% glucose/2% citric acid, and 20% glucose/2% citric acid extrudates had the least amount of lactose remaining after extrusion

Conclusions

Lactose formulas were efficiently polymerized to produce oligosaccharides by a continuous, twin-screw extrusion process. A higher citric acid concentration (2% versus 1%) resulted in an increase in polymerization, while glucose concentration had variable effects. Further evaluation of optimum acid catalyst levels, characterization of the soluble fiber for types of bonds and sugars present, an assessment of the prebiotic potential, and development of clean up and concentration procedures could lead to the production of a high-value soluble fiber ingredient from lactose.

References

Atra, R., G. Vatai, E. Bekassy-Molnar, and A. Balint. 2005. Investigation of ultra- and nanofiltration for utilization of whey protein and lactose. J Food Eng. 67:325-332.

Barreteau, H., C. Delattre, and P. Michaud. 2006. Production of oligosaccharides as promising new food additive generation. Food Technol. Biotechnology 44:323-333.

Buera, M. P., J. Chirife, S. L. Resnik, and R. D. Lozano. 1987. Nonenzymatic browning in liquid model systems of high water activity: Kinetics of color changes due to caramelization of various single sugars. J. Food Sci. 52:1059-1062.

Colonna, P., J. Tayeb, and C. Mercier. 1989. Extrusion cooking of starch and starchy products. Pages 247-320 in Extrusion Cooking. C. Mercier, P. Linko, and J. M. Harper ed. American Association of Cereal Chemists, Inc., St. Paul, MN.

Färber, E. inventor. 1936. Method of polymerizing monosaccharides. Anonymous 2027904.

Fanaro, S., Boehm, G., Garssen, J., Knol, J., Mosca, F., & Stahl, B. (2005). *Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: A review*. Oslo: Scandinavian University Press.

Fetzer, W. R., E. K. Crosby, C. E. Engel, and L. C. Kirst. 1953. Effect of acid and heat on dextrose and dextrose polymers. Ind. Eng. Chem. 45:1075-1083.

Fichtali, J., and F. R. van de Voort. 1989. Fundamental and practical aspects of twin screw extrusion. Cereal Foods World. 34:921-929.

Food Quality and Standards Service (AGNS) and Food and Agriculture Organization of the United Nations (FAO). 2007. FAO technical meeting on prebiotics.

Frost and Sullivan. 2009. U.S. digestive health ingredients markets.

Ghebre-Sellassie, I., and C. Martin. 2003. Pharmaceutical Extrusion Technology. Marcel Dekker, New York.

Gibson, G. R., Rabiu, B., Rycroft, C. E., & Rastall, R. A. (2004). Trans-galactooligosaccharides as prebiotics. In C. Shortt, & J. O'Brien (Eds.), *Handbook of functional dairy products* (pp. 91-108). Boca Raton: CRC Press.

Gillot, H. 1904. Properties of mixtures: melting points of some mixtures of sugars. Page 804 in Journal of the Chemical Society. Vol. 86 Part 2. Chemical Society, Royal Society of Chemistry, Great Britain.

Rastall, R. (2010). Functional oligosaccharides: Application and manufacture. *Annual Review of Food Science and Technology, 1*(1), 305-339.

Rudolfova, J., & Curda, L. (2005). Galactooligosaccharides as prebiotics and their production from lactose. *Chemické Listy, 99*(3), 168-174.

Schafer, R. 2010. Process variables & parameters. In Food Extrusion Workshop. Buhler, University of Minnesota.

Shallenberger, R. S. 1993. Taste Chemistry. 1st ed. Blackie Academic & Professional, London; New York.

Smithers, G. 2008. Whey and whey proteins - from 'gutter-to-gold'. Int. Dairy J. 18:695-704.

Taniguchi, H. (2005). Carbohydrate active enzymes for the production of oligosaccharides. In C. T. Hou (Ed.), *Handbook of industrial catalysts* (pp. 20-1). Boca Raton, FL: Taylor &

Francis/CRC.

Vrese, M., and J. Schrezenmeir. 2008. Probiotics, prebiotics, and synbiotics. Adv. Biochem. Eng. Biotechnol. 111:1-66.

Wu, W., H. Huff, and F. Hsieh. 2007. Processing and properties of extruded flaxseed-corn puff. J. Food Process. Preserv. 31:211-226.

TABLE 1.

THE EFFECT OF INDEPENDENT VARIABLES AND INTERACTION BETWEEN THE INDEPENDENT VARIABLES ON PROCESS RESPONSES

Variable	Process responses[1]			
	SME[2] (W/kg)	Motor torque (Nm)	Die temperature (°C)	Product temperature[3] (°C)
Glucose concentration	**[4]	**	NSD[5]	NSD
Citric acid concentration	NSD	NSD	NSD	NSD
Glucose concentration x citric acid concentration	NSD	NSD	NSD	NSD

[1]Number of observations for each process response, per treatment, per rep= 2

[2]Specific mechanical energy

[3]Product temperature measured at the end of heating zone 3

[4]Significant difference $p < 0.001$

[5]No significant difference

TABLE 2.

AVERAGE VALUES FOR EACH TREATMENT FOR SELECT RESPONSES

Glucose concentration (% by weight of dry mix)[1]	Citric acid concentration (% by weight of dry mix)	SME[2] (W/kg)	Motor torque (Nm)	Temperature (°C)		Moisture (%)[3]		Color			Original lactose remaining after extrusion (%)
				Product	Die	Raw mix	Extrudates	*L* value	*a* value	*b* value	
0	1	222[a]	126[a]	182.1[a]	165[a]	4.44[b]	0.91[a]	67.5[b]	2.7[a]	16.7[a]	50.7[bc]
0	2	205[a]	118[a]	179.5[a]	164[a]	4.55[ab]	1.19[a]	70.5[ab]	1.9[a]	17.2[a]	29.9[a]
10	1	113[b]	67[b]	179.6[a]	165[a]	5.18[ac]	0.91[a]	71.1[ab]	1.2[a]	15.9[a]	54.6[b]
10	2	107[b]	62[b]	179.5[a]	164[a]	5.20[ac]	1.14[a]	75.0[a]	1.2[a]	16.1[a]	35.8[ac]
20	1	89[b]	54[b]	179.0[a]	161[a]	5.52[c]	1.16[a]	77.1[a]	1.1[a]	16.1[a]	41.1[abc]
20	2	84[b]	54[b]	179.4[a]	165[a]	5.39[c]	1.23[a]	74.4[ab]	1.2[a]	16.1[a]	31.3[a]
SD between sample replicates[4]		6	4	0.5	0	0.12	0.03	0.2	0.2	0.3	2.9

[1]The remaining % of dry mix consisted of citric acid and lactose. Dry mix fed at 15 kg/h

[2]Specific mechanical energy

[3]Determined on a wet basis, g/100 g

[3]Number of observations for each response, per treatment, per rep: SME= 2; Motor torque= 2; Temperature= 2; Moisture= 2; Color= 5; % Original lactose remaining after extrusion= 2

[a-c]Means without a common superscript letter within the same column are significantly different ($p < 0.05$)

TABLE 3.

THE EFFECT OF THE INDEPENDENT VARIABLES AND INTERACTION BETWEEN THE INDEPENDENT VARIABLES ON PRODUCT RESPONSES

Variable	Product responses[1]				
			Color[2]		
	Soluble dietary fiber[3]	% Original lactose remaining after extrusion[4]	*L* value	*a* value	*b* value
Glucose concentration	NSD[5]	*[6]	*	*	NSD
Citric acid concentration	*	**[7]	NSD	NSD	NSD
Glucose concentration x citric acid concentration	NSD	NSD	NSD	NSD	NSD

[1]Number of observations for each product response, per treatment, per rep: Soluble dietary fiber= 1; % Original lactose remaining after extrusion= 2; Color= 5

[2]Color determined by measuring *L*, *a* and *b* values of crushed extrudates on a HunterLab colorimeter

[3]Measured with Megazyme integrated dietary fiber assay procedure and calculated as % by weight of un-extruded mix

[4]Measured with Megazyme lactose and D-galactose assay procedure and calculated as g/100 g of sample

[5]No significant difference

[6]Significant difference $p < 0.05$

[7]Significant difference $p < 0.001$

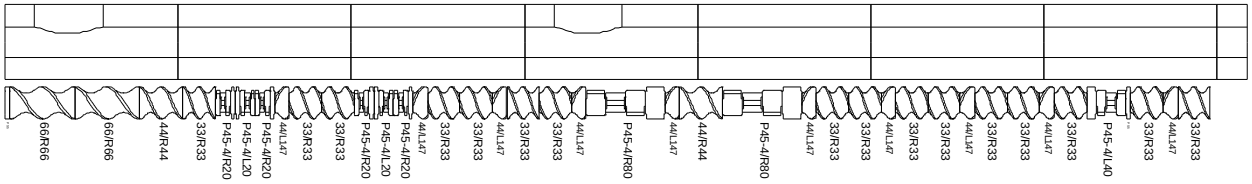

Figure 1. Screw design used on a Buhler 44mm co-rotating twin-screw extruder for the manufacture of lactose formulations containing varying concentrations of glucose and citric acid

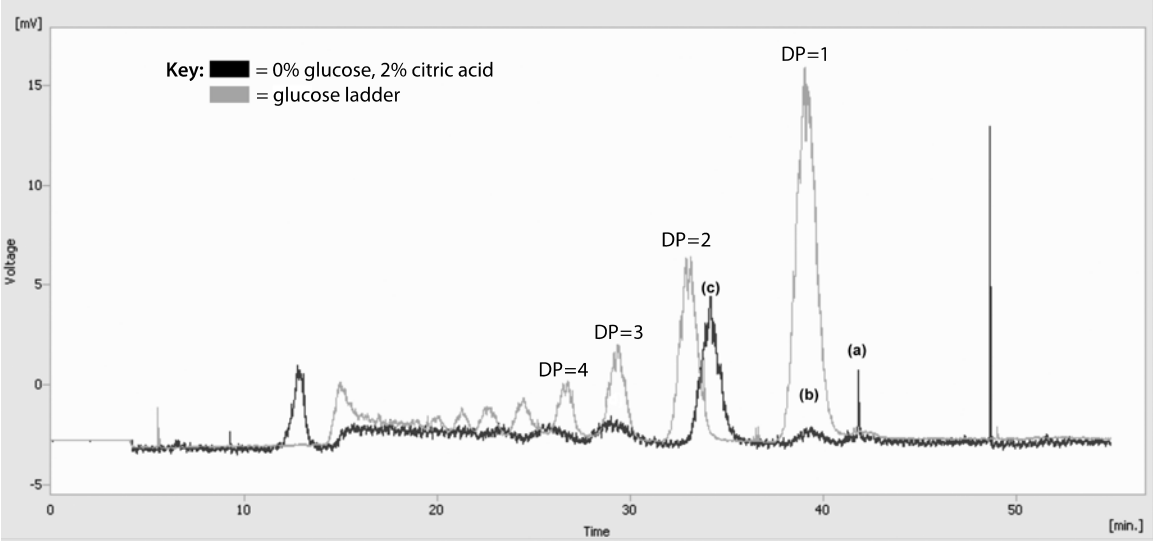

Figure 2. HPLC chromatogram of the degree of polymerization (DP) for extrudates with 0% glucose and 2% citric acid, overlaid with a glucose ladder. (a) galactose (b) glucose (c) lactose