Polymerization of lactose by twin-screw extrusion to produce indigestible oligosaccharides

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A B S T R A C T

Soluble dietary fiber is a growing food ingredient market. Twin-screw extrusion of lactose with an acid catalyst has been reported as a way to polymerize lactose to oligomers which are indigestible and analyze as dietary fiber. Lactose was dry blended with citric acid at two different concentrations, 1 and 2%. Glucose was added to raw mixes at 0, 10, or 20% (w/w). Samples with 2% citric acid yielded a higher concentration of indigestible oligosaccharides (52.3–99.8%, w/w) than 1% citric acid samples (37.1–49.9%). Glucose did not affect the yield. Glucose addition was beneficial and reduced the motor torque and specific mechanical energy of the extruder, and extruded products were lighter in color. The generated oligomers had a degree of polymerization that ranged from 3 to 5, as determined by mass spectrometry. Testing if the oligomers confer a beneficial effect (to be classified as dietary fiber), is still required.

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1. Introduction

Lactose is the major solid component in sweet whey produced as a result of renneted cheese making. It is currently used as an ingredient in foods and pharmaceuticals (Jelen, 2009), and to standardize skim milk powder to a desired protein content (Sikand, Tong, & Walker, 2010). Current value-added derivatives of lactose include galactose, lactulose, lactitol, lactobionic acid, lactic acid, galacto-oligosaccharides (GOS), lactosucrose and tagatose (Paterson & Kellam, 2009).

Food fibers are a rapidly growing ingredient market for formulating food and beverages world-wide, and market revenue in the USA alone was projected to grow from US$285 million in 2012 to US$512 million by 2017 (Reddy, 2012). Soluble dietary fibers are very popular in food formulations because of their ability to be incorporated with little effect on flavor and texture attributes, and because there have been studies demonstrating health benefits such as prebiotic potential (Barreto, Delattre, & Michaud, 2006; Góñi, Díaz-Rubio, & Saura-Calixto, 2009; Vrese & Schrezenmeir, 2008). The most common soluble food fiber ingredient sources are inulin, oligofructose and beta glucan (Reddy, 2012).

The classification of a material as dietary fiber varies around the world. The Codex Committee on Nutrition and Foods for Special Dietary Uses recommended that dietary fiber be defined to include carbohydrates with a degree of polymerization (DP) > 10, that are not hydrolyzed by enzymes in the small intestine of humans (Codex Alimentarius, 2008). They also commented that decisions to include carbohydrates with 3–9 DP should reside with national labeling authorities. Many authorities do include DP 3–9 and most agree with Codex that when a carbohydrate is derived from a raw food material by physical, enzymatic or chemical means, or has been synthetically produced, it should have a demonstrated physiological beneficial effect in humans (Codex Alimentarius, 2008; de Menezes, Giuntini, Dan, Sarda, & Lajolo, 2013).

Soluble fiber market projections illustrate an opportunity to develop methods of oligosaccharide production capable of meeting demand. Oligosaccharides can be extracted from natural sources (such as in the case of inulin) or produced enzymatically (i.e., GOS). Enzymatic synthesis of oligosaccharides is associated with production challenges that include long reaction times, yields that often do not exceed 50 percent by weight of the raw material, enzyme inactivation, and the inability to reuse the enzyme (Gosling, Stevens, Barber, Kentish, & Gras, 2010; Hwang, Kim, & Kim, 1998; Playne & Crittenden, 2004).

It has been demonstrated that sugars can be polymerized in the presence of heat and acid to form oligomers (Farber, 1936; Fetzer, Crosby, Engel, & Kirst, 1953; Leuck, 1943, 1948; Manley-Harris & Richards, 1993, 1994). Polydextrose is produced by polymerizing glucose or maltose with an acid catalyst and sugar alcohol to create branched oligomers that are used in food as bulking agents and considered soluble fiber. It is typically produced by a batch process under vacuum to minimize browning (Rennhard, 1973). Acid
catalyst concentration, the temperature and time of the reaction, and moisture affect polymerization when producing polydextrose (Rennhard, 1973).

Hwang, Kim, and Kim (1997) and Hwang et al. (1998) investigated the continuous polymerization of sugars to oligomers by twin-screw extrusion to produce polydextrose or lactose-oligomers. They reported that glucose and lactose were polymerized up to 93.7% and 45.9%, respectively, during extrusion processing at 200 °C with the addition of an acid catalyst (Hwang et al., 1997). The authors hypothesized that lactose was less polymerized due to its disaccharide structure. They also used a higher citric acid concentration for their glucose experiments, and had less glucose, so the molar ratios of acid to sugar were different between the two sugars.

Twin-screw extrusion provides for continues feeding of reactants through a heated high shear mixer that can create pressure and shear conditions that allow for reactions that are not as easily obtained at the same temperature during atmospheric mixing. The screw elements can be varied on the shafts to change the amount of shear on the product, the volume held in the extruder (by the use of elements that are flighted in the reverse of the product flow), and the rate at which a particle flows through (by changing the flow characteristics and volume filling the extruder). The temperature to which the material is exposed is varied by both the temperature applied to the barrel by heaters, and the shear applied to the material by the combination of the screw revolution rate, element configuration of the screw, and the viscosity of the material.

Because extrusion could provide a continuous way to produce oligosaccharides from lactose that could potentially be categorized as dietary fiber, we investigated the polymerization of lactose by extrusion. We hypothesized that by including glucose in the formula we could increase the amount of lactose polymerized because its molecular weight is lower, leading to more possibilities to form new glycosidic linkages on a weight basis. We also expected that the concentration of acid catalyst would affect the amount of lactose polymerized to oligomers. Our objectives, therefore, were to investigate the effect of varying concentrations of glucose (0, 10 or 20%, w/w, of the formula) and citric acid catalyst concentrations (1 or 2%, w/w, of the formula) on the yield of oligomers from lactose during twin-screw extrusion.

2. Material and methods

2.1. Materials

Refined edible fine grind lactose, >99% purity (Davisco Foods International, Inc., Eden Prairie, MN, USA), dextrose (Roquette Granulated XX Dri Sweet, Roquette America, Inc., Geneva, IL, USA) (more than 73% of material retained on a 0.5 mm screen and 47% retained on a 0.85 mm screen by the Ro-tap method (W. S. Tyler, Mentor, OH, USA)) and citric acid (Gadot Biochemical Industries Ltd., Haifa Bay, Israel) (greater than 97% retained on a 0.5 mm screen and 12% retained on a 0.85 mm screen by the Ro-tap method) were used in all extrusion trials.

2.2. Pretrial experiments to determine extrusion conditions

Three pretrial extrusion runs took place to determine the extruder processing conditions required to melt the lactose. The effect of granulation size of the lactose was evaluated by comparing the fine and regular grinds from the same supplier; we found that we were unable to run the regular grind alone with citric acid on our system due to the material physically locking in our funnel and auger feed system above a certain head pressure. We measured the particle size of both lactose ingredients on a Malvern Mastersizer laser light scattering instrument (Malvern Instruments, Inc., Westborough, MA, USA) in isopropyl alcohol and the mean of the regular grind was approximately 0.173 mm while the fine grind had two main distributions (0.044 and 0.0034 mm). All subsequent evaluations were with the fine grind. Barrel temperatures, screw design, feed rate and screw speed were varied on a Buhler 44 mm co-rotating twin-screw extruder DNDL 44 with a length to diameter ratio of 28 (Bühler AG, Uzwil, Switzerland). The rheological properties of the extruded product were measured by torque and specific mechanical energy (SME). SME was calculated by the automation software by measuring the amps drawn by the motor to calculate the energy per kg of product fed to the extruder. The screw design was optimized to melt the lactose as early as possible in the screw, which was difficult to do when no glucose was added. This was accomplished by using reverse elements and kneading blocks extensively in the earlier barrel zones but still including them throughout. Of the 39 elements, the final configuration had 29 reverse elements, 9 kneading blocks (some forward, some reverse) and 10 conveying elements.

2.3. Experimental design and preparation of raw materials

The experimental design consisted of three glucose concentrations (0, 10 or 20%, w/w, of the formula) and two citric acid concentrations (1 or 2%, w/w, of the formula), 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 a separate processing day. The order in which samples were run was randomized for each set of product replicates.

Lactose, glucose and citric acid were mixed in 13.6 kg batches with a ribbon blender model IMS-1 (Bepex International LLC, Minneapolis, MN, USA) in forward and reverse directions for 2 min each. The mix was processed on the extruder and the four barrel zones were set at 230 °C, 238 °C, 238 °C, and no heating from inlet to outlet, respectively. 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, USA). Dry feed was conveyed 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 and extruder shaft torque reached steady state, and then allowed to cool and solidify at room temperature. Process responses (product temperature (measured with a probe inserted into the flowing product in the last barrel (barrel 7) approximately 3.4 cm prior to the end of the extruder barrel), exit temperature (measured by a probe in the product at the point immediately prior to where a die plate would be installed), motor torque and SME) were collected at the beginning and end of sample collection for each treatment. Sample collection commenced when the product appearance and process responses had reached steady state. The product was ground into a fine powder with a coffee grinder and then stored at room temperature in glass jars until analyzed.

2.4. Chemical and physical analysis

2.4.1. Moisture content

Moisture content of the non-extruded and extruded samples was determined by the vacuum oven method (APD, 2002). Between 1 and 1.5 g of the samples were dried to constant weight at 100 °C and ~91 kPa for 5 h, in duplicate.

2.4.2. Color

The color of crushed extrudates was determined using the method described by Wu, Huff, and Hsieh (2007). Briefly, the
powdered extrudate 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, USA). A total of five measurements were recorded by rotating the Petri dish less than a quarter of a turn after each reading.

2.4.3. Lactose and glucose
The amount of lactose and glucose in the extruded samples was quantified by the lactose/glucose enzymatic assay procedure item number: K-LACSU 01/12 (Megazyme, Bray, Ireland) using a Beckman DU6500 Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA) for measuring absorbance as per the method.

2.4.4. Dietary fiber
The non-digestible oligomers were quantified by using the integrated total dietary fiber assay procedure (Megazyme), based on AOAC Method 2009.01, with minor modifications as follows. α-Ribose (Sigma, St. Louis, MO, USA) was utilized as the internal standard instead of α-sorbitol to enable greater resolution from other monosaccharides. An oligosaccharide (DP ladder (ALO-3038, Phenomenex, Torrance, CA, USA)) was used to determine the demarcation point between DP 2 and DP 3 sugars. All high performance liquid chromatography (HPLC) analyses were performed with a Transgenomics CHO-411 column (Omaha, NE, USA) with detection by low temperature evaporative light scattering (ELSD-LT, Shimadzu Corporation, Kyoto, Japan) instead of refractive index detection. The column temperature was 80 °C, the eluent was double distilled water and the flow rate was 0.3 mL min⁻¹. The ELSD nebulizer was maintained at 40 °C and had a nitrogen pressure of 250 kPa.

2.4.5. Degree of polymerization
Samples that were digested and cleaned up according to the integrated total dietary fiber method and had previously been analyzed by HPLC-ELSD were subsequently analyzed by HPLC-mass spectrometry (MS) with a Shimadzu LC-10AD chromatograph coupled to a Waters micromass ZQ (Waters Corporation, Milford, MA, USA) using Mass Lynx 4.1 software. The HPLC system also included a Shimadzu CTO-10A oven, SCL-10A system controller and Degassit 6324 degasser. The same column, oven temperature, eluent, and flow rate as for HPLC-ELSD analyses were used. The mass spectrometer was operated in positive electrospray ionization mode. Optimum ionization conditions were determined by tuning a solution of lactose in water and were as follows: source temperature 140 °C, desolvation temperature 400 °C, cone gas flow 100 L h⁻¹, desolvation gas flow 600 L h⁻¹, capillary voltage 4 kV, cone voltage 75 V, extractor voltage 2 V, RF lens voltage 0.2. Samples were analyzed using selective ion recording mode and a list of the following ions was monitored: m/z 203, 365, 527, 689, 851, 1013, 1175, 1337, 1499, corresponding to sodium adducts of hexose-derived oligosaccharides with DPs of 1–9.

2.5. 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, USA). 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).

3. Results and discussion
3.1. Formula effects on process measurements and product parameters
As documented in Table 1, the motor torque and SME were greatest during the processing of the 0% glucose formulae and were significantly different from the other samples; however, there was no statistical difference between the 10 and 20% glucose formulae (although values were numerically lower at 20% addition). Despite differences in motor torque and SME between formulae with and without glucose, the exit and product temperature did not differ among treatments. The concentration of citric acid did not affect any of the extruder process responses.

Heat transfer during extrusion processing is due to both thermal and mechanical energy (Lei, Fulcher, Ruan, & Lengerich, 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, de Carvalho, Ascheri, Ascheri, & de Andrade, 2010; Schaer, 2010). Torque is described as the effectiveness of a force to produce rotation (Ghebre-Sellassie & Martin, 2003) and is a direct measurement of the power consumed by the electric motor turning the extruder screws. The torque required to rotate the screw is related to its speed, material fill in the extruder, as well as to the viscosity of the food material in the barrel (Colonna, Tayeb, & Mercier, 1989).

The higher motor torques and SME of 0% glucose formulae indicate a higher viscosity of this formula compared with those with glucose, and possibly more material filling the extruder which would result in a longer residence time for this formula. There are several reasons why the addition of glucose could have contributed to changing the viscosity and flow characteristics in the extruder. These include the effect on the melting point of the combined ingredients because of the difference in colligative properties.

Table 1
Effect of extrusion starting formula on conditions during extrusion.¹

<table>
<thead>
<tr>
<th>Formula (% w/w)</th>
<th>Specific mechanical energy (W h kg⁻¹)</th>
<th>Motor torque (N m)</th>
<th>Product temperature (°C)</th>
<th>Exit temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Citric acid Lactose</td>
<td>125 ± 13A</td>
<td>182.1 ± 0.3A</td>
<td>165.3 ± 2.8A</td>
<td>0 1 99 222 ± 13A</td>
</tr>
<tr>
<td>0 2 98 205 ± 15A</td>
<td>0 2 98 205 ± 15A</td>
<td>0 2 98 205 ± 15A</td>
<td>0 2 98 205 ± 15A</td>
<td>0 2 98 205 ± 15A</td>
</tr>
<tr>
<td>10 1 89 112 ± 20A</td>
<td>67 ± 18 79.6 ± 1.7A</td>
<td>165.0 ± 2.5A</td>
<td>10 1 89 112 ± 20A</td>
<td>10 1 89 112 ± 20A</td>
</tr>
<tr>
<td>10 2 88 107 ± 10B</td>
<td>62 ± 16 79.5 ± 1.5A</td>
<td>164.9 ± 1.6A</td>
<td>10 2 88 107 ± 10B</td>
<td>10 2 88 107 ± 10B</td>
</tr>
<tr>
<td>20 1 79 89 ± 8A</td>
<td>54 ± 2A 179.0 ± 1.6A</td>
<td>161.3 ± 4.4A</td>
<td>20 1 79 89 ± 8A</td>
<td>20 1 79 89 ± 8A</td>
</tr>
<tr>
<td>20 2 78 84 ± 22A</td>
<td>54 ± 2A 179.4 ± 1.5A</td>
<td>164.5 ± 2.9A</td>
<td>20 2 78 84 ± 22A</td>
<td>20 2 78 84 ± 22A</td>
</tr>
</tbody>
</table>

¹ Specific mechanical energy was calculated by the automation software by measuring the amps drawn by the motor to calculate the energy per kg of product fed to the extruder. Product temperature was measured 3.4 cm from end of extruder barrel with a probe inserted into product flow; exit temperature was measured at exit point of molten product, immediately prior to die plate installation location; with probe inserted into product flow. Values are means ± standard deviation; means without a common superscript letter within the same column are significantly different (p < 0.05; 95% confidence interval using a T-distribution).
deliquescence, the moisture brought in by the glucose, the viscosity differences between mono and disaccharides in solution, and the particle size differences between lactose and glucose.

The melting point of crystalline lactose monohydrate is reported variously from 160 to 214 °C depending on the study (Anonymous, 2006; Raemy, Hurrel, & Loeliger, 1983; Raemy & Schweizer, 1983; Roos, 1993), while glucose has been reported to have a melting point varying from 130 to 176 °C (Lee, Thomas, & Schmidt, 2011). Our commercial sugar ingredients could also have contained some amorphous sugar. These would then start to become rubbery and flow above their glass transition temperature (Tg). The onset Tg for α-lactose monohydrate and d-glucose has been reported to be 101 °C and 31 °C, respectively (Roos, 1993). Additional factors that complicate understanding the melting temperature of a commercial crystallized sugar ingredient include the presence of impurities, the water content, and other solvents present (Roos et al., 2012, 2013). Known impurities of edible lactose that are reduced in the production of pharmaceutical grade lactose include riboflavin, protein, lactose phosphate and lactic acid (Paterson, 2009). Water content similarly affects Tg and it is reduced with increasing water content due to plastization (Roos, 1993). The citric acid we added as a catalyst would be considered an impurity in terms of how it affected the melting temperature. The prior handling of crystalline sugars can also affect crystal integrity, which affects the temperature at which one would start to see flow in the material. The mechanical stress induced by grinding crystalline lactose has been reported as a way to destroy crystallinity to produce a non-crystalline solid (Otsuka, Ohtani, Kaneniwa, & Higuchi, 1991; Otsuka, Ohtani, Otsuka, & Kaneniwa, 1993). Our lactose used in this study was milled after manufacturing to produce its fine particle size. We did not measure the crystallinity prior to extrusion, however.

Having glucose with lactose, with the lower melting temperature of glucose, could lead to a reduction in the melting temperature of lactose. One reason this would occur is due to having a material in a liquid state surrounding one in a crystalline state, acting as a solvent (Roos et al., 2012, 2013). Additionally, the decomposition of a sugar occurs near its melting point, and in our case, we also added an acid catalyst to polymerize the sugars. Since polymerization is a condensation reaction, water is released. Water reduces the melting point of crystalline sugars (Roos et al., 2013). Water released from decomposing glucose or anhydrous lactose would increase the dissolution of the remaining crystalline material by reducing its melting point. The glucose ingredient also had a higher moisture content (Table 2) and this in and of itself would affect the melting point of lactose. But additionally, simply combing three materials that deliquesce (citric acid, lactose and glucose) and having them in intimate contact, reduces the relative humidity and temperature of deliquescence. Deliquescence is a phase transformation of crystalline materials where crystalline materials absorb water at their surface, leading to the dissolution of the crystals to a liquid state (Kwok, Maurer, & Taylor, 2010; Maurer & Taylor, 2010; Salameh, Maurer, & Taylor, 2005). As the materials deliquesce, there is more bulk-phase water as the water associated with the crystal is released and this leads to increased solubilization of other crystalline material. In the study reported by Salameh et al. (2005), the additive effect of combining deliquescing compounds was reported. The researchers evaluated individual sugars alone, with citric acid, and in combinations of up to three sugars and acid. The relative humidity of deliquescence of the combined formulae were always lower as more deliquescing compounds were added. So flow (evident by reduced viscosity) could have started at a lower temperature in our lactose, glucose, citric acid blends than the lactose, citric acid formula alone. Additionally, the lactose, citric acid formula should have solubilized and started to flow at temperatures lower than that required to melt pure lactose, because of deliquescence.

Another reason for the difference in viscosity we observed during extrusion when glucose was added is because the viscosity of sugar solutions is directly related to the molecular weight of the sugars (Chirife & Buera, 1997). So the addition of a monosaccharide with the concurrent reduction of the disaccharide present would reduce the viscosity after both sugars were in a liquid state.

The effect of the particle size of seed materials has also been evaluated for its effect on viscosity as measured by extruder motor torque. Garber, Hsieh, and Huff (1997) found that when the particle size of cereal grain materials increased above approximately 1 mm, the torque in the extruder decreased. By contrast, Onwulata and Konstancé (2006) found that smaller particle size corn starch fractions caused higher viscosity. Karunanithy and Muthukumarappan (2011) found the results varied depending on the material they analyzed and the rps they ran their extruder at. However, because we do not have a material with starch, protein and fiber components which will absorb water and increase viscosity as they are developed or gelatinized, as is the case in these aforementioned studies, it is unknown if the reduction we saw on torque and SME when glucose was added is related to its larger particle size than the lactose used.

In typical extrusion processes, both the product temperature and motor torque are dependent upon the shear applied by the screw, moisture content, flow rate, and barrel temperature profile in the extruder (Fichtall & van de Voort, 1989; Lei, Ruan, Fulcher, & Lengerich, 2005). The lack of differences in both product and exit temperatures lower than that required to melt pure lactose, because of deliquescence.

### Table 2

<table>
<thead>
<tr>
<th>Formula (%, w/w)</th>
<th>Color</th>
<th>a Value</th>
<th>b Value</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Citric acid Lactose</td>
<td>L value</td>
<td>Raw mix</td>
<td>Extrudate</td>
<td>Raw mix</td>
</tr>
<tr>
<td>0 1</td>
<td>99</td>
<td>67.46 ± 0.30A</td>
<td>2.70 ± 0.17A</td>
<td>16.65 ± 0.46A</td>
</tr>
<tr>
<td>0 2</td>
<td>98</td>
<td>70.48 ± 0.12A</td>
<td>1.90 ± 0.13A</td>
<td>17.15 ± 0.61A</td>
</tr>
<tr>
<td>10 1</td>
<td>89</td>
<td>71.12 ± 0.72A</td>
<td>1.15 ± 0.51A</td>
<td>15.87 ± 0.72A</td>
</tr>
<tr>
<td>10 2</td>
<td>88</td>
<td>75.02 ± 1.06A</td>
<td>1.15 ± 0.29A</td>
<td>16.07 ± 0.71A</td>
</tr>
<tr>
<td>20 1</td>
<td>79</td>
<td>77.06 ± 1.02A</td>
<td>1.08 ± 0.25A</td>
<td>16.07 ± 0.95A</td>
</tr>
<tr>
<td>20 2</td>
<td>78</td>
<td>74.33 ± 1.41A</td>
<td>1.23 ± 0.24A</td>
<td>16.12 ± 0.82A</td>
</tr>
</tbody>
</table>

A Values are means ± standard deviation (n = 10 for color values; n = 4 for % moisture); means without a common superscript letter within the same column are significantly different (p < 0.05; 95% confidence interval using a t-distribution).

B Measured by vacuum oven at 100 °C.
“product” to “exit” temperature. These monitoring locations are the typical points monitored when extruding expanded products. With a die plate in place, both the temperature and pressure prior to the exit through the die are correlated with the expansion properties achieved in the material and used to evaluate changes in the process. For our purposes, however, it was not the ideal location to monitor product temperature when no die plate is used. Overall, the higher height and torque indicate more energy was needed to run formulæ without glucose, and the addition of glucose improved the run-ability of formulæ. Reduced torque and SME could allow for increased feed rates with the combined sugar formulæ over lactose only formulæ.

3.2. Color analysis

The extruded samples were all of light reddish-brown color, and the measured Hunter L, a, and b values are shown in Table 2. While b values were not significantly different between treatments, samples with different glucose concentrations in the raw mixes differed values were not significant. Since the starting materials were white and did not contain protein, it is evident that these color changes arose due to caramelization. The addition of glucose had a beneficial effect on product color. Similarly, in the study of Hwang et al. (1997), the researchers noted that when they extruded lactose with citric acid, their extrudate had a much darker brown color than glucose-citric acid extrudates.

The rate and extent of caramelization in sugars is dependent on temperature, pH and the time of the reaction, and the heat induced decomposition reactions this produces (Buera, Chirife, Resnik, & Lozano, 1987). Buera et al. (1987) studied the kinetics of caramelization in glucose and lactose solutions and assigned both to the same kinetic model for caramelization. However, they found that the rate of color development of lactose was greater than the rate for glucose at pH 5, the lowest pH at which lactose was tested in their study. As for our study, since the 0% glucose formulæ had higher lactose concentrations and a higher SME, the material received greater energy input that could lead to more extreme browning. The higher viscosity could also indicate greater extruder filling of the 0% glucose formulæ that would mean this material had a longer residence time in the extruder.

3.3. Composition of the extrudates

As shown in Table 3, between 15.4 and 44% of the original lactose remained, depending on treatment and trial run. Samples containing 2% citric acid had less lactose remaining in the extrudates. Glucose concentration, on the other hand, did not correlate with the citric acid catalyst percent as clearly as lactose did. The extrude of 20% glucose formulæ contained more residual glucose than formulæ with 0% or 10%. Within the 20% formulæ, 2% citric acid concentrations resulted in less residual glucose and lactose than 1% citric acid. However, both had the same amount of non-digestible oligosaccharides. Because the extrude of 0% added glucose formulæ contained glucose, it was evidently released through the hydrolysis of lactose during extrusion. We measured galactose enzymatically (data not shown) and the quantities measured were less than 1%, indicating it was most likely incorporated into the oligosaccharides formed.

Since caramelization involves condensation reactions (Golon & Kuhnert, 2012; Shallenberger, 1993), and we generated steam during the extrusion process, it is not surprising that the extruded samples were lower in moisture than the raw mixes (Table 2).

While the raw mixes were measured from 4.4 to 5.5% moisture, the extrudates contained only 0.9–1.2%. During extrusion, water was lost as steam, through decomposition, dehydration and/or polymerization reactions. We observed black smoke during the extrusion runs, indicating that some amount of decomposition occurred. The lower moisture of the extrudates in our study indicates that the process of condensation polymerization of the sugars occurred, as will be discussed below in terms of indigestible oligosaccharides we measured.

Twin-screw extrusion of the dry sugar and citric acid formulæ was successful in generating between 37.1 and 59.8% (w/w, of starting material) indigestible oligosaccharides (Table 3). Most of the oligomers (90.5–99.7%, with a mean of 96.5%) analyzed as low molecular weight soluble dietary fiber (LMWSDF), as indicated by its solubility in 80% ethanol. Formulæ with 1% citric acid and no glucose or 10% glucose were less polymerized than other samples (Table 3). The amount of citric acid had the greatest effect on the percent of indigestible oligosaccharides in the extrudate in our experiments. Hwang et al. (1997) polymerized lactose by twin-screw extrusion with the addition of 1% citric acid as a catalyst. They reported that varying the temperature of extrusion affected the yield of dietary fiber. The temperatures they used on their extruder were lower than in our experiment. They investigated temperatures of 160, 180 and 200 °C and reported yields of polymerized material (dietary fiber plus oligosaccharides) of 26.5%, 38.2% and 45.9%, respectively. We had three zones that were maintained at 230 °C or greater. This was necessary for us to melt the lactose, and to not have granular, un-melted lactose coming out at the end of the extruder. However, since their extruder barrel was longer (they had the same diameter but greater screw length) than ours, and they ran at lower screw rpms (which affects residence time and fill in the extruder), and there is no indication of screw elements used in their study, it is possible that these extrusion differences resulted in a polymerization percentage for their 200 °C treatment as our 1% citric acid, no glucose treatment. However, their methods for determination of oligosaccharides and dietary fiber were much different from ours and did not include clean-up procedures to remove non-carbohydrate compounds created during caramelization reactions, so direct comparison between our studies is difficult.

HPLC chromatograms of a raw mixture containing 20% glucose, 2% citric acid and 78% lactose, the water soluble extract of the extrudate from that formula, and this extrudate after enzymatic digestion and clean-up as per the dietary fiber assay visualize the polymerization that occurred (Fig. 1). While only the lactose and glucose peaks are present in the raw mix (Fig. 1(a)), the extruded sample (Fig. 1(b) and (c)) contained many peaks that eluted before

Table 3

<table>
<thead>
<tr>
<th>Formula</th>
<th>Glucose</th>
<th>Citric acid</th>
<th>Lactose</th>
<th>Non-digestible oligomers</th>
<th>Lactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 1 19</td>
<td>37.1 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.8 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>0 2 98</td>
<td>52.3 ± 6.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.0 ± 5.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.5 ± 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>1 1 89</td>
<td>37.4 ± 7.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.0 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.5 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>1 2 88</td>
<td>53.4 ± 10.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.2 ± 6.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>20 1 79</td>
<td>49.9 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.5 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4 ± 0.4&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>20 2 78</td>
<td>58.8 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.5 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>a</sup>Non-digestible oligomers quantified as dietary fiber using the integrated total dietary fiber assay procedure (Megazyme, Bray, Ireland), based on AOAC Method 2009.01; lactose and glucose quantified by the lactose/glucose enzymatic assay procedure (Megazyme); Values (x, w/w, dry basis) are means ± standard deviation (n = 4); means without a common superscript letter within the same column are significantly different (p < 0.05; 95% confidence interval using a T-distribution is shown).

...
The DPs of digested and cleaned up extrudates were determined using a mass spectrometer operating in positive electrospray mode instead of the ELSD as detector following HPLC under the same conditions as for the dietary fiber quantification. Ions corresponding to sodium adducts of hexose-based oligosaccharides with DPs between 1 and 9 were monitored. Fig. 2 shows the chromatograms of extrudates with 0, 10 or 20% glucose in the raw mix. Aside from DP 2 representing lactose, the main components in all three samples were trisaccharides, tetrasaccharides and pentasaccharides. In the study by Hwang et al. (1997), the average molecular mass was characterized based on gel permeation chromatography. As their extrusion temperatures increased from 180, 200 and 220 °C, their reported average molecular masses increased (302, 559 and 732 Da, respectively). We cannot relate our MS results directly to this report as all their peaks were combined into an average. Our m/z ratio showed peaks with a mass of 365 (lactose) and tri, tetra and pentasaccharides with m/z ratios of 527, 689 and 851, respectively.

A field of study where sugars are polymerized with an acid catalyst is in the production of polydextrose. Polydextrose is added as a low-calorie bulking agent and fiber to foods. Production of polydextrose from glucose or maltose involves the use of food grade acids (i.e., citric) as a catalyst and cross-linker at concentrations reported to be from 0.5 to 5% of the sugar polymerized, and the addition of a sugar alcohol (i.e., sorbitol) that acts as a plasticizer and chain terminator (Mora & Pacsu, 1955; Renhard, 1973; Shah, Craig, Morrill, & Wuesthoff, 2003). This reaction is conducted at the melt temperature of the sugar but, unlike the process described in our study, under negative pressure to minimize color formation and to remove water to allow for polymerization and minimize hydrolysis reactions (Renhard, 1973). As acid catalyst concentrations are increased in polydextrose production, the acid induced cross-linking increases as well as the concentration of water insoluble polymers (Renhard, 1973). The extrudate from our experiments was water soluble, and the majority analyzed as LMWSDF. While we saw a difference in the quantity of polymerized material with an increase in acid concentration, we did not see water insoluble material. We did see evidence of hydrolysis of lactose to glucose in the treatment with no glucose added (Table 3), however the samples with the highest initial moisture did not show higher rates of sugar hydrolysis or less polymerization to soluble fiber, unlike what is demonstrated in polydextrose production.

4. Conclusion

Lactose formulae were successfully polymerized to produce indigestible oligosaccharides by a continuous, twin-screw extrusion process. A higher citric acid concentration (2% versus 1%) resulted in higher yields of dietary fiber (up to 60%), which consisted mostly of LMWSDF. Added glucose in the raw mix resulted in lower extruder torque and SME, and extrusion products of lighter color. This would have the beneficial effects in reducing energy consumption and possibly increasing throughput. Given these promising results, further evaluation of the optimum citric acid catalyst concentrations for glucose/lactose mixtures for maximum oligosaccharide yield is warranted. The further characterization of the generated soluble fiber for types of bonds, sugars present and purification and concentration protocols is warranted. Additional research to determine if a beneficial effect of this ingredient is observed in humans could allow for the indigestible oligosaccharides to be categorized as dietary fiber, and allow for the commercialization of a high-value, “polylactose” fiber food ingredient from lactose.
Acknowledgments

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References


Chirife, J., & Buera, M. P. (1997). A simple model for predicting the viscosity of sugar with 0% (a), 10% (b) and 20% (c) glucose and 2% citric acid after digestion and purification according to AOAC 2009.01; the ions with m/z 851, 689, 527 and 365 correspond to penta-, tetra-, tri- and disaccharides, respectively.


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