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Processing effects on four prebiotic carbohydrates supplemented in an extruded cereal and a low pH drink

Rebecca M. Duar^{1,2}, Pei Tze Ang¹, Michelle Hoffman¹, Randy Wehling¹, Robert Hutkins¹ and Vicki Schlegel^{1*}

Abstract: Prebiotic carbohydrates are added as functional ingredients to a variety of processed foods. Data on the stability of prebiotics during food processing in complex matrices remain limited. The objective of this project was to determine the stability of fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS), and resistant starch (RS₂), when added as ingredients (1% w/w) to an extruded cereal and a low pH drink. The cereal was prepared using different screw speeds and barrel temperatures. GOS was not affected by any of the extrusion conditions, whereas inulin decreased significantly at 140 and 170°C. FOS levels decreased in all extrusion conditions, while resistant starch (RS) unexpectedly increased for each of the parameters. The low pH drink was prepared with different sucrose to corn syrup solids (S:CSS) ratios (1:2, 1:1, 2:1) at pH 3.0, 3.5, and 4.0. The 1:1 S:CSS drink at pH 3.0, negatively impacted FOS and inulin. Moreover, FOS levels decreased when exposed to 1:2 S:CSS (pH 3.5 and 4.0) and 1:1 S:CSS (pH 3.0). GOS and RS were unaffected by any drink formulations. As different conditions impact the stability of prebiotics differently, this study addresses the importance of developing product specific processes for each prebiotic when supplemented into a processed food.

Subjects: Food Additives & Ingredients; Food Chemistry; Beverages; Carbohydrates; Food Analysis; Food Laws & Regulations

Keywords: prebiotics; GOS; FOS; inulin; resistant starch; extrusion; low pH

ABOUT THE AUTHORS

The researchers' interests include the characterization of small molecules with potential health benefits to facilitate the development of functional foods and nutraceuticals. In particular, the authors are interested in characterizing carbohydrates with prebiotic potential, including the evaluation of the effects of processing technologies on the chemistry of the oligosaccharides, and the understanding of the molecular basis for on how prebiotic oligosaccharides shift the intestinal microbiota in humans and animals.

PUBLIC INTEREST STATEMENT

This research provides information on the stability on four different prebiotic oligosaccharides when supplemented in two distinct food matrices. Information provided could hereby serve as a basis to select prebiotics to be added as functional ingredients into health-promoting foods.



Rebecca M. Duar

1. Introduction

Prebiotics are defined as non-digestible carbohydrates that upon consumption confer beneficial effects to the host by selectively inducing changes in the makeup and/or activity of the intestinal microbiota (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). Several potential health benefits have been attributed to these carbohydrates, including improving bowel function, preventing of colon cancer, promoting growth of selective beneficial microorganisms of the microbiota, lowering of serum cholesterol, improving mineral absorption, and enhancing immune function (Crittenden & Playne, 2006; Roberfroid et al., 2010). As an outcome, there is an increased interest in the use of prebiotics as preventative and potentially therapeutic interventions for various diseases such as ulcerative colitis, colon cancer, coronary heart disease, allergy, and osteoporosis. The mechanisms by which these health-promoting events occur are not entirely understood and are still under scientific debate (Roberfroid et al., 2010). Further studies in human subjects are therefore needed to substantiate these claims, as declared by the European Food Safety Authority (2010). Nonetheless, inulin, fructooligosaccharides (FOS) and galactooligosaccharides (GOS) are currently classified as prebiotics (Roberfroid, 2007). Resistant starch (RS) is considered a potential prebiotic as its consumption has been associated with changes in the composition of the gut microbiota (Martínez, Kim, Duffy, Schlegel, & Walter, 2010) and several health benefits have been documented in animal models (Nugent, 2005).

Prebiotics carbohydrates must resist hydrolysis by human digestive enzymes and reach the large intestine intact to be fermented by the colonic bacteria into short chain fatty acids (Roberfroid et al., 2010). From a structural point of view, inulin and fructooligosaccharides (FOS) are composed of β -(2 \rightarrow 1) linked D-fructofuranose monomers with an α -(1 \rightarrow 2) linked D-glucosyl residue at the end of the chain. The degree of polymerization (DP) of commercial inulin and FOS ranges from 20–60 units to 2–10 units, respectively (Niness, 1999). GOS are composed of galactose monomers linked by β -(1 \rightarrow 3), β -(1 \rightarrow 4), or β -(1 \rightarrow 6) bonds with a terminal glucose (Nauta, Bakker-Zierkze, & Schoterman, 2009). Lastly, RS is the portion of starch that is not broken down by digestive enzymes within 120 min of consumption, making it available for fermentation in the colon (Englyst & Kingman, 1990). RS is classified into four subtypes, including RS₁, RS₂, RS₃, and RS₄. Types 1, 2, and 3 occur naturally in foods, while type 4 is produced synthetically (Nugent, 2005).

Prebiotics have attracted considerable interest in the food industry and are incorporated into dairy products, fruit juices, baked goods, and other foods (Charalampopoulos & Rastall, 2012). Published reports remain limited on the interaction of prebiotics with complex food matrices and their stability when exposed to food-processing conditions, such as extrusion, high temperatures, and low pH. Stability of prebiotics during food processing is an essential requirement given that their biological activity likely depends on their structural integrity. For example, hydrolyzed or degraded prebiotics caused by processing may no longer be active (Huebner, Wehling, Parkhurst, & Hutkins, 2008). Therefore, the objective of this study was to determine the impact of two very different processes (extrusion and low pH) on the total levels of four prebiotic carbohydrates when added as ingredients into two types of processed foods (low pH drink and an extruded cereal). It is expected that the results presented herein will be helpful to select prebiotics that could be added to extruded foods and low pH beverages.

2. Materials and methods

2.1. Prototype foods

Processed foods used for this study included an extruded breakfast cereal and a low pH drink. These products were chosen as they represent foods typically subjected to relatively severe, but distinct processing treatments. All products were prepared at 1% w/w final concentration each of the following prebiotics: (1) Purimune™ GOS 92%, (2) NutraFlora® P-95 FOS from GTC Nutrition (Colorado USA), (3) Orafiti® inulin \geq 92% oligofructose from BENE0 (Tienen, Belgium), and (4) Hi-maize™ 260 \geq 60% RS₂ from National Starch Food Innovation (New Jersey, USA). The backbone of RS₂ was composed of approximately 260 monosaccharide units. The degree of polymerization (DP) of inulin and GOS ranged

between 3–60 and 3–6, respectively. FOS contained 1-kestose 33.8%, DP 3; nystose 50.1%, DP 4; and fructosyl nystose 11.6%, DP 5.

The breakfast cereal contained oat flour (800 g), corn flour (1010 g), sucrose (140 g), sodium chloride (20 g), calcium carbonate (10 g), and the prebiotic (20 g). The mix was equilibrated overnight with appropriate additions of distilled water to a final moisture content of 17% prior to extrusion. A conical twin-screw laboratory extruder with a barrel diameter of 1.9 cm and a length–diameter ratio of 20:1 was used for the extrusion process (C.W. Brabender Model 2003 GR-8). To achieve optimal expansion, the screw speed and barrel temperature were adjusted to 170 rpm and to 140°C, respectively. The stability of the prebiotics to extrusion was evaluated by subjecting the breakfast cereal at two additional barrel temperatures and screw speeds using a completely randomized design. Temperature was adjusted to 120 and 170°C, while holding the screw speed at 170 rpm. Likewise, screw speeds of 120 and 220 rpm were tested at 140°C. Product batch formulations were prepared in triplicate.

To determine the effects of reducing sugars and pH on the stability of each prebiotic, the drink was prepared at various ratios of sucrose to corn syrup solids (S:CSS, 1:2, 1:1, 2:1) and adjusted to pH to 3.0, 3.5, and 4.0 with citric acid using a randomized 2³ factorial split plot design. The drink mixture was prepared in 10 L batches at a final sweetener concentration of 50 g/L. The prebiotic was added with the other remaining ingredients, which included sodium chloride (10 g), sodium citrate (1 g), and red food coloring. The ingredients were stirred and heated to a temperature of 79 ± 1°C using a stir/hot plate. The drink product was hot-filled into PET bottles and allowed to cool prior to analysis. Product batch formulations were prepared in triplicate.

2.2. Prebiotics extraction and analysis

Different analytical methods are reported throughout the literature to detect non-digestible carbohydrates. The vast majority have only been applied to the analysis of pure prebiotics (Courtin, Swennen, Verjans, & Delcour, 2009). The objective of this project was to evaluate the stability of FOS, GOS, inulin, and RS₂ when added to an extruded breakfast cereal and a low pH drink by measuring total yields before and after the process. For this purpose, we selected robust analytical methods capable of accurately measuring these prebiotics in the presence of complex matrices. Prebiotics were measured with optimized and validated extraction and analytical methods that included UV-Vis spectroscopy (inulin and RS₂), GC (GOS), and HPLC (FOS). Each method was validated in terms of accuracy, precision, ruggedness, and linearity for a given matrix according to the US Pharmacopia (1995).

FOS was extracted from the cereal by stirring 10.0 ± 0.05 g product with 50% ethanol for 30 min at 22–25°C. The suspension was then centrifuged (10,000 × g for 20 min) to remove large particles. Extracts were vacuum-dried and resuspended in distilled water to a final volume of 1.0 ml. A specific procedure was not needed to extract FOS from the low pH drink. Samples were filtered (0.45 µm) and analyzed by HPLC as described by Sheu, Lio, Chen, Lin, & Duan (2001) with some modifications. FOS was identified and quantified using a 3.9 × 300 mm amino-bonded phase carbohydrate column (Waters, Massachusetts, USA) heated to 35°C and interfaced to a refractive index detector. Carbohydrates were eluted in 75% acetonitrile in order of increasing monosaccharide chain length. The concentration of FOS was calculated based on calibration curves constructed from standards.

Inulin was measured using an adapted version of the Total Fructan AACC Method 32-32 (2001), with a kit purchased from Megazyme International© (K-FRUC 5/2008, Ireland Ltd., County Wicklow Ireland). Briefly, inulin was extracted from 1.0 ± 0.05 g of cereal or 1.0 ± 0.05 ml of drink with 50 ± 0.1 ml of water at 80°C for 15 min followed by enzymatic hydrolysis as indicated by the manufacturer. Total fructose derived from inulin was measured spectrometrically at 410 nm after reaction with p-hydroxybenzoic acid hydrazide and calculated against a standard curve constructed with known amounts on inulin and prepared the same as the samples.

To determine GOS levels, the breakfast cereal (0.50 ± 0.05 g) was combined with 50 ± 0.1 ml of water and stirred for 30 min at $25\text{--}30^\circ\text{C}$. The sample was then centrifuged at $10,000 \times g$ for 20 min and pellet discarded. GOS was extracted from the supernatant according to AACC Method 32-25.01 method (1994). To extract GOS from the drink, 1.00 ± 0.05 ml of sample was mixed with 10.0 ± 0.1 ml of water at $25\text{--}30^\circ\text{C}$ for 30 min with no other additional steps. Residues of GOS were determined as alditol acetates by gas liquid chromatography (GC) as described by Courtin, Van den Broeck, and Delcour (2000). The derivatized sugars were resolved on an Elite 225 $30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ film column (Perkin Elmer, Waltham, MA) interfaced to a gas chromatograph (Agilent Technologies 7820A, Santa Clara, CA) with splitter injection port (split ratio 1:20) and flame ionization detector. Helium served as the carrier gas, and the separation and detection temperatures were 220 and 240°C , respectively.

Sugar losses due to hydrolysis, derivatization, and to different GC responses were accounted by using an internal standard (*myo*-inositol) and calculating the following correction factor (*CF*) obtained from external standards of glucose and galactose:

$$CF = \frac{A_{Std} \times W_{MS}}{A_{MS} \times W_{Std}}$$

where A_{MS} is the peak area and W_{MS} is the weight (mg) of the monosaccharide (glucose and galactose). A_{Std} and W_{Std} are the peak area and weight (mg) of internal standard, respectively.

GOS was calculated from galactose as anhydrosugar (AS) based on the following equation:

$$AS = \frac{CF \times A_{MS} \times W_{Std} \times F_m}{A_{Std} \times S}$$

where F_m is the calculation factor for individual monosaccharides to polysaccharide residues (0.90 for hexoses), and S is the weight (mg dry matter) of original sample.

The amount of galactose derived from GOS was determined by analyzing standards of known concentrations analyzed with the same procedure used for the food samples. The content of free galactose (non-derived from GOS) was also determined for both control and prebiotic-containing prototype food samples. It should be noted that this GC method was used instead of the official AOAC 2001.1 method for determining *trans*-galactooligosaccharides in food, which is based on high-performance anion-exchange chromatography with pulsed amperometric detection, because salts and other components present in the food matrices greatly affected method accuracy.

RS content was measured using a kit purchased from Megazyme International© (K-RSAR 08/11) based on the AACC Method 32-40.01. In brief, non-resistant starch was solubilized and hydrolyzed into glucose and discarded. RS was obtained in the pellet by centrifugation and subsequently solubilized with 2 M KOH. Finally, RS was enzymatically hydrolyzed to glucose with amyloglucosidase followed by the addition of glucose oxidase–peroxidase. The absorbance of each solution was measured at 510 nm against a D-glucose standard curve. RS content (% dry weight basis) was calculated as follows:

$$RS \text{ (g/100 g sample)} = (RS \times V \times F) \times (100/M)$$

where RS is the amount in mg/mL calculated from the calibration curve, V is the final volume in ml, F is the 162/180 factor to convert from free D-glucose to anhydro-D-glucose as occurs in starch, and M is the moisture content of the sample.

As the complexity of the food matrix may affect the ability to detect a given prebiotic, the previously cited tests were validated and optimized using prototype foods spiked with known amounts of a given prebiotic according to the United States Pharmacopeial Convention (1995) (data

not provided). Percent recovery was calculated as the percent change in probiotic concentration in the a test sample relative to a control sample spiked with prebiotic at 1% w/w and analyzed under the same conditions.

2.3. Statistical analysis

All data are shown as the mean \pm standard errors of the mean (SEM). Percent recoveries were calculated from three independent analyses. Treatments were compared to each other by ANOVA and Tukey's *post hoc* test at 5% level of significance. Statistical analyses were completed using GraphPad Prism® version 6.04 (GraphPad Software California, USA).

3. Results

The stability of the prebiotics in the breakfast cereal during extrusion was evaluated using different screw speeds and barrel temperatures. As shown in Figure 1, extrusion in general resulted in reduced FOS levels. The lowest amounts were recovered from the process using the highest temperature (170°C). More than 50% of the supplemented FOS was degraded during optimum expansion conditions (170 rpm and 140°C). Inulin levels supplemented in the breakfast cereal were not significantly affected by either the optimal or the low-temperature extruding conditions. However, variations in the screw speed resulted in recoveries, 25% at low (120 rpm) and 34% at high speed (170 rpm), respectively (Figure 2). Low levels of inulin (35%) were also recovered from high-temperature (170°C) extrusion. GOS showed a high stability at all the extrusion conditions tested. As shown in Figure 3, recoveries of 115, 105, 102, and 99% were obtained using the high speed, low speed, low barrel temperature, and the optimal conditions, respectively. The lowest levels of GOS (81%) were recovered when the breakfast cereal was extruded at the highest temperature; however, the results were not significantly different ($p > 0.05$) from the optimal conditions. Recovery of RS₂ in the breakfast cereal resulted in percentages

Figure 1. Percent recovery of FOS supplemented into extruded breakfast cereal prepared under different screw speeds and barrel temperature.

Notes: Results are shown as mean \pm standard error of the mean ($n = 3$). Bars with different letters are statistically different ($p > 0.05$).

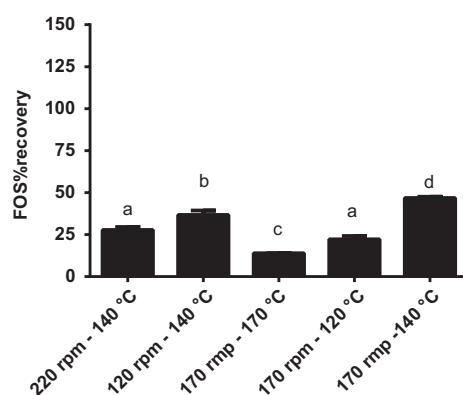
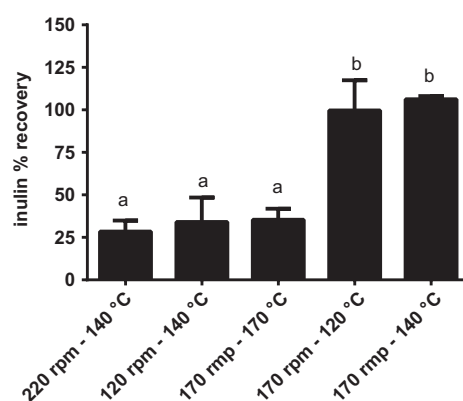


Figure 2. Percent recovery of inulin supplemented into extruded breakfast cereal prepared under different screw speeds and barrel temperature.

Notes: Results are shown as mean \pm standard error of the mean ($n = 3$). Bars with different letters are statistically different ($p > 0.05$).



higher than expected, i.e. ~200% for all the parameters used (Figure 4). Analysis of the non-supplemented cereal subjected to the extrusion process contained only approximately 0.24% w/w RS₂ (data not shown), whereas the prebiotic-supplemented extruded products resulted in RS₂ levels of approximately 2.30 ± 0.33%.

The effects of temperature, pH, and reducing sugars on the prebiotic stability were also analyzed. FOS was stable at the highest pH (4.0), regardless of the sweetener composition (Figure 5). However, recoveries of less than 70% were obtained at pH 3.0 and 3.5 independent of the sweetener composition. In particular, FOS decreased significantly (to 37%) when exposed to pH 3.0 and 1:1 sucrose and corn syrup solids. Inulin was stable regardless of the pH or sweetener composition (Figure 6). The lowest inulin was

Figure 3. Percent recovery of GOS supplemented into extruded breakfast cereal prepared under different screw speeds and barrel temperature.

Notes: Results are shown as mean ± standard error of the mean (n = 3). Bars with different letters are statistically different (p > 0.05).

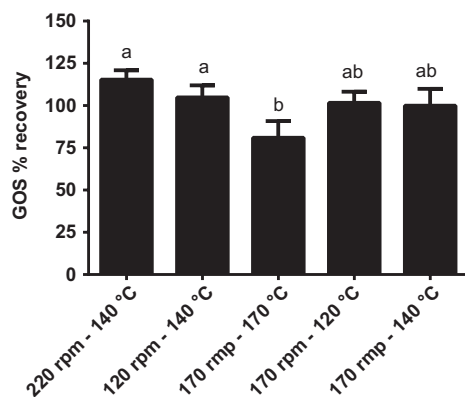


Figure 4. Percent recovery of RS supplemented into extruded breakfast cereal prepared under different screw speeds and barrel temperature.

Notes: Results are shown as mean ± standard error of the mean (n = 3). Bars with different letters are statistically different (p > 0.05).

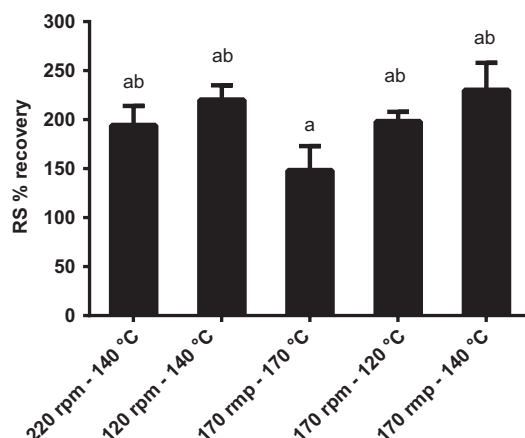
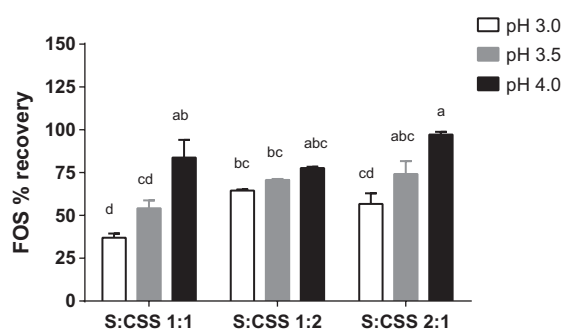


Figure 5. Percent recovery of FOS supplemented in a low pH drink prepared under different pH and sweetener ratios (sucrose:corn syrup solids (S:CSS)).

Notes: Results are shown as mean ± standard error of the mean (n = 3). Bars with different letters are statistically different (p > 0.05).



recovered (90%) at 1:1 sucrose and corn syrup solids ratio, pH 3.0, which was statistically different ($p > 0.05$) from the results obtained for the pH 3.5 and pH 4.0 formulations at the same sweetener compositions. GOS was stable at all pH values and sweetener conditions with recoveries greater than 95% at pH 3.0 and pH 3.5 (Figure 7). Although lower levels were obtained at pH 4.0, this outcome was not significantly different ($p > 0.05$) from the other treatments. Lastly, RS was relatively stable to the different low pH drink preparations. As shown in Figure 8, the lowest RS recovery (90%) occurred at pH 4.0 and a 1:1 sucrose and corn syrup solids ratio.

4. Discussion

Interest in prebiotics as added ingredients in processed foods is increasing due to their potential to modulate specific members of the intestinal microbiota and confer health benefits. During processing, carbohydrates can undergo different changes, such as Maillard-reaction, caramelization, and hydrolysis. Oligosaccharides that have been enzymatically or chemically hydrolyzed are not expected to retain prebiotic activity considering that released sugars would be absorbed in the gastrointestinal tract or metabolized by the general commensal microbiota (Huebner et al., 2008). As such, it is essential

Figure 6. Percent recovery of inulin supplemented in low pH drink prepared under different pH and sweetener ratios (sucrose:corn syrup solids (S:CSS)).

Notes: Results are shown as mean \pm standard error of the mean ($n = 3$). Bars with different letters are statistically different ($p > 0.05$).

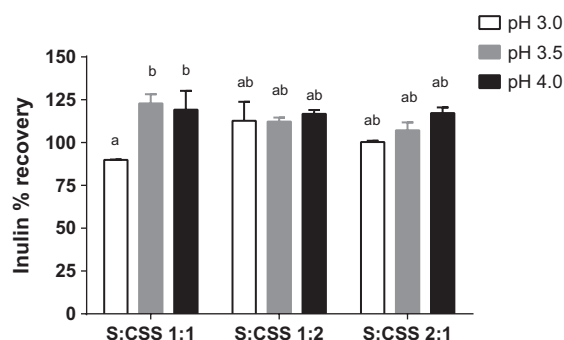


Figure 7. Percent recovery of GOS supplemented in low pH drink prepared under different pH and sweetener ratios (sucrose:corn syrup solids (S:CSS)).

Notes: Results are shown as mean \pm standard error of the mean ($n = 3$).

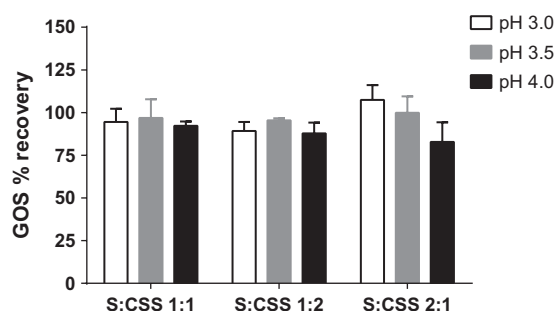
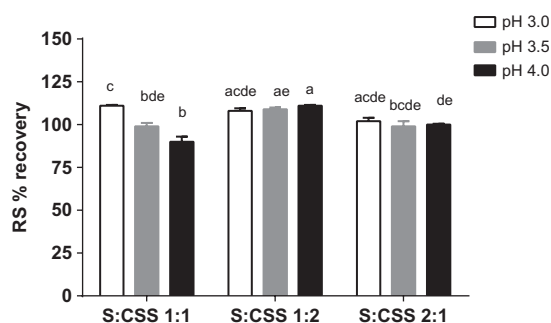


Figure 8. Percent recovery of RS supplemented in low pH drink prepared under different pH and sweetener ratios (sucrose:corn syrup solids (S:CSS)).

Notes: Results are shown as mean \pm standard error of the mean ($n = 3$). Bars with different letters are statistically different ($p > 0.05$).



that prebiotics remain stable under typical food-manufacturing conditions. Thus, the interaction of the prebiotic with the surrounding matrix and the effect that processing might have on their stability must be considered in food applications.

Several studies have evaluated the chemical stability of various oligosaccharides during thermal and acidic conditions (Courtin et al., 2009; Huebner et al., 2008; Klewicki, 2007; L'homme, Arbelot, Puigserver, & Biagini, 2003; Matusek, Merész, Le, & Örsi, 2008; Wang, Sun, Cao, Tian, & Wang, 2009). For example, Courtin et al. (2009) studied the kinetics of glycosidic bond hydrolysis at low pH and high temperature, and during long-term stability for arabinoxylooligosaccharides, xylooligosaccharides (XOS), and FOS. However, published data remain limited on the stability of prebiotics exposed to different processes in a complex food matrix. In this study, we determined the processing stability of FOS, inulin, GOS, and RS₂ during extrusion, high temperature, presence of reducing sugars, and low pH. Stability was determined by applying optimized extraction and analytical methods to prototype food products supplemented with 1% w/w prebiotic.

Extrusion is a widely utilized process to manufacture, cereals, snacks, pasta, etc. The combination of heat and pressure produces significant conformational changes to molecules in the food product (Harper & Clark, 2009). This study indicates that FOS underwent substantial degradation during this process, while inulin was only affected with variations in the screw speed. Several mechanisms could be involved in the degradation of carbohydrates during these processes. The high screw speed exposes the polymers to sheer stress. At lower speeds, the pressure within the extruder increases due to a higher screw fill exposing the molecules to high temperature and pressure for a longer time. This can result in chemical bond breakage (Gualberto, Bergman, Kazemzadeh, & Weber, 1997). High-temperature degradation of inulin (Böhm, Kaiser, Trebstein, & Henle, 2004) and FOS (Courtin et al., 2009) has been previously reported. However, information is limited regarding the chemical stability of FOS and inulin during extrusion.

High-temperature heating is a typical process used in the food industry to pasteurize beverages and food products. Collective data from this study and previously published reports substantiate the liability of FOS at typical pasteurization temperatures, especially in combination with low pH (Huebner et al., 2008; Wang et al., 2009). Breakage of the glycosidic bonds β -(2→1) between fructose units in FOS occurs rapidly at low pH and high temperature (Courtin et al., 2009). Hydrolysis is most likely caused by oxygen protonation in the glycosidic bond during acid hydrolysis (Voragen, 1998). Thermolysis of inulin in the presence of citric acid has been reported to occur at 160°C, while FOS completely degrades at 120°C (Christian, Manley-Harris, Field, & Parker, 2000; L'homme et al., 2003). In accordance, our results indicate that inulin has a higher thermostability at low pH compared to FOS.

GOS was stable at both high temperature and low pH when supplemented in the cereal and drink. Other researchers have reported on GOS stability in response to heat and acid conditions. For example, Klewicki (2007) showed that GOS does not undergo hydrolysis at pH 3.0 with heating at 100°C for 10 min, and only low losses occur (5%) at a pH of 2.0. The thermostability GOS at low pH has been attributed to the presence of the β -glycosidic linkages (Voragen, 1998).

Although RS was not affected by either high temperature or low pH as an ingredient in the drink, the extrusion process itself resulted in unexpected high levels, which is not clearly understood at this point. We hypothesize that this unexpected increase in RS could be due to gelatinization of the high-amylose starches at high temperatures, such as those reached during extrusion. Upon cooling, starch undergoes a retrogradation process where the molecules re-associate and form tightly packed structures by hydrogen bonding. This form of starch is thermally stable and resistant to amylase, which is known as RS₃ (Haralampu, 2000). Previous studies conducted in our laboratory have shown that the test method used for detecting RS₂ is unreliable for RS₃. As such, the higher numbers could be an artifact of the analytical method used, albeit more studies are needed to test this hypothesis. Another possibility is that RS₁ encapsulated in the plant structure was liberated during the extrusion process (Alsaffar, 2011).

However, the grains used for this study were milled flour, which typically reconfigures the RS₁ structure to the RS₂ (Alsaaffar, 2011). Nonetheless, these results indicate that process did affect the RS₂ prebiotic, either in total amount and/or conformation.

This work provides important insights into the effects of processing on prebiotics supplemented into two very different food systems. However, further studies are needed with extended time periods and storage conditions commonly used for an extruded product and a low pH drink to derive shelf life durations. Nonetheless, it is expected that the information presented herein together with previously published reports will aid manufacturers in to develop suitable food processes and to select adequate prebiotics for the manufacture of foods with health-promoting purposes.

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Cover image

Effects of prebiotic recovery at different pH and sucrose:corn syrup solids (S:CSS).
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