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Formation of type 4 resistant starch and maltodextrins from amylose and amylopectin upon dry heating: A model study

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ABSTRACT

Starch is one of the main components of human diet. During food processing, starch is submitted to high temperatures in the presence or absence of water. Thus, the main goal of this work was to identify structural modifications caused by dry heating in starch polysaccharides (amylose and amylopectin) and structurally related oligosaccharides, maltotetraose (M4) and glucosyl-maltotriose (GM3), simulating processing conditions. The structural modifications were evaluated by methylation analysis, electrospray mass spectrometry (ESI-MS), tandem mass spectrometry (ESI-MS/MS) and anionic chromatography after *in vitro* enzymatic digestion. Dry heating promoted dehydration, depolymerization, as well as changes in Glc glycosidic linkage positions and anomeric configuration. In oligosaccharides, polymerization was also observed. All these changes resulted in a lower *in vitro* digestibility, suggesting that dry heating of starch polysaccharides and related oligosaccharides may be associated with the formation of type 4 resistant starch and maltodextrins, non-digestible carbohydrates that are responsible for beneficial effects in human intestinal tract.

1. Introduction

Starch is the major source of carbohydrate in human diet (Ratnayake & Jackson, 2008). Processing of foods rich in carbohydrates for human consumption normally involves thermal treatment, resulting in significant physical and chemical modifications (Liu, Xie, Yu, Chen, & Li, 2009a). Depending on the modifications, enzymatic availability and digestion can be increased due to gelatinization and/or dextrinization. The formation of starch–lipid complexes (Guraya, Kadan, & Champagne, 1997), starch retrogradation upon cooling (Sajilata, Singhal, & Kulkarni, 2006), starch protein interactions or chemical modification of starches (Mangala, Udayasankar, & Tharanathan, 1999) are factors that reduced it digestibility. The interest in non-digestible starch fractions, called "resistant starches", has been raised (Cummings & Englyst, 1991; Englyst, Kingman, Cummings, & Seibel, 1993). They have physiological functions similar to those of dietary fibre, and possess nutritional value (Björck & Asp, 1994; Eerlingen & Delcour, 1995; Haralampu, 2000). The formation of resistant starch during food processing has been mainly studied under cooking conditions that promotes starch gelatinization, amylose leaching and, upon storage, mainly amylose retrogradation. Baking has also been implicated in the formation of resistant starch due to the formation of bread crust (Baiano, Romaniello, Lamacchia, & Notte, 2009; Liljeberg, Åkerberg, & Björck, 1996; Pizzoferrato, Rotilio, & Paci, 1999; Tovar & Melito, 1996). The decrease in available starch due to dry heating of starch is not accompanied by any increase in dietary fibre, suggesting that the fragments remaining after enzymatic digestion have low molecular weight (Siljeström, Björck, & Westerlund, 1989). Thermal decomposition of starch has been the aim of several studies (Liu, Xie, et al., 2009; Liu, Yu, Liu, Chen, & Li, 2009; Tomasik, Wiejak, & Pałasiński, 1989), but many of these studies did not use reaction conditions mimicking food processing. This process engages several reactions and depends on many factors, such as temperature, presence or absence of air or any inert gas, their pressure, moisture content, and the amylose/amylopectin ratio (Aggarwal & Dollimore, 2000; Liu, Yu, et al., 2009). Depending on these factors, different products of thermal degradation can be formed (Ciesielski & Tomasik, 1996; Tomasik et al., 1989; Zhang, Golding, & Burgar, 2002).

It has been frequently reported that starch with different amylose/amylopectin ratios have a strong influence on the formation of resistant starch, and this has been linked to the increase of amylose. Nevertheless, due to the different structural features

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of both polysaccharides, we hypothesized that amylose and amylopectin can have additionally different behaviour during starch dry heating, which is an issue that has not been yet addressed. Structural changes of starch that contribute to their resistance during digestion (Lehmann & Robin, 2007) include: formation of new glycosidic bonds not hydrolysed by α-amylase, like (1 → 2), (1 → 3) and new (1 → 6) bonds, formation of modified residues at the reducing end, and formation of β-anomers (Siljeström et al., 1989). Also, the formation of maltodextrins during the initial high moisture heat treatment of starch (Hoseney, 1991), and further heating during dry heat treatment of starch can contribute to the formation of resistant maltodextrins. The formation of resistant maltodextrins by acid catalyzed pyrodextrination of starch has been used to commercially produce resistant starch by prolonged heating and enzymatic hydrolysis of starch (Cho & Samuel, 2009). If occurring during dry heating food processing, the formation of resistant maltodextrins could change the nutritional profile of foods, as well as physiological activities that include reduction of glycaemia (Brouns et al., 2007), acceleration of lipid metabolism, reducing visceral fat, and maintenance of healthy intestinal regularity (Hashizume et al., 2012).

Resistant starch is classified in five different types. Type I is physically inaccessible starch synthesized in the endosperm of cereal grains or seeds, where starch granules are surrounded by protein matrix and cell wall material. Granular starch with the B- or C-type polymorphism, such as uncooked potato starch and green banana starch, among others, is highly resistant to enzymatic hydrolysis and is classified as type II resistant starch. Type III is retrograded starch formed during processing when heating and subsequent cooling of starch renders the molecules of amylose and amylopectin inaccessible to enzymatic hydrolysis. Type IV is chemically modified starch resistant to enzymatic hydrolysis in consequence of the modifications. Type V is amylose–lipid complexed starch (Birt et al., 2013).

The purpose of this work was to study the mechanism of starch degradation during dry heating at conditions of low water content, approaching those of food processing. To obtain an overall view on this subject and, simultaneously, to have new insights on the structural modifications in starch polysaccharides (PS), model systems with amylose and amylopectin, and also with structurally related oligosaccharides (OS), maltotetraose (M4) and glucosyl-maltotriose (GM3), were submitted to dry thermal treatment, mimicking food processing conditions, and further analyzed by methylation analysis, electrospray mass spectrometry (ESI-MS), tandem mass spectrometry (ESI-MS/MS) and anionic chromatography after *in vitro* enzymatic digestion. This allows studying the possibility of formation of type 4 resistant starch and maltodextrins, namely the contribution of amylose and amylopectin for their formation. The mechanism of formation of resistant maltodextrins is also addressed.

2. Materials and methods

2.1. Samples

Maltotetraose (M4), amylose (from corn) and amylopectin (from potato) were purchased from Sigma–Aldrich (St. Louis, MO). Glucosyl-maltotriose (GM3) was purchased from Megazyme (County Wicklow, Ireland) and α-amylase (EC 3.2.1.1) from Fluka (St. Louis, MO, USA).

2.2. Thermal treatment of samples

The thermal treatments were performed with a thermogravimetric analyser, model TGA-50 (Shimadzu, Kyoto, Japan), operating with a controlled air flow of 20 mL/min and a heating rate of 10 °C/min. All the experiments were conducted using a platinum sample cell and an initial sample mass of 4–15 mg. As used in previous studies with arabinosyl and mannosyl oligosaccharides (OS) (Moreira, Coimbra, Nunes, & Domingues, 2013; Moreira, Coimbra, Nunes, Simões, & Domingues, 2011), three independent samples of each OS, M4 and GM3, and polysaccharide (PS), amylose and amylopectin, were heated from room temperature (RT) up to 200 °C, and maintained at 200 °C for different periods of time: 0 (200T1), 30 (200T2), and 60 (200T3) min. To study their thermal stability, a sample of each PS was heated from RT up to 600 °C. Considering their thermal stability, two samples of each PS were also heated from RT up to 265 °C and maintained at 265 °C for different periods of time: 0 (265T1) and 30 (265T2) min. The temperatures used for dry thermal treatments resemble the baking conditions during food processing.

The OS submitted to the thermal treatments at 200 °C and PS submitted to the thermal treatments at 265 °C were recovered, weighed, suspended in ultrapure water (5 mg/mL), and vortex mixed. To facilitate the dissolution of the PS samples, they were then stirred at 50 °C for 1 h. The water-soluble and water-insoluble fractions were then separated by centrifugation and kept frozen at −20 °C for further analysis. Solutions (1 mg/mL in ultrapure water) of each untreated OS (T0) samples were also prepared and kept frozen at −20 °C for MS analysis.

2.3. Neutral sugars analysis

Neutral sugars were converted to alditol acetates, as described in Supplementary Data, and then analyzed by gas chromatography (GC) on a PerkinElmer Clarus 400 chromatograph (Waltham, MA) equipped with a flame ionization detector (FID) and a DB-225 column with 30 m of length, 0.25 mm of internal diameter, and 0.15 μm of film thickness (J&W Scientific, Folsom, CA). Hydrogen was used as carrier gas with 1 mL/min of flow. The follow oven temperature programme used was: initial temperature 200 °C, temperature increase of 40 °C/min to 220 °C, maintained at 220 °C for 7 min, followed by an increase of 20 °C/min up to 230 °C, holding for 1 min. The temperature of injector and detector was 220 and 230 °C, respectively. The quantification of neutral sugars was obtained using 2-deoxyglucose as an internal standard.

2.4. Methylation analysis

For determination of glycosidic linkage composition, sugars were converted to partially O-methylated alditol acetates, as described in Supplementary Data, and then analyzed by gas chromatography mass spectrometry (GC–MS) on an Agilent Technologies 6890N Network (Santa Clara, CA). The GC was equipped with a DB-1 column with 30 m of length, 0.25 mm of internal diameter, and 0.1 μm of film thickness (J&W Scientific, Folsom, CA). The samples were injected with the injector operating at 220 °C and using the following temperature programme: initial temperature, 50 °C, raised at a rate of 10 °C/min until 140 °C, standing for 5 min at this temperature, followed by rate of 0.5 °C/min to 170 °C, and then followed by a rate of 15 °C/min to 280 °C, with a further 5 min at 280 °C. The helium carrier gas had a flow rate of 0.2 mL/min. The GC was connected to an Agilent 5973 mass quadrupole selective detector operating with an electron impact mode at 70 eV and scanning the m/z range 40–500 in a 1 s cycle in full-scan mode acquisition.

2.5. Enzymatic hydrolysis and size-exclusion chromatography (Bio-Gel P-2)

Samples (1 mg) of amylose and amylopectin (unroasted and roasted at 265 °C) were hydrolysed with 2 U of pure