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Evaluation of oligosaccharide profiles in selected cooked tubers and roots subjected to *in vitro* digestion

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ABSTRACT

The influence of the preparation method and the effect of *in vitro* digestion on the fructo-oligosaccharides (FOS) and malto-oligosaccharides (MALTOS) of sweet potato (*Ipomoea batatas* Lam), *mandioquinha* (*Arracacia xanthorrhiza*), white yam (*Dioscorea alata* L.), cassava (*Manihot esculenta* Crantz), and taro (*Colocasia esculenta*) were evaluated. Tubers/roots were cooked in two ways: whole/unpeeled and peeled/in cubes. *In vitro* digestion was performed on whole/unpeeled cooked vegetables. An oligosaccharide analysis was carried out by HPAE-PAD. The largest amounts of oligosaccharides were found in the whole/unpeeled cooked tubers/roots. Sweet potato showed the highest content of FOS (29.08 ± 1.60 mg/100 g) and the highest concentrations of MALTOS were observed in white yam (113.70 ± 3.41 mg/100 g). *In vitro* digestion promoted an increase in maltotriose in all tubers/roots. Sweet potato FOS were not changed by digestion. However, in *mandioquinha*, white yam, and cassava, FOS levels were higher after digestion. The increase in MALTOS after *in vitro* digestion may be associated with both the hydrolysis of oligosaccharides with a higher degree of polymerization and starch. FOS contents increased after digestion possibly by hydrolysis of inulin or release from the food matrix. Sweet potato, cassava, and white yams can contribute to an increased intake of prebiotics.

1. Introduction

Oligosaccharides are food components consisting of 3–10 sugar residues linked by glycosidic bonds, which can be hydrolysed to their monosaccharide units by acids or specific enzymes (Crittenden & Playne, 1996). These compounds may be naturally present in some foods, but not in a pure form. They are usually present as a combination of oligosaccharides with different degrees of polymerization (Crittenden & Playne, 1996; Judprasong, Tanjor, Puwastien, & Sungpuag, 2011; Moongngarm, Trachoo, & Sirigungwan, 2011). Additionally, the oligosaccharides may be obtained by controlled hydrolysis of carbohydrates with a high degree of polymerization or may be synthesized from simple sugars (i.e., sucrose or lactose) by transglycosylation reactions. As examples of oligosaccharides we can include galacto-oligosaccharides, malto-oligosaccharides (MALTOS) and isomalto-oligosaccharides, xylo-oligosaccharides and fructo-oligosaccharides (FOS) (Crittenden & Playne, 1996). According to their physiological properties, oligosaccharides can be classified as digestible and non-digestible (Voragen, 1998).

MALTOS, which are formed by glucose residues linked by α (1 → 4) glycosidic bonds, belong to the group of digestible oligosaccharides. After ingestion, they are hydrolysed by carbohydrases and are subsequently absorbed in the small intestine (Crittenden & Playne, 1996; Moongngarm et al., 2011). MALTOS may be found in tubers, roots, and bulbs in addition to as products resulting from starch hydrolysis (Moongngarm et al., 2011). Examples of MALTOS include maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6), and maltoheptaose (G7).

FOS consist of fructose molecules linked with each other by β (2 → 1) glycosidic bonds. There is also the possibility of a glucose molecule binding at the end of the chain through an α (1 → 2) linkage. The main FOS are 1-kestose (GF2), nystose (GF3), and 1-fructofuranosylnystose (GF4) (L'homme, Peschet, Puigserver, & Biagini, 2001). The β linkages are not hydrolysed by the digestive enzymes, and only at very low pH conditions, they may eventually

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undergo hydrolysis. FOS are classic examples of non-digestible oligosaccharides (Mussatto & Mancilha, 2007). They occur naturally in vegetables such as yacon, Jerusalem artichoke, chicory, onion, garlic, leek, and artichoke; in fruits such as banana, apple and pear, and also in honey (L'homme et al., 2001; Mussatto & Mancilha, 2007). However, other foods may also contain low concentrations of FOS (Judprasong et al., 2011).

FOS are recognized as prebiotics because they are not hydrolysed and absorbed in the upper gastrointestinal tract. They may serve as a substrate for beneficial bacteria (bifidobacteria and lactobacilli) and contribute to a healthier colonic microbiota, generating some beneficial systemic effects (Gibson & Roberfroid, 1995; Mussatto & Mancilha, 2007; Rastall & Gibson, 2015). Numerous positive effects are associated with the consumption of prebiotics, mainly the reduced risk of intestinal cancer; protection against gastrointestinal infections; increased absorption of minerals with an emphasis on calcium; and the improvement in the metabolism of carbohydrates and cholesterol with consequent reduction in the risk of diabetes and obesity (Mussatto & Mancilha, 2007; Rastall & Gibson, 2015; Roberfroid et al., 2010).

Most often, these compounds are consumed in the form of supplements or by FOS-enriched foods, such as dairy products, baked goods and beverages (Charalampopoulos & Rastall, 2012; Voragen, 1998). However, small amounts of FOS are also present in foods present in a normal diet, but which are not traditionally recognized as sources of this component.

Tuber/root crops are carbohydrate-rich foods, produced and consumed worldwide as they represent an important source of energy, (Hoover, 2001). In addition to the high starch content, these vegetables may further include other carbohydrates such as MALTOS, FOS and inulin (Judprasong et al., 2011; Moongngarm et al., 2011). Studies regarding oligosaccharides in tubers/roots are often conducted with raw vegetables, but humans consume cooked forms of these foods. It is important to stress that thermal treatment could lead to substantial changes in the compounds present in foods and in their concentrations (Lai, Huang, Chan, Lien, & Liao, 2013). In addition, the process of food preparation prior to cooking can influence these changes.

Despite the generally accepted concept that FOS pass through the upper gastrointestinal tract without modifications (Gibson & Roberfroid, 1995; Mussatto & Mancilha, 2007; Rastall & Gibson, 2015; Roberfroid et al., 2010), there is a lack of information about the conditions of these compounds at the end of gastrointestinal digestion. *In vitro* digestion is an alternative method for the evaluation of transformations that different compounds undergo during gastrointestinal digestion. This method has some limitations, such as the absence of hormonal and neural controls that are responsible for the regulation of important digestive processes such as gastric emptying, intestinal transit, and secretion of digestive fluids (Guerra et al., 2012). However, its main positive characteristics are the simplicity and speed of the method in addition to the absence of ethical restrictions found in the *in vivo* assays. Thus, it is used in several studies including the evaluation of polysaccharides (Dona, Pages, Gilbert, & Kuchel, 2010; Villemejane et al., 2016; Yin et al., 2012).

This study has two main objectives: i) to evaluate the influence of the preparation mode (thermal treatment with and without peeling and cutting) on the oligosaccharide contents in sweet potato (*Ipomoea batatas* Lam), *mandioquinha* (*Arracacia xanthorrhiza*), white yam (*Dioscorea alata* L.), taro (*Colocasia esculenta*), and cassava (*Manihot esculenta* Crantz); and ii) to investigate the effect of *in vitro* digestion on oligosaccharides in the cooked tubers/roots previously cited.

2. Materials and methods

2.1. Materials

Approximately 1 kg of sweet potato, *mandioquinha*, and taro and 1.5 kg of white yam and cassava were acquired from local markets (Campinas, São Paulo, Brazil) in June 2015.

Pepsin from porcine gastric mucosa (EC 3.4.23.1, P-7000), pancreatin from porcine pancreas (4xUSP-US Pharmacopeia specifications, P-1750), and bile extract from porcine (B-8631) were purchased from Sigma-Aldrich Co. (St. Louis, USA). Standards of 1-kestose (GF2), nystose (GF3), and 1-fructofuranosylnystose (GF4) were obtained from Wako Pure Chemical Industries (Osaka, Japan), and standards of maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6), and maltoheptaose (G7) were provided by Supelco (Bellefont, PA, USA). Sodium hydroxide and sodium acetate for HPLC were obtained from Merck (Darmstadt, Germany). The water was purified by the Milli-Q system (Millipore, Bedford, USA). All other chemicals were of analytical grade.

2.2. Processing methods

2.2.1. Sample preparation

Initially, the different varieties of fresh tubers/roots were washed, and each type was separated into two batches. One batch was hand peeled and cut into cubes of about 2.0 cm (in piece) with sharp stainless steel knife, and the other was maintained whole and unpeeled (whole). Only cassava was prepared exclusively peeled and in pieces because it is very difficult to cook this root unpeeled.

2.2.2. Thermal treatment

Each individual preparation of tubers/roots was autoclaved in distilled water at 120 °C in a tubers/roots:water ratio of 1:3 (w:v) for different periods of time (Table 1). After cooking, the water was discarded and the whole-type samples were peeled for further processing.

2.2.3. Homogenate

Fifty grams of each cooked vegetable and 100 mL of distilled water were homogenized in a domestic mixer (Black & Decker, Brazil) for 2 min and centrifuged (10 min, 3800 *g*, 10 °C). The supernatant was removed, aliquoted and stored at −80 °C for subsequent analysis.

2.3. In vitro gastrointestinal digestion procedure

Samples were digested *in vitro* as described by Faller, Fialho, and Liu (2012) with slight modifications. Briefly, 1 mL of each homogenate was mixed using a vortex whit 3.5 mL of saline solution (140 mM NaCl, 5 mM KCl) to obtain a final volume of 4.5 mL. The samples were acidified to pH 2.0 with 6 M HCl, and 125 μL of porcine pepsin solution (200 mg pepsin in 5 mL 0.1 M HCl) was added. The solution was incubated at 37 °C in a shaking water bath for 1 h under continuous agitation (130 rpm). After gastric digestion, the pH was increased to 6.8 with 1 M $NaHCO_3$, followed by the addition of 625 μL of pancreatin-bile solution (225 mg of bile extract and 37 mg of pancreatin in 18.7 mL of 0.1 M $NaHCO_3$) and incubation in a shaker at 37 °C for 2 h (130 rpm). At the end of intestinal digestion, samples were cooled in an ice bath, the digested volume was adjusted to 5.5 mL with saline solution and stored at −80 °C for further experiments. A blank sample consisting of phosphate buffered saline at pH 7.0 was subjected to the digestion procedures to eliminate any interferences coming from the reagents.

Oligosaccharide standards were digested under the same