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Characterization of cell wall polysaccharides from *Sicana odorifera* fruit and structural analysis of a galactan-rich fraction pectins as side chains

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ABSTRACT

Sicana odorifera is a Brazilian native fruit. In this work, cell wall polysaccharides from *S. odorifera* pulp were isolated by sequential extraction with water, citric acid, and sodium hydroxide solutions. The monosaccharide composition of crude polysaccharide fractions was determined. The aqueous fractions displayed the highest yields and they were constituted by pectins, having mainly galactans as side chains. The citric acid fraction (SCA) had galactose as the main component. The hemicellulosic fractions consisted mainly of xylose, mannose, galactose, and glucose, suggesting the presence of xyloglucans, xylans and mannans. The SCA fraction was further purified, resulting in a linear galactan (SCAI2). NMR and methylation analysis showed that SCAI2 was a β-(1→4) D-galactan with molar mass of 17,560 g/mol, determined by light scattering. The presence of a linear galactan in free form in fruits is unusual because these polymers usually occur as side chains of type I rhamnogalacturonans.

1. Introduction

Sicana odorifera (Vell.) Naudin, popularly known as *sikana*, *musk cucumber*, *melón de olor*, *cruá*, *croá*, *cassabanana* or *jamelão*, is a tropical member of the Cucurbitaceae family (Morton, 1987; Parada, Duque, & Fujimoto, 2000). This specie is believed native to Brazil, but it is also found in other countries of tropical America, such as Colombia and Peru (Bussmann et al., 2011; Contreras-Calderón, Calderón-Jaimes, Guerra-Hernández, & García-Villanova, 2011; Jaramillo, Dawid, Hofmann, Fujimoto, & Osorio, 2011). It is a perennial climbing plant and fast growing and the fruit is ellipsoid or nearly cylindrical, 30–60 cm long and 7–11 cm in diameter. A picture of the plant can be seen at Madeira (2016). The peel is hard and the color varies from orange-red, maroon, dark-purple until entirely jet-black and the pulp is soft with numerous oval and flat seeds (Morton, 1987). The fruit is consumed fresh or used in culinary preparations like jam, preserves and soup. Its long-lasting fragrance is used to perfume clothes and houses and also it is known as moth repellent (Jaramillo et al., 2011; Parada et al., 2000). Few studies were found in the literature about *S. odorifera* fruit. Due to its intense and pleasant aroma, the volatile compounds from the pulp have been investigated (Parada et al., 2000). Secondary metabolites from the peel and seeds have been described, such as triterpenes and flavonols (Jaramillo et al., 2011; Nakano, Fujimoto, & Takaishi, 2004). Contreras-Calderón et al. (2011) evaluated the antioxidant capacity of extracts of *S. odorifera* pulp, seeds and peel and compared with other 23 exotic fruits. The extracts of *S. odorifera* showed low antioxidant capacity by FRAP and ABTS assays compared to the other fruits.

The study of fruits is important because they contain essential nutrients for human nutrition, such as minerals, vitamins, phenolic compounds and dietary fibers (Rufino et al., 2010). Concerning dietary fibers, they are complex carbohydrates obtained from plants that are not digested by human intestine. They are essential for the correct function of gastrointestinal tract and they have also been correlated with the prevention of many diseases, such as, coronary heart disease, diabetes and cancer (McDougall, Morrison, Stewart, & Hillman, 1996). Plant cell walls are the major source of dietary fibers, which can be obtained from vegetables, fruits, nuts and seeds (McDougall et al., 1996). The walls of growing plant cells (primary cell wall) is constituted predominantly by polysaccharides, which are typically classified as cellulose, hemicelluloses and pectins (McCann & Roberts, 1991; Mcneil, Darvill, Albersheim, & Fry, 1984). In some fruits, pectins are the main cell wall polysaccharides. Pectins are a very complex family of polysaccharides which had galacturonic acid as the main monosaccharide (Ridley, O'Neill, & Mohnen, 2001). The hemicelluloses represent about 30% of primary cell walls, and they are characterized by a β-(1→4)-linked backbone with an equatorial configuration at C1 and C4 (Scheller & Ulvskov, 2010). Cellulose, the microfibrillar component of plant cell wall, constitutes about 20–30% of cell wall (Mcneil et al., 1984).

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Plant cell wall polysaccharides have attracted a great deal of attention because they can be used in the food industry to offer physiological functionalities to products (Lopes da Silva & Rao, 2006) and due to their health benefits such as antibacterial (Qian, 2014), anti-cancer (Delphi & Sepehri, 2016) and immunomodulatory activities (Amorim, Vriesmann, Petkowicz, Martinez, & Noleto, 2016).

The physicochemical and biological properties of polysaccharides depend on their chemical structural (Sila, Van Buggenhout, Duvetter, Van Loey, & Hendrickx, 2009). To date, no reports were found concerning the composition of the polysaccharides from *S. odorifera* fruit. Therefore, the aim of this study was to obtain information about the polysaccharides present in the pulp of *S. odorifera* fruit. A purified fraction isolated with citric acid was characterized. The information about the polysaccharides present in the *S. odorifera* fruit can contribute to find out new applications to the fruit and add value to this specie.

2. Experimental

2.1. Chemicals

Total starch assay kit was purchased from Megazyme (Megazyme, Bray, Ireland). Sulfuric acid, dimethyl sulfoxide (DMSO), acetone, phenol, sodium nitrite and sodium azide were obtained from Merck (Merck, Darmstadt, Germany). Trifluoroacetic acid (TFA), powdered NaOH, methyl iodide, deuterium oxide (D_2O), sodium borohydride, m-hydroxydiphenyl, monosaccharide standards and dialysis tubing were purchased from Sigma–Aldrich (Sigma–Aldrich Chemical Co., St. Louis, MO, USA). Barium carbonate ($BaCO_3$), ethanol, citric acid, sodium hydroxide, formic acid, pyridine and acetic anhydride were obtained from Synth (Labsynth, Diadema, Brazil).

2.2. Plant material

Fresh fruit of *Sicana odorifera* (2.5 kg) was purchased from Municipal Market of Curitiba in October 2012. Fruit pulp (1.845 g), free of peel (462.5 g) and seeds (192.5 g), was cut into small pieces, stored at −20 °C and freeze-dried. The dried pulp (150 g) was grounded to a fine powder with a mortar and pestle and treated with absolute ethanol (1.5 L) under reflux for 20 min giving rise to the alcohol insoluble residue (AIR; yield 54.3%).

2.3. Proximate composition of S. odorifera pulp

Moisture, ash, protein (N × 6.25) and lipid were determined according to the Association of Official Analytical Chemists AOAC (2005). The pulp (0.5 mg) was submitted to a total hydrolysis with 72% (w/w) H_2SO_4 (0.5 mL) for 1 h at 0–4 ∘C, diluted to 8% and kept at 100 °C for 15 h. The hydrolysate was neutralized with $BaCO_3$, and after salt removal it was used for determining the total carbohydrate content. Total carbohydrate was assayed by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using galactose as standard. The content of starch was determined using a Megazyme kit (Megazyme, Bray, Ireland) according to the manufacturer's instructions.

2.4. Extraction of polysaccharides

The AIR (80 g) was submitted to sequential extractions following the scheme in Fig. 1. Sequential extractions were performed using water at 25 °C (SW fraction; yield 6.72 g), boiling water (SHW fraction; yield 4.72 g), boiling 0.5% citric acid (SCA fraction; yield 1.44 g), 2 M NaOH at 60 °C (S2M fraction; yield 0.32 g) and 6 M NaOH at 60 °C (S6M fraction; yield 0.40 g) (Fig. 1). For each extraction, 800 mL of solvent was used. The extracts from aqueous extraction were centrifuged (8000 rpm, 20 min) in a Hitachi Himac CR 21E equipment (Hitachi Ltd, Tokyo, Japan), the supernatant concentrated under reduced pressure (temperature < 60 °C) in a Fisatom rotary evaporator (Fisatom, São Paulo, SP, Brazil) and treated with ethanol (3:1, v/v) in order to obtain the polysaccharides, which were then washed three times with ethanol (15 mL) and dried under vacuum. After acid and alkaline extractions the resulting supernatants were precipitated with ethanol and the polysaccharides dissolved in distilled water and dialyzed for 48 h against tap water. $NaBH_4$ (0.8 g) was used in the alkaline extractions to avoid alkaline degradation.

2.5. Purification of fraction SCA

Fraction SCA (1 g), extracted with 0.5% citric acid, was dissolved in 200 mL distilled water (5 mg/mL) and submitted to freeze-thawing process (freeze-thawing I). The procedure resulted in a fraction soluble in cold water and an insoluble fraction (SCAI1). The soluble fraction was submitted to an additional freeze-thawing process (freeze-thawing II), yielding a soluble fraction in cold water (SCAS) and a second insoluble fraction (SCAI2) (Fig. 1).

2.6. Monosaccharide composition

Polysaccharides (2 mg) were hydrolyzed with 2M TFA (1 mL) for 8 h, at 100 °C. The hydrolysates were evaporated to dryness, dissolved in distilled water (10 mL) and reduced with $NaBH_4$ (2 mg). (Wolfrom & Thompson, 1963b). Then, they were acetylated with pyridine–acetic anhydride (1:1 v/v, 0.5 mL, 24 h, at 25 °C) (Wolfrom & Thompson, 1963a). The final insoluble residue (5 mg) was hydrolyzed with 72% (w/w) H_2SO_4 (0.5 mL) for 1 h at 0 °C, diluted to 8% and kept at 100 °C for 15 h (Biermann, 1989). The hydrolysate was neutralized with $BaCO_3$ and the insoluble material removed by filtration. Monosaccharides were reduced and acetylated as described above. The resulting alditol acetates were examined by gas-liquid chromatography (GLC) using a Thermo Trace GC Ultra gas chromatograph (Thermo Scientific, Waltham, Massachusetts, USA) equipped with a Ross injector and a DB-225 capillary column (0.25 mm internal diameter x 30 m). The flame ionization detector and injector temperatures were 300 °C and 250 °C, respectively. The oven temperature was programmed from 100 to 220 °C at a rate of 40 °C/min with helium as the carrier gas (1.0 mL/min). Uronic acid was estimated by the m-hydroxydiphenyl colorimetric method (Blumenkrantz & Asboe-Hansen, 1973) using galacturonic acid as standard.

2.7. High-performance size-exclusion chromatography (HPSEC)

The polysaccharides were analyzed by HPSEC using a Waters unit (Waters Corporation, Milford, Massachusetts, USA) coupled to a refractive index (RI) and a Wyatt Technology Dawn-F multi-angle laser light scattering (MALLS) detector (Wyatt Technology, Santa Barbara, CA, USA). Four Waters Ultrahydrogel columns (2000; 500; 250; 120) were connected in series and coupled to the multidetection instrument. A solution of 0.1 M $NaNO_2$ and 0.02% NaN_3 was used as eluent at a flux of 0.6 mL/min. Prior to the analyses, the samples (1.5 mg/mL) were filtered through a 0.22 μm cellulose acetate membrane. All the analyses were carried out at 25 °C. The refractive index increment of the solvent–solute solution with respect to a change in solute concentration (dn/dc) was determined using a Waters 2410 differential refractometer. The average molecular mass (M_w) was calculated using a Wyatt Technology ASTRA software (Wyatt Technology, Santa Barbara, CA,USA).

2.8. Nuclear magnetic resonance spectroscopy (NMR)

^1H, ^{13}C NMR, ^1H-^{13}C HSQC (heteronuclear single quantum coherence), ^1H-^{13}C HMBC (heteronuclear multiple bond coherence) and NOESY (nuclear Overhauser effect spectroscopy) spectra were carried out on a Bruker Avance III 400 MHz spectrometer (Bruker, Karlsruhe, Germany). The sample was dissolved in D_2O (20 mg/mL) and the