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Cell wall polysaccharides from pulp and peel of cubiu: A pectin-rich fruit

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ABSTRACT

To investigate the polysaccharides of cubiu (*Solanum sessiliflorum* D.) fruits, pulp and peel were subjected to sequential extractions using different solvents, giving rise to pectins heterogeneous with respect to their structural characteristics, xylan, arabinogalactoxyloglucans and glucomannans. Chemical, physicochemical and spectroscopic analyses were used to characterize the polysaccharides obtained. Pectins with uronic acid content of commercial grade were obtained from the peel of cubiu fruits using water (25 °C and 100 °C) and EDTA and from its pulp using water (100 °C). The fraction with the highest yield (9.6%) and uronic acid content (79.0%) extracted from the peel using water at 100 °C was chemically and structurally characterized. This fraction consists predominantly of a mixture of a homogalacturonan (degree of methyl-esterification 56.9%) and a small amount of rhamnogalacturonan I branched by type I and type II arabinogalactans. The results suggest that cubiu is a promising source of pectins with high uronic acid content extractable by cheaper and environmentally friendly method.

1. Introduction

Amazon hosts the greatest diversity of plant species in the world. Although a large number of species have been already known and studied, particularly for the use of their secondary metabolites, our understanding of the chemical diversity of Amazonian species still represents only a fraction of about 30,000 plant species existing in this biome (Skirycz, Kierszniowska, Méret, Willmitzer, & Tzotzos, 2016).

One of the Amazonian species still little known is cubiu (*Solanum sessiliflorum* Dunal). Cubiu belongs to Solanaceae family and is widely distributed in the equatorial regions of Brazil, Colombia and Peru (Mascato et al., 2015). Its pulp is edible and has a characteristic acid taste, which has been described as close to a lemon and a tomato (Agudelo, Igual, Moraga, & Martínez-Navarrete, 2016). Amazon population uses cubiu in preparations such as alcoholic beverages, jellies, jams, juices, and sauces (Mascato et al., 2015).

Dried and milled cubiu has been sold in Brazil as a nutritional supplement and 16 cubiu products have been registered in the National Health Surveillance Agency until 2016. These products are often recommended for control of diabetes, cholesterol, triglycerides, uric acid and hypertension, among others. However, few studies were found in literature demonstrating the effects of cubiu in health.

According to Pires, Silva, Nardelli, Gomes, and Ramos (2006), cubiu is rich in pectin. The National Institute for Amazonian Research – INPA (2014) reported that some Brazilian farmers have been cultivating cubiu to export the fruit to Japan, where they are used to extract pectins. However, no study was found in literature concerning pectins or other polysaccharides from cubiu.

Fruits are a major source of dietary fibers which mainly consists of cell wall polysaccharides. Cell wall polysaccharides are categorized into three main groups: pectins, hemicelluloses and cellulose (Cosgrove, 2005).

Cellulose is the most abundant polysaccharide in plant cell wall forming mechanically strong crystalline microfibrils (Cosgrove, 2005; Caffall & Mohnen, 2009).

Associated with the cellulose microfibrils, are hemicelluloses, which are quite heterogeneous with respect to chemical composition and structure and comprise xyloglucans, mannans, xylans and the mixed-linkage β-glucans (Ebringerová, Hromádková, & Heinze, 2005).

Pectins form a matrix that embedded the network formed by the association of cellulose and hemicellulose in the cell wall and are also the main component of the middle lamella (Cosgrove, 2005). They include three main classes of acidic polymers: homo-

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galacturonan, rhamnogalacturonan I and rhamnogalacturonan II (Mohnen, 2008; Voragen, Coenen, Verhoef, & Schols, 2009; Yapo, 2011). Homogalacturonan is the most abundant pectic polysaccharide and is formed by a linear chain of 1,4-linked α-D-GalA residues, which can be partially methyl esterified at C-6 carboxyl and *O*-acetylated at *O*-2 or *O*-3 (Mohnen, 2008). In rhamnogalacturonan I, α-D-GalA units are interspersed by 1,2-linked α-L-*R*ha residues which may contain neutral side chains, mainly arabinan, galactan or arabinogalactan attached to *O*-4 (Voragen et al., 2009; Yapo, 2011). Rhamnogalacturonan II is the most structurally complex pectin, and is composed of an homogalacturonan backbone with side chains containing 12 types of sugars, including some rare sugars, in over 20 different linkages (Mohnen, 2008). Pectin is considered a safe food ingredient (De Cindio, Gabriele, & Lupi, 2016), widely used by food industry due to its ability to form gels (Willats, Knox , & Mikkelsen, 2006).

Together, these polysaccharides form the main structure of the cell wall, ensuring the integrity and support of plants and regulating life processes (Cosgrove, 2005). In addition to critical functions *in situ*, plant cell wall polysaccharides also have found different applications, either due to their physico-chemical properties that allow them to be applied in different segments of industry (Harris & Smith, 2006), or due to their biological effects, that lead to possibilities for therapeutic applications (Paulsen, 2001; Schepetkin & Quinn, 2006; Zong, Cao, & Wang, 2012). Both industrial and biological applications depend on the composition and chemical structure of polysaccharide.

In order to gain information about the cell wall polysaccharides from cubiu the aim of this study was to fractionate the polysaccharides from the pulp and peel of cubiu fruits through sequential extractions and subsequently characterize these fractions.

2. Materials and methods

2.1. Plant material

Cubiu fruits were obtained from General Warehouses Company of São Paulo, São Paulo, Brazil. Its pulp and peel were separated and freeze-dried. The dried materials were ground into powder using an analytical mill (IKA A-11; IKA, Germany) and then treated with absolute ethanol (1:10 w/v) for 20 min under reflux affording the alcohol insoluble residue of pulp (AIR-P) and the alcohol insoluble residue of peel (skin) (AIR-S). AIR-P and AIR-S were subjected to a pretreatment with DMSO 90% for 12 h under mechanical stirring for starch removal. The resulting AIRs, free of starch, were then used for polysaccharide extractions.

2.2. Starch content

Total starch was determined by a colorimetric method using the K-TSTA 07/11 kit (Megazyme, Ireland).

2.3. Sequential extraction of cell wall polysaccharides

After starch removal, AIR-P and AIR-S were sequentially extracted with water, EDTA and citric acid to isolate pectins (Carpita & Kanabus, 1987; Voragen et al., 1995). 2 M and 4 M NaOH were employed to solubilize hemicelluloses (Lindblad & Albertsson, 2005).

Sequential extractions were based on the works of Bochicchio, Petkowicz, Alquini, Busato, and Reicher (2006) and Vriesmann and Petkowicz (2009), and were carried out according to the flowchart in Fig. 2.

All extractions were carried out with each solvent under mechanical stirring using a liquid-solid ratio of 10 ml/g. After extraction, the extract was centrifuged (10,000 rpm for 20 min at 4 °C) and the residue was used for the next extraction. For water, EDTA and citric acid extractions, the polysaccharides from the extract were directly precipitated using ethanol (3:1 v/v). In the citric acid extraction, a solution of 0.1% citric acid was used to adjust the extraction pH to 2.5. For the alkaline extractions, the extracts were first precipitated by neutralization using 50% (v/v) acetic acid and centrifuged (resulting in hemicellulose A). The resultant supernatant were dialyzed (12,000 Da, 3 days against tap water) and precipitated as described above (resulting in hemicelulloses B). All polysaccharides were recovered by centrifugation (10,000 rpm for 20 min at 4 °C), washed three times with ethanol and dried under vacuum. Water extractions were performed at 25 °C for 15 h and then at 100 °C for 2 h, and afforded fractions W and HW, respectively. Then, 0.05 M EDTA was employed for 4 h yielding fractions EDTA and citric acid, pH 2.5 at 70 °C for 30 min yielded fractions CA. Alkaline extractions were then performed using 2 M and 4 M NaOH in the presence of 20 mM $NaBH_4$ at 25 °C for 16 h and provided fractions HA2, HB2, HA4 and HB4. Polysaccharide fractions from pulp carried letter P at the end of sample name, while those obtained from the skin carried letter S.

2.4. Monosaccharide composition

Neutral monosaccharides were determined after total acid hydrolysis with 2 M trifluoroacetic acid (6 h, 100 °C). The monosaccharides, obtained on evaporation to dryness, were reduced with $NaBH_4$ (Wolfrom & Thompson, 1963b) and then acetylated with pyridine-acetic anhydride (1:1 v/v, 16 h, at 25 °C) (Wolfrom & Thompson, 1963a). The resulting alditol acetates were extracted with $CHCl_3$ and analyzed by GC–MS (Varian, Saturn 2000R – 3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer), using a DB-225-MS column (0.32 mm internal diameter × 30 m × film thickness 0.25 μm) programmed from 50 to 220 °C at 40 °C/min, with He as carrier gas at 1 ml/min.

Uronic acids content was quantified by colorimetric *m*-hydroxybiphenyl method, according to Blumenkrantz and Asboe-Hansen (1973), using galacturonic acid as standard. The identity of the uronic acid was determined by anion exchange chromatography with pulse amperometric detection (HPAEC-PAD). After hydrolysis with 2 M TFA (8 h, 100 °C), drying and repeated washing until total removal of the acid, the sample (1 mg/mL) was filtered through a membrane of 0.22 μm, injected in a Thermo Scientific Dionex ICS-5000 chromatograph (Thermo Fisher Scientific, USA) with CarboPac PA20 column (3 × 150 mm) using gradient of 1 M NaOH and 1 M NaOAc as eluent (Nagel, Sirisakulwat, Carle, & Neidhart, 2014) in N_2 atmosphere in a flow of 0.2 ml/min at 30 °C. Analyses were carried out in triplicate. Data were collected and analyzed using the Chromeleon™ 7.2 Chromatography Data System software.

For AIR, the sample (10 mg) was treated with 72% (w/w) H_2SO_4 for 1 h in an ice bath, diluted to 8% and kept at 100 °C for 15 h (Saeman, Moore, Mitchell, & Millet, 1954). The hydrolysate was filtered and divided into two aliquots: one was used for determination of uronic acids contents by the *m*-hydroxybiphenyl method; the other one was neutralized with $BaCO_3$, the insoluble material was removed by filtration and the solution was reduced, acetylated and analyzed by GC–MS as described above.

2.5. Degree of methyl esterification

The Fourier transform-infrared (FT-IR) spectra of pectins were collected at the absorbance mode in the frequency range of 4000–400 cm^{-1} using a Vertex 70 spectrophotometer (Bruker, Germany), at 4 cm^{-1} resolution. Spectroscopic grade KBr powder was used and discs were prepared using a 99:1 salt/sample proportion. The degree of methyl-esterification was determined by