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Research article

A comparative study of mucilage and pulp polysaccharides from tamarillo fruit (*Solanum betaceum* Cav.)

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ABSTRACT

A comparative study of mucilage (locular tissue) and pulp polysaccharides from ripe tamarillo fruits (*Solanum betaceum* Cav.) was carried out. After aqueous and alkaline extractions and various purification steps (freeze–thaw and α-amylase - EC 3.2.1.1 treatments, Fehling precipitation and ultrafiltration through 50 kDa cut-off membrane), the obtained fractions from mucilage were analyzed by sugar composition, HPSEC, and NMR spectroscopy analyses. The results showed that the mucilage of tamarillo contains a highly methoxylated homogalacturonans mixed with type I arabinogalactans, a linear (1 → 5)-linked α-L-arabinan, and a linear (1 → 4)-β-D-xylan. A comparison with polysaccharides extracted from the pulp revealed that differences were observed in the yield and in the ratio of extracted polysaccharides. Moreover, structural differences between pulp and mucilage polysaccharides were also observed, such as in the length of side chains of the pectins, and in the degree of branching of the xylans.

1. Introduction

Tamarillo (*Solanum betaceum* Cav. syn *Cyphomandra betacea* Sendt.) is a small tree native from the Andes and belongs to same taxonomic genus of tomato. Tamarillo fruits look much like an oval-shaped tomato, but their succulent flesh has unique flavor quite unlike that of its famous cousin (Popeone et al., 1989). Tamarillo cultivars are distinguished based mainly on fruit colors, which are solid deep-purple, blood-red, orange or yellow, or red and-yellow (Acosta-Quezada et al., 2011; Morton, 1987). The flesh color of tamarillo fruits depends on their content of chlorophylls, carotenoids, and anthocyanins, providing significant amounts of these bioactive components (Acosta-Quezada et al., 2015; Espin et al., 2016; Mertz et al., 2010; Osorio et al., 2012).

Concerning the carbohydrates content, tamarillo fruits contain low levels of sugars (fructose, glucose and sucrose) compared to other tropical fruits and contain approximately 3% of fiber (Boyes and Strübi, 1997; Vasco et al., 2009). Gannasin et al. (2015) studied the hydrocolloids from pulp and mucilage of tamarillo and characterized them as high molecular weight hemicellulosic polysaccharide and as arabinogalactan-protein associated with pectin, respectively. In previous work, we described the chemical structure of a type I arabinogalactan and of a glucuronoarabinoxylan extracted from tamarillo pulp (do Nascimento et al., 2015; do Nascimento et al., 2013). Herein, we investigate the structure of polysaccharides obtained from the mucilage (locular tissue) that surround the seeds of orange tamarillo fruits and compare them with the structure of polysaccharides obtained from the pulp.

2. Materials and methods

2.1. Plant material

Ripe fruits of tamarillo (*Solanum betaceum* Cav), orange type, were collected in the town of Prudentópolis, State of Paraná (PR), Brazil. A voucher specimen was deposited in the UPCB (Herbarium of the Federal University of Paraná), registration number 72896.

2.2. Polysaccharide extraction and fractionation

Seeds surrounded by mucilage (locular tissue) were manually separated from pulp (pericarp) with the aid of a knife, yielding 2.3 kg of material. For removal of the seeds, distilled water was added to this material and it was strained with the aid of a sieve. The strained material (1 L) was maintained overnight at room

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temperature with stirring, and then centrifuged (12,000*g*, 20 min at 25 °C) (Fig. 1). Distilled water (1 L) was again added to the residue and it was maintained overnight at room temperature with stirring, followed by centrifugation. This process was repeated once more. The obtained supernatants were combined and concentrated under reduced pressure. The polysaccharides were precipitated with ethanol (3 vol), collected by centrifugation (12,000*g*, 20 min at 4 °C), dialyzed with tap water (12–14 kDa cut-off membrane) and freeze-dried, giving fraction MWC. The retained seeds on the sieve were lyophilized giving 172 g of material.

The mucilage residue (Residue 1, Fig. 1) was then exhaustively extracted with hot water (1 L each, x 6, at 100 °C) under reflux for 2 h. The hot water extracts were obtained from the supernatants after centrifugation (12,000*g*, 20 min at 25 °C), joined and concentrated under reduced pressure. Then, the polysaccharides were precipitated by ethanol (3 vol), centrifuged, dialyzed and freeze-dried, giving fraction MWH. Finally, the remaining residue (Residue 2, Fig. 1) was submitted to alkaline extraction with aq. 10% KOH (1 L each, x 3, at 100 °C) under reflux for 2 h. The alkaline extracts were neutralized with acetic acid, dialyzed for 48 h with tap water, concentrated under reduced pressure and freeze–dried, giving fraction MK.

As a first purification step, a freeze-thaw treatment was applied in fractions MWC, MWH and MK. In this procedure, the sample is frozen and then thaw at room temperature followed by centrifugation (Gorin and Iacomini, 1984). The freeze-thaw treatment was repeated until no more precipitate appeared, to give cold-water soluble (SMWC, SMWH and SMK, respectively) and insoluble fractions (PMWC, PMWH and PMK, respectively) (Fig. 1). In order to remove starch, these fractions were extensively treated with α-amylase (EC 3.2.1.1, from *Bacillus licheniformis*, Sigma A3403, 100 units/ml, 24 h at 37 °C) and dialyzed.

The fraction SMWC was submitted to ultrafiltration through membrane with cut-off of 50 kDa (14650-47D, Sartorius), yielding eluted (SMWC-50E) and retained (SMWC-50R) fractions on the membrane. On the other side, fraction SMWH was dissolved in distilled water and then treated with Fehling's solutions Jones and Stoodley (1965), resulting in an insoluble polysaccharide-copper complex (SMWH-PF) and a soluble fraction (SMWH-SF), which were separated by centrifugation (12,000*g*, 15 min at 15 °C). Each fraction was neutralized with acetic acid (HOAc), dialyzed against tap water and deionized with a cation exchange resin (Fig. 1).

The yields of polysaccharide fractions were expressed as percent based on the weight of dried tamarillo mucilage that was submitted to extraction (298 g), whereas the moisture was expressed as percent based on the weight of wet tamarillo mucilage plus seeds (2.3 kg).

2.3. Sugar composition

Neutral monosaccharide components of the polysaccharides (2 mg) and their ratios were determined by hydrolysis with 2 M TFA (1 ml) for 8 h at 100 °C. The acid was evaporated and the residue was dissolved in water (1 ml), then the hydrolyzates were converted to alditol acetates by successive sodium borohydride reduction, followed by acetylation with acetic anhydride/pyridine (1/1, v/v, 1 ml) at 100 °C for 30 min. The resulting alditol acetates were analyzed by GC–MS using a Varian gas chromatograph and mass spectrometer, model Saturn 2000R, with He as carrier gas. A capillary column (30 m × 0.25 mm i.d.) of DB-225, held at 50 °C during injection for 1 min, then programmed at 40 °C/min to 220 °C and held at this constant temperature for 19.75 min was used for the quantitative analysis. The alditol acetates were identified by their retention times and typical electron impact breakdown profiles.

Uronic acid contents were determined spectrophotometrically using the *m*-hydroxybiphenyl method (Filisetti-Cozzi and Carpita, 1991), using galacturonic acid as standard.

2.4. Carboxy-reduction of polysaccharides

Fraction SMWC-50E was carboxy-reduced by the carbodiimide method (Taylor and Conrad, 1972), using sodium borohydride as the reducing agent, giving products with the COOH groups of their

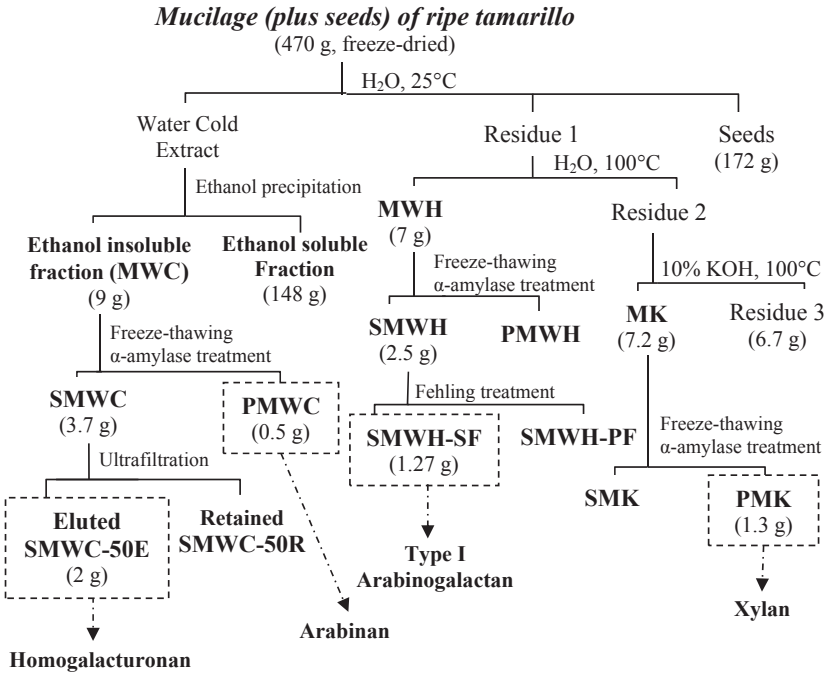

Fig. 1. Scheme of extraction and fractionation of pectic and hemicellulosic polysaccharides from mucilage of tamarillo fruits *(Solanum betaceum Cav.)*.