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Acidic Polysaccharides from *Psidium cattleianum* (Araçá)

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ABSTRACT

The aim of this work was to study the acidic polysaccharides of Psidium cattleianum*. Pectic fractions were obtained by successive extractions with water, EDTA and sodium carbonate (4 and 25ºC). Monosaccharide composition, total carbohydrates, uronic acids and protein contents were determined for each fraction. The yield of water and EDTA-soluble fractions and high content of uronic acid were consistent with the presence of pectins probably arising from the middle lamellae. On the other hand, sodium carbonate-soluble pectins had a higher neutral sugar content, indicating highly branched polysaccharides in these fractions, consistent with pectins from cell wall.*

Key words: Pectins, acidic polysaccharides, *Psidium cattleianum*, cell wall

INTRODUCTION

Pectins are structural polysaccharides, involved with many aspects of plant physiology and pathology. They are commercially important due to their gelling power under certain conditions and due to this property, they are used in jams, jellies and marmalade, confectioneries and mild acidic products. They have been well studied for applications in acidic food products, because of their stability at low pH values (May, 1990; Pilnik and Voragen, 1992; Ridley et al., 2001; Rolin, 1993). Pectins are also used for different pharmaceutical purposes and other applications such as dentistry, cosmetics, cigarette manufacture, etc (Endress, 1991).

Pectic polysaccharides are probably the most complex and variable of cell wall polysaccharides (Bacic et al., 1988). They include a family of acidic polymers such as homogalacturonans and rhamnogalacturonans. Homogalacturonans are defined as polymers consisting predominantly of α-(1→4) galacturonan residues. Pure homogalacutronan have been rarely reported. Rhamnogalacturonans comprise two types: rhamnogalacturonans I (RG-I) and rhamnogalacturonans II (RG-II) (O'Neill et al., 1990). They are characterized by a backbone in which "smooth" α-(1→4) galacturonan regions are interrupted by branches rhamnogalacturonan regions, highly substituted by neutral sugar-rich, side chains. It has been suggested that only up 10% of the galacturonosyl residues occur in the ramified ("hairy") regions, while these regions contain almost all of the neutral sugar residues. Characteristic of RG-II is the presence of rare sugars such as 2-*O*-methyl-xylose, apiose, 2-*O*-methyl-fucose, aceric acid, Kdo (2-keto-3-deoxi-lixo-2-heptolosaric acid) next to more common sugar residues (De Vries et al., 1982). Moreover,

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the galacturonic acid residues present in pectins can be partly methyl esterified at C-6 (Pilnik and Voragen, 1970), and the hydroxyl groups at position O-2 and/or O-3 partly acetyl-esterified (Roumbouts and Thibault, 1986). According to the degree of methylation (DM or DE), pectins are classified as high-methoxyl (HM) with DM > 50 or low-methoxyl (LM) with DM < 50.

Gelation of pectins is affected by several parameters such as the DM, molecular weight and charge distribution along the backbone. Pectins can form gel by two different mechanisms: high-methoxyl pectins (HM) require a relatively high concentration of soluble solids (sucrose) and a low pH. LM pectins require only the presence of divalent cations, particularly Ca^{+2} and can be used in low calory products (Rolin, 1993; Ridley et al., 2001).

Pectins have a number of applications and new sources of these polysaccharides are constantly sought (May, 1990). Although pectin commonly occurs in most plant tissues, as a cementing substance in the middle lamellae and as a thickening on the cell wall, the number of sources commercially used is quite limited (Simpson et al, 1984). Commercial pectins are almost exclusively derived from either citrus peels or apple pomace (Canteri-Schemin et al., 2005; May, 1990).

In Brazil, there is such an abundance of fruits, many of which are not collected, thus left rotting in the field. These fruits could contribute as a source of raw materials for the industry. The fruits of araçazeiro (*P. cattleianum*), popularly called araçá, are used in domestic jam and jelly production. In view of the utility of pectins, these native Brazilian species are under investigation. Thus, the aim of this work was to study the chemistry and the monosaccharide composition from fruit mesocarp fractions.

MATERIALS AND METHODS

Fruit samples

The fruits of araçazeiro (*P. cattleianum*), family Myrtaceae, were colleted at initial stage of ripening in December 2002 from trees in Ponta Grossa-PR. To minimize the time that fruit was exposed to ambient temperature, it was processed within 24 h of harvesting. The seeds were removed and the mesocarp or flesh (edible portion of the fruit) was stored at -10 ºC.

Cell-wall material preparation and isolation of alcohol insoluble residues (AIR)

The fruit mesocarp (~500 g) was processed in liquefier blender and submitted to enzyme inactivation with ethanol under reflux for 20 min, immediately cooled to room temperature and centrifuged at 5000 x g for 15 min. The alcohol insoluble residue (AIR) was centrifuged at 20000 x g for 20 min, washed three times with ethanol (96%) and dried under vacuum at 40 ºC for 6 h. This fraction was obtained in duplicate and the yield was determined in relation to mesocarp.

Polysaccharide fractionation

AIR from araçá was subjected to sequential fractionation. About 80 g of AIR was mixed with 1000 mL of water at 25 ºC for 3h. Suspension was centrifuged at 20000 x g for 20 min. Supernatant was separated, concentrated in rotary evaporator and precipitated with 2 volumes of ethanol (96%). After centrifugation, the precipitated polysaccharides were dried under vacuum at 40 ºC for 4 h and a water soluble fraction was obtained. Afterwards, the water-insoluble residue was treated with 1000 mL of 2% (w/v) EDTA solution at 25 ºC for 6 h and centrifuged at 20000 x g for 20 min. Supernatant was treated in accordance with the former experimental procedure and one EDTA-soluble fraction was obtained. The EDTA-insoluble pellet was submitted at two consecutive extractions with 1000 mL of 50 mM Na_2CO_3 solution containing 20 mM $NaBH_4$, each step. The first step was done at 4 ºC for 4 h and the second step at room temperature for other 4h. After centrifugation at 20000 x g for 20 min, supernatants resulting from each step were separated and pH of the extracts was adjusted to 6.0 with 50% acetic acid. The solution was dialyzed against distilled water for 24 h at 25 ºC, concentrated, precipitated and the final solid was the carbonate soluble fractions. Finally, the carbonate-insoluble residue was extracted with 1000 mL of 2M KOH solution containing 20 mM $NaBH_4$ at 14 ºC for 2h. After centrifugation at 20000 x g for 20 min, alkaline extract was separated, neutralized, extensively dialyzed, concentrated, precipitated and a alkali soluble fraction was obtained. The remaining residue was washed until neutral pH and dried. The sequential extractions were performed in duplicate. The fractions yields were expressed in relation to AIR.

General methods

Polysaccharide fractions were hydrolyzed with 1M trifluoroacetic acid solution (5 h, 100 ºC); the hydrolyzates were evaporated and the residues were reduced with $NaBH_4$. Then the products were acetylated with pyridine-acetic anhydride (1:1 v/v, 16 h, at 25 ºC). The resulting alditol acetates were analyzed by gas-liquid chromatography (GLC) using a 5890 S II HP Gas Chromatograph at 220 ºC (FID and injector temperature, 250 ºC) with a DB-210 capillary column (0,25 mm i.d. x 30m), film thickness 0.25 µm, the carrier gas being nitrogen (Sloneker, 1972).

Paper chromatography (PC) of the hydrolizates was conducted by the ascending method on Whatman no 1 paper using benzene:1-butanol:pyridine:water (1:5:3:3 v/v; upper phase) solvent system. Sugars were detected using alkaline $AgNO_3$, dip reagent (Trevelyan et al., 1950). Total sugars were determined according to the phenol-sulphuric acid (Dubois et al., 1956), and their values were calculated using glucose as external standard. Uronic acid was estimated by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973), using galacturonic acid as standard and protein content were determined by the Hartree method (1972) using BSA as standard. All fractions were analyzed in triplicate by colorimetric methods as above.

RESULTS AND DISCUSSION

During cell wall preparation, it is indispensable to inactivate the hydrolytic enzymes presents in the fruit which could act on component polysaccharides. In the present work, extraction of free solids was performed with boiling ethanol because of the proven inactivation effect that ethanol exerted against polygalacturonases and other hydrolases (Sáenz et al., 2000; Koch and Nevins, 1989; Rose et al., 1998). Also, this treatment eliminated the autolytic formation of reducing end groups on polysaccharides from subsequent cell wall preparations (Carrington et al., 1993). Pectins are spatially localized in cell-wall domains and its composition varies with the source from which they are isolated, as well with the conditions applied during isolation and purification (Rolin, 1993).

Four pectin fractions soluble in water, EDTA, cold dilute sodium carbonate and sodium carbonate at room temperature were isolated (Table 1).

Table 1 - Determination* of carbohydrates and proteins of fractions from araçá (*P. cattleianum*).

Fraction	Yield[a]	Total sugar[b]	Protein[c]
Water	3.8	49.6	8.5
EDTA	1.0	51.0	6.9
Na_2CO_3, 4ºC	1.1	45.1	7.3
Na_2CO_3, 25ºC	1.2	78.2	6.2
KOH	0.6	60.2	11.1

*: Values determined in %.
a: in relation to AIR.
b: Determined by phenol-sulfuric method (Dubois et al., 1956).
c: Determined by Hartree method, 1972.

Sequential extractions of AIR afforded fractions with yields between 0.6 to 3.8 %. These values were lower than those ones obtained for other fruits using similar methods (Redgwell et al., 1992; Beleski-Carneiro et al., 2000). This could be due the presence of brachysclereides petrous cells in the fruits mesocarp, as observed by photonic microscopy (Fig. 1). The molecular weight of Na_2CO_3 extractable pectins were higher than those extracted with EDTA (McCann and Roberts, 1991).

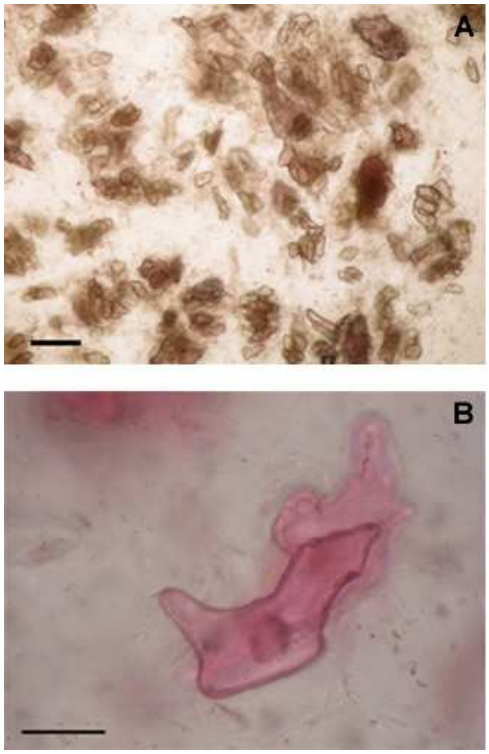

Figure 1 - Brachysclereides petrous cells from araçá pulp. Bars: A = 200 μm, B = 50 μm.

It is known that pectins are very susceptible to chemical treatments. In basic or neutral media, polygalacturonate chains can suffer scission via β-elimination (Kravtchenko et al., 1992). It has been well established that treatments involving alkaline pH and/or the use of heat promote this reaction of pectins (Kiss, 1974). In the present work, AIR yielded 22% and was the raw material for sequential extractions.

Total sugar content was higher in de-esterified fractions. The protein content (8.5 to 11.0%) determined by a modification of the Lowry procedure was overestimated by the presence of phenolic compounds, detected in all the fractions by ultraviolet spectroscopy (at 280 nm), which interfered in the method used. However, the protein content was also determined by the Peterson method (1977) which eliminated the phenolic compound interference and the value fell to 2.3 to 3.4%. In the walls of sugar beet and spinach, some pectic arabinosyl and galactosyl residues are esterified with ferulic or coumaric acid and these phenolic residues are potential sites for cross-linking *via* oxidative coupling. An unidentified phenolic material has been reported to be responsible by hemicellulose-pectin cross-linking in the walls of cauliflower (Ridley et al., 2001). The monosaccharide composition of the pectins and hemicellulosic fractions are shown in Table 2.

Table 2 - Monosaccharide composition[a] of polysaccharides obtained from the mesocarp of araçá (*P. cattleianum*).

Fraction	Uronic acid[b]	Ara	Gal	Rha	Man	Glc	Fuc	Xyl
Water	30.0	50.3	10.4	0.1	-	-	4.1	5.1
EDTA	42.6	37.8	8.8	2.7	0.7	4.3	1.0	3.1
Na_2CO_3, 4 ºC	20.0	34.2	16.4	4.2	4.1	12.5	1.3	7.4
Na_2CO_3, 25 ºC	21.6	37.2	17.9	4.6	3.7	11.2	-	3.8
KOH	19.8	2.8	2.5	1.0	-	4.1	-	69.1

a: determined by GLC, Mol %

b: determined by BLUMENKRANTZ and ASBOE-HANSEN method, 1973.

Different pectin subpopulations can be distinguished by their sugar composition and uronic acid amount (Redgwell and Selvendran, 1986). Paper chromatography showed the qualitative profile of the pectic fractions, which was similar to that described for apple pectin by Schols et al. (1995). In the present work, non-associated cell-wall polysaccharides were solubilized with water. Colorimetric data showed the presence of 30% of uronic acids in this fraction, and by GLC analysis, this fraction showed appreciable arabinose content (50.3 %). A portion of pectin with the highest content of uronic acid (42.6%) was solubilized by treatment with aqueous EDTA. This fraction is believed to originate from pectins that are not covalently linked to the cell wall and are only associated by calcium. The high proportion of uronic acid in both fractions was consistent with predominance of "smooth" regions, typically found in pectins components of middle lamella (McCann and Roberts, 1991). Pectins that were covalently cross-linked to the cell wall were extracted by Na_2CO_3, which broke interpolimeric ester linkages, and were removed with 50 mM Na_2CO_3 in two steps; the first one at low temperature (4 ºC) and the second one at ambient temperature (25 ºC). This stepwise procedure and the use of 20 mM $NaBH_4$ in extraction solution reduces pectin degradation by β-elimination (Fischer et al., 1994).These fractions have about 20% of uronic acid and high content of neutral sugars, mainly arabinose and galactose, suggesting the presence of arabinans and galactans as side chains. The highest ratio of rhamnose: uronic acid was also observed for these fractions, indicating the presence of rhamnose rich zones that carried neutral oligosaccharide side chains, "hairy" regions.

Hemicelluloses (called cross-linking glycans) which were hydrogen-bond to cellulose microfibrils (Carpita and McCann, 2000; Redgwell et al., 1986), were extracted with 2 M KOH, yielding a fraction composed mainly of xylose and uronic acid, probably an acidic xylan. In this case, $NaBH_4$ was included as a reducing agent of chain ending aldehyde groups in order to minimize alkaline degradation.

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RESUMO

Psidium cattleianum, árvore nativa do sul do Brasil, produz frutos popularmente conhecidos como araçá e que muitas vezes não são colhidos e estragam nos campos. Neste trabalho, os frutos de *P. cattleianum* foram utilizados como fonte de polissacarídeos ácidos. Frações de pectinas foram obtidas por extrações seqüenciais com água, agente quelante (EDTA) e carbonato de sódio (4 e 25ºC). A composição monossacarídica, os teores de açúcar total, ácidos urônicos e proteínas foram determinados para cada fração. O rendimento das frações solúveis em água e EDTA e o elevado teor de ácidos urônicos são consistentes com a presença de pectinas oriundas provavelmente da lamela média. Por outro lado, as pectinas solúveis em carbonato de sódio têm o maior teor de açúcares neutros, indicando a presença de polissacarídeos altamente ramificados nestas frações, consistentes com pectinas da parede celular.

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