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Structural characterization of a rhamnogalacturonan I-arabinan-type I arabinogalactan macromolecule from starfruit (*Averrhoa carambola* L.)

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ABSTRACT

A structural characterization of polysaccharides obtained from edible tropical fruit named starfruit (*Averrhoa carambola* L.) was carried out. After fractionation by freeze–thaw and Fehling precipitation, a pectic polysaccharide was obtained. It was composed of rhamnose, arabinose, galactose and uronic acid in the 5.0:72.5:12.1:10.4 molar ratios, respectively. A combination of monosaccharide, GPC, methylation and NMR analysis and enzymatic hydrolysis with endo-β-(1 → 4)-D-galactanase showed the presence of a rhamnogalacturonan I to which a branched arabinan and a type I arabinogalactan are attached. The arabinan moiety was formed by (1 → 5)-linked α-L-Ara*f* units in the backbone, branched only at *O*-3 by (1 → 2)- and (1 → 3)-linked α-L-Ara*f* units, while the type I arabinogalactan was formed by (1 → 4)- and (1 → 4,6)-linked β-D-Gal*p* units in the backbone with (1 → 5)-, (1 → 3,5)- and (1 → 3)-linked α-L-Ara*f* units as side chains.

1. Introduction

Pectins are a family of closely associated complex polysaccharides present in all plant primary cell walls and intercellular regions of higher plants (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). They have common features, but are extremely diverse in their fine structures (Ridley, O'Neill, & Mohnen, 2001). Homogalacturonan (HG), rhamnogalacturonan I (RG-I) and substituted galacturonans such as rhamnogalacturonan II (RG-II), xylogalacturonan (XGA), apiogalacturonan (A_pGA), galactogalacturonan (GGA), arabinogalacturonan (A_rGA) and galacturonogalacturonan (GaGA) are the pectic polysaccharides in all primary cell walls that have been studied. Rhamnogalacturonan I is composed by a backbone with repeating units of alternating rhamnose (Rha) and galacturonic acid (GalA) linked [→ 4-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 →]. The side chains, mainly linked to the *O*-4 of some of the α-L-Rha*p* units, could be arabinans, galactans, type I and/or type II arabinogalactans (AG) (Yapo, 2011).

RG-I have been reported in some fruits up to now, as for example, in apple cellulosic residue (Oechslin, Lutz, & Amadò, 2003), in caja fruits (*Spondias cytherea*) (Iacomini et al., 2005), in citrus peels (Yapo, Lerouge, Thibault, & Ralet, 2007), in the pulp of *Argania spinosa* (Aboughe-Angone et al., 2008), in albedo cell walls of orange (*Citrus sinensis*) (Prabasari, Pettolino, Liao, & Bacic, 2011) and in prunes (*Prunus domestica*) (Cantu-Jungles et al., 2014).

Averrhoa carambola L., also known as starfruit or carambola, is an edible tropical fruit of the Oxalidaceae family, it has an oblong shape with three to six longitudinal ribs, resulting in a star-shaped cross-section when cut. This specie is native of Malaysia and cultivated in various other Asian countries and the tropical areas of America, including Brazil (Manda, Vyas, Pandya, & Singhal, 2012; O'Hare, 1993; Soncini et al., 2011).

Carbohydrates are the main macronutrient present in starfruit (Manda et al., 2012), however, there is no studies dealing with the identification and chemical characterization of starfruit's polysaccharides. Thus, this paper reports the isolation, composition and structural features of a pectin, formed by a rhamnogalacturonan I to which a branched arabinan and a type I arabinogalactan are attached.

2. Materials and methods

2.1. Plant material

Ripe fruits of starfruit (*A. carambola* L.) from cultivar B10 were purchased in a local market in Curitiba, State of Paraná, Brazil.

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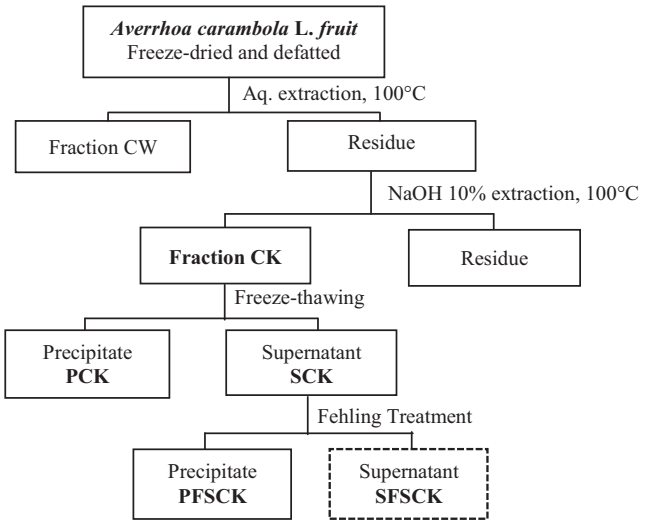

Fig. 1. Scheme of extraction and fractionation of fraction SFSCK from the fruit of starfruit (*Averrhoa carambola* L.).

2.2. General analytical methods

All solutions were evaporated below 60 °C under reduced pressure. Dialysis was performed using 12–14 kDa cut-off membrane.

The total lipid quantification was performed by extraction employing chloroform–methanol (1:1) as solvent through Soxhlet apparatus.

The fraction was carboxy-reduced by the carbodiimide method (Taylor & Conrad, 1972), using $NaBH_4$ as the reducing agent, giving products with the –COOH groups of its uronic acid residues reduced to $–CH_2OH$.

2.3. Extraction and purification of polysaccharides

The fruits were washed, cut and the seeds were manually removed. The fruits were freeze-dried and milled. Dried pulp powder (310.7 g) was defatted with chloroform–methanol (1:1), yielding a moisture and nonpolar compounds content of approximately 88% and 4%, respectively. Polysaccharides were extracted from the residue with boiling water under reflux for 2 h (×6, 1 L each). The aqueous extracts were obtained by centrifugation (12,000 × *g*, 20 min at 10 °C), combined and concentrated under reduced pressure. The polysaccharides were precipitated with ethanol (3 vol.), collected by centrifugation (12,000 × *g*, 20 min at 10 °C), and freeze-dried, giving fraction CW (7% yield).

The remaining residue was then extracted with aq. 10% NaOH under reflux at 100 °C for 2 h (×6, 1 L each) in the presence of $NaBH_4$. The alkaline extracts were obtained by centrifugation (12,000 × *g*, 20 min at 10 °C), neutralized with acetic acid (HOAc), dialyzed against tap water, concentrated under reduced pressure and freeze-dried, giving fraction CK, with 5% yield (Fig. 1). A freeze–thaw treatment was applied in fraction CK, to give cold-water soluble and insoluble fractions SCK and PCK, respectively. In this procedure, the sample was frozen and then thawed at room temperature followed by centrifugation, until no more precipitate appeared.

Fraction SCK was dissolved in distilled water and then treated with Fehling's solutions (Jones & Stoodley, 1965), resulting in a soluble fraction SFSCK and a precipitated fraction PFSCK, which were separated by centrifugation (12,000 × *g*, 20 min at 10 °C). Each fraction was neutralized with acetic acid (HOAc), dialyzed against tap water and deionized with cation exchange resin.

The yields of polysaccharides were expressed as % based on weight of dried starfruit that was submitted to extraction (310.7 g).

2.4. Monosaccharide analyses

Neutral monosaccharide components and their ratios were determined after hydrolysis of the polysaccharides with 2 M trifluoroacetic acid (TFA) for 8 h at 100 °C, followed by conversion to alditol acetates by reduction with $NaBH_4$ and acetylation with acetic anhydride-pyridine (1:1, v/v, at 100 °C for 30 min). These 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 uronic acid contents were determined using the m-hydroxybiphenyl colorimetric method (Filisetti-Cozzi & Carpita, 1991), using galacturonic acid as standard.

2.5. Determination of homogeneity and molecular weight of polysaccharides

The homogeneity and molecular weight of water soluble polysaccharides were determined by gel permeation chromatography (GPC), using a refractometer and a ultraviolet detector (at 280 nm) as detection equipments. Four columns were used in series, with exclusion sizes of 7×10^6 Da (Ultrahydrogel 2000, Waters), 4×10^5 Da (Ultrahydrogel 500, Waters), 8×10^4 Da (Ultrahydrogel 250, Waters) and 5×10^3 Da (Ultrahydrogel 120, Waters). The eluent was 0.1 M aq. $NaNO_2$ containing 200 ppm aq. NaN_3 at 0.6 ml/min. The samples, previously filtered through a membrane (0.22 μm, Millipore), were injected at a concentration of 1 mg/ml. To obtain the molecular weight, standard dextrans (487, 266, 124, 72.2, 40.2, 17.2 and 9.4 kDa, from Sigma) were employed to obtain the calibration curve. The molecular weight of the samples was calculated according to the calibration curve.

2.6. Methylation analysis of polysaccharide

Prior to glycosyl linkage analysis, uronic acids were reduced using carboxyl reduction method of Taylor and Conrad (1972). Then the carboxyl-reduced sample was *O*-methylated according to the method of Ciucanu and Kerek (1984), using powdered NaOH in DMSO-MeI. The per-*O*-methylated polysaccharide was then submitted to methanolysis in 3% HCl–MeOH (at 80 °C, 2 h) followed by hydrolysis with H_2SO_4 (0.5 M, 12 h, at 100 °C) and neutralization with $BaCO_3$. The resulting mixture of partially *O*-methylated monosaccharides was successively reduced with $NaBD_4$ and acetylated with acetic anhydride–pyridine. The products (partially *O*-methylated alditol acetates) were examined by capillary GC–MS. 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 210 °C and held at this temperature for 31 min was used for separation. The partially *O*-methylated alditol acetates were identified by their typical electron impact breakdown profiles and retention times (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005; Sassaki, Iacomini, & Gorin, 2005).

2.7. Nuclear magnetic resonance (NMR) spectroscopy

^{13}C $\{^1H\}$ NMR spectra were acquired at 50 °C on a Bruker AVANCE III 400 NMR spectrometer, operating at 9,5 T, observing ^{13}C at 100.61 MHz, equipped with a 5-mm multinuclear inverse detection probe with z-gradient. The water soluble samples were acquired in D_2O. Chemical shifts were expressed as δ ppm relative to CH_3 signal from acetone at δ 30.2 as internal reference.

Table 1
Monosaccharide composition of fractions obtained from the fruit of starfruit (*Averrhoa carambola* L.).

Fractions	Monosaccharide composition (%)[a]						
	Rha	Fuc	Ara	Xyl	Gal	Glc	Uronic acid[b]
CK	1.4	–	77.5	7.2	8.7	5.2	nd[c]
SCK	1.7	tr[d]	69.1	7.4	8.7	8.0	5.0
PFSCK	–	2.3	10.2	37.8	9.6	33.3	6.8
SFSCK	5.0	–	72.5	–	12.1	–	10.4
SFSCK-GAL5	5.0	–	83.6	–	4.4	–	7.0
SFSCK-GAL10	4.5	–	83.3	–	5.2	–	7.0
SFSCK-GAL5S	–	–	70.3	–	29.7	–	–

[a] % of peak area relative to total peak areas, determined by GC–MS.
[b] Determined spectrofotometrically using the m-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991).
[c] Not determined.
[d] Trace amounts.

2.8. Enzymatic hydrolysis with endo-β-(1 → 4)-D-galactanase

Fraction SFSCK was degraded with an endo-β-(1 → 4)-D-galactanase (EC No. 3.2.1.89) from *Aspergillus niger* (Megazyme International, Ireland). A sample material (100 mg) was dissolved in 30 ml 0.02 M sodium acetate buffer (pH 4.4) and incubated with 75 μl enzyme solution at 45 °C, at two incubation times (5 and 10 h). The enzyme was inactivated at 100 °C for 10 min and after cooling down to room temperature, the reaction mixture was precipitated with cold ethanol (3 vol.) and centrifuged. Thus, the precipitates contained the enzyme resistant polymers remaining after 5 h (SFSCK-GAL5, 80 mg) and 10 h (SFSCK-GAL10, 77 mg) of incubation and the supernatants contained the fragments released by the enzymatic hydrolysis at 5 h (SFSCK-GAL5S, 20 mg) and 10 h (SFSCK-GAL10S, 23 mg) of incubation.

3. Results and discussion

Fractions CW and CK were obtained from ripe fruits of *A. carambola* employing water and aq. 10% NaOH at 100 °C, respectively. Fraction CK was chosen for further purification due its GPC elution profile, which demonstrated the presence of fewer peaks than fraction CW (data not shown). Monosaccharide analysis of the fraction CK indicated arabinose as main neutral monosaccharide (Table 1), together with galactose, xylose, glucose and rhamnose. The content of uronic acid was not determined.

A freeze–thaw treatment was applied in fraction CK, giving cold-water soluble polysaccharides (SCK, 3.5% yield) and cold-water insoluble polysaccharides (PCK, 1.4% yield). Monosaccharide analysis demonstrated that fraction SCK (Table 1) has similar composition with CK, having arabinose as major neutral monosaccharide and 5.0% of uronic acids.

The ^{13}C NMR spectrum of SCK can be seen in Fig. 2A and showed signals at δ 108.1 and δ 107.5 corresponding to anomeric carbons of α-L-Ara*f* units and a signal at δ 104.3 corresponding to β-D-Gal*p* units. These assignments could suggest the presence of an pectic arabinogalactan (Cipriani, Mellinger, Gorin, & Iacomini, 2004; Cipriani et al., 2009; Cordeiro et al., 2012; Thude & Classen, 2005; Xu, Dong, Qiu, Cong, & Ding, 2010). Although SCK contained 5% of uronic acid, no signal was observed for the carboxyl groups, due to spectral conditions. Moreover, the spectrum also showed anomeric signals of small intensity between δ 98.3 and 103.2, indicative the presence of another polysaccharide, present in small amount. This mixture was also observed in the analysis of gel permeation chromatography (GPC) of this fraction, which showed a heterogeneous elution profile (Fig. 3A).

In order to purify the arabinogalactan (AG), fraction SCK was submitted to Fehling precipitation (Fig. 1), once arabinogalactans do not complex with Cu^{++} and remain soluble in this treatment (Cantu-Jungles et al., 2014). This strategy was highly efficient, as it produced one homogeneous fraction (SFSCK, 1.9% yield), as could be seen by its elution profile on GPC analysis (Fig. 3A). Its molecular weight was 44 kDa. This fraction showed no absorption at 280 nm, indicating that it was free of protein. Moreover, it could be seen by monosaccharide analysis that all the xylose, glucose and fucose present in fraction SCK appeared in the Fehling precipitate fraction (PFSCK). PFSCK was composed by fucose, arabinose, xylose, galactose and glucose as neutral sugars (Table 1) and 6.8% of uronic acids.

Monosaccharide analysis of fraction SFSCK showed rhamnose, arabinose, galactose and uronic acid (Table 1). In order to confirm the identity of the uronic acid present in SFSCK, an aliquot of the sample was carboxy-reduced and submitted to monosaccharide analysis by GC–MS. In this procedure, the uronic acid is converted to its corresponding neutral sugar. It was observed an increase in the percentage of galactose in the GC–MS analysis of carboxy-reduced SFSCK indicating that the uronic acid present is the galacturonic acid. The presence of rhamnose and galacturonic acid probably suggested that the AG present in fraction SFSCK is linked to type I rhamnogalacturonan backbone.

The ^{13}C NMR spectrum of SFSCK is showed in the Fig. 2B and demonstrated the presence of typical signals of α-L-arabinofuranosyl units, with C-1 signals at δ 108.0, δ 107.6 and δ 107.2. Signals of the (1 → 4)-linked β-D-Gal*p* could be seen at δ 104.3 (C-1), δ 77.6 (substituted C-4), δ 74.5 (C-5), δ 73.5 (C-3), δ 72.0 (C-2) and δ 60.9 (C-6). These assignments are in agreement with published literature data (Cipriani et al., 2004, 2009; Cordeiro et al., 2012; Thude & Classen, 2005; Xu, Dong, Qiu, Cong & Ding, 2010). Furthermore, it can be observed in the spectrum, signals compatible with a rhamnogalacturonan structure (RG-I), with C-1 signals at δ 98.3 and δ 97.7 from α-L-Rha*p* and α-D-Gal*p*A units, respectively, and the presence of C-6 of Rha*p* units at δ 16.6 (Colquhoun, Ruiter, Schols, & Voragen, 1990; Renard, Lahaye, Mutter, Voragen, & Thibault, 1998). Although SFSCK contained 10.4% of galacturonic acid, the signal at δ 174.0–175.0 for carboxy groups did not appear, due to spectral conditions.

Methylation analysis of SFSCK (Table 2) was performed in the carboxy-reduced sample due to the presence of galacturonic acid. The nonreducing terminals consisted of Ara*f* (10.8%) and a small amount of Gal*p* (3.5%). The main observed derivatives were those of arabinose, which was in agreement with monosaccharide analysis. It could be seen in Table 2 the presence of high amounts of 2,3-Me_2-Ara-ol acetate, indicating the presence of (1 → 5)-linked Ara*f* units. The presence of 3-Me- and 2-Me-Ara-ol acetates indicated that this arabinan was branched at *O*-2 and *O*-3, respectively. The derivatives 3,5-Me_2- and 2,5-Me_2-Ara-ol acetates indicated (1 → 2)- and (1 → 3)-linked Ara*f* units, respectively, probably present as side chains. Arabinopyranose 2-*O*-substituted was also observed in small amounts (1.8%). The methylated derivatives of galactose were 2,3,6-Me_3- and 2,3-Me_2-Gal-ol acetates, wich correspond to (1 → 4)- and (1 → 4,6)-linked Gal*p* units. These data indicate a (1 → 4)-linked galactan as main chain, carrying branches exclusively at *O*-6, suggesting the presence of type I arabinogalactan (AG-I).

This was also observed for arabinogalactans extracted from leaves of *M. ilicifolia* (Cipriani et al., 2004), from caja fruits (Iacomini et al., 2005) and pulp of tamarillo fruits (do Nascimento et al., 2015). The derivative 3,4-Me_2-Rha-ol acetate demonstrated the presence of (1 → 2)-linked Rha*p* units. Moreover, the 3-Me-Rha-ol acetate indicated the presence of 2,4-di-*O*-substituted Rha*p* units, which are the insertion point of AG-I in the rhamnogalacturonan backbone. Comparing the methylation analysis of native (data not show) and carboxy-reduced SFSCK, it was possible to observed the increase of the 2,3,6-Me_3-Gal-ol acetate of the carboxy-reduced

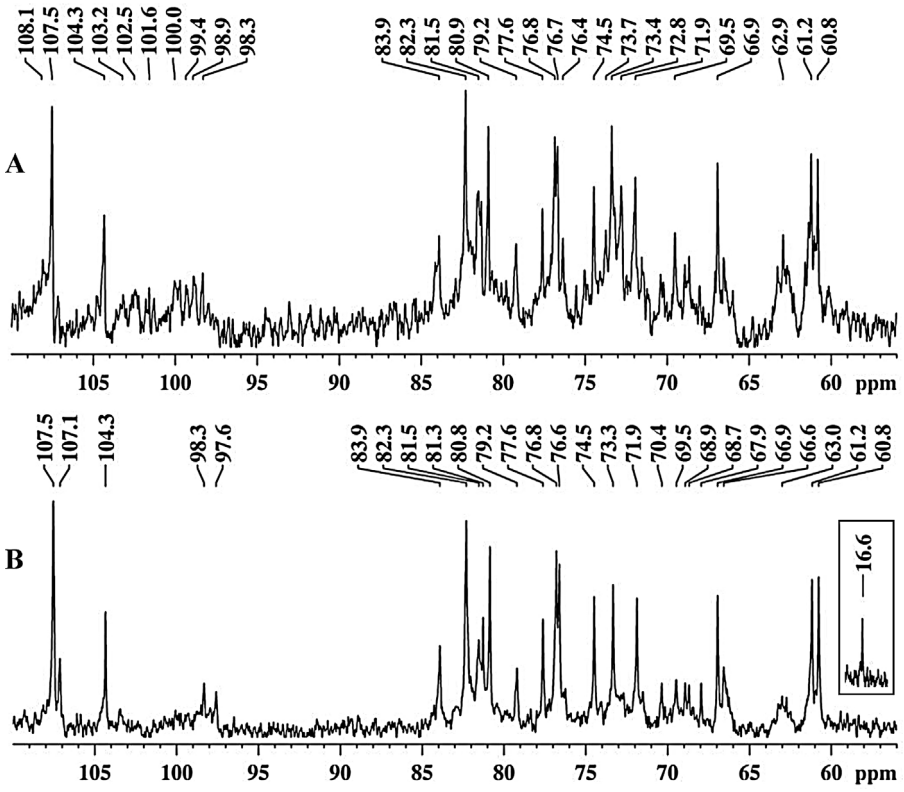

Fig. 2. ^{13}C NMR spectra of fraction SCK (A) and fraction SFSCK (B), in D_2O at 50 °C.

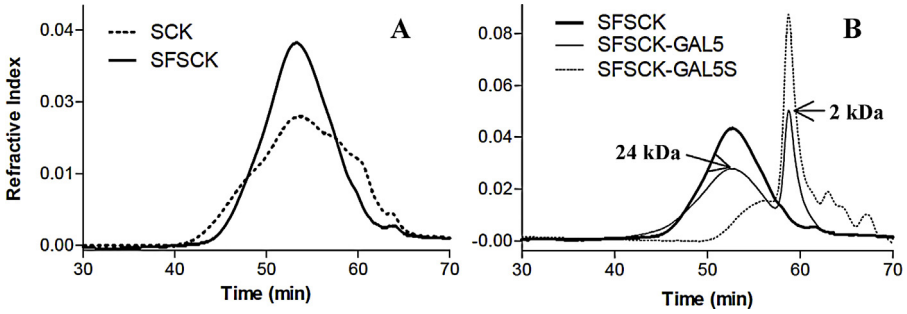

Fig. 3. GPC elution profile of (A) fractions SCK and SFSCK and (B) fraction SFSCK before and after incubation with endo-galactanase (fractions SFSCK-GAL5 and SFSCK-GAL5S). Refractive index detector.

Table 2
Linkage types based on analysis of partially *O*-methyl alditol acetates of fractions SFSCK, SFSCK-GAL5 and SFSCK-GAL5S.

Partially *O*-methylalditol acetate	Linkage type[b]	SFSCK[c]	SFSCK-GAL5	SFSCK-GAL5S
2,3,5-Me_3-Ara[a]	Ara*f*-(1→	10.8	10.8	11.9
3,5-Me_2-Ara	→2)-Ara*f*-(1→	8.3	12.4	–
2,5-Me_2-Ara	→3)-Ara*f*-(1→	7.8	8.0	7.3
2,3-Me_2-Ara	→5)-Ara*f*-(1→	35.7	37.3	34.8
3,4-Me_2-Ara	→2)-Ara*p*-(1→	1.8	–	–
2-Me-Ara	→3,5)-Ara*f*-(1→	9.3	15.1	8.2
3-Me-Ara	→2,5)-Ara*f*-(1→	1.1	–	–
2,3,4,6-Me_4-Gal	Gal*p*-(1→	3.5	6.7	27.0
2,3,6-Me_3-Gal	→4)-Gal*p*-(1→	13.0[d]	2.2	8.5
2,3,4-Me_3-Gal	→6)-Gal*p*-(1→	1.1	0.9	–
2,3-Me_2-Gal	→4,6)-Gal*p*-(1→	1.7	–	2.3
2,3,4-Me_3-Rha	Rha*p*-(1→	1.1	–	–
3,4-Me_2-Rha	→2)-Rha*p*-(1→	3.8	3.4	–
3-Me-Rha	→2,4)-Rha*p*-(1→	1.2	3.4	–

[a] 2,3,5-Me_3-Ara = 2,3,5-tri-*O*-Methylarabinitolacetate, etc.
[b] Based on derived *O*-methylalditol acetates.
[c] % of peak area of *O*-methylalditol acetates relative to total area, determined by GC–MS. Samples were carboxy-reduced by the carbodiimide method (Taylor & Conrad, 1972), prior to methylation analysis.
[d] Also includes the percentage of carboxy-reduced Gal*p*A (10.4%).

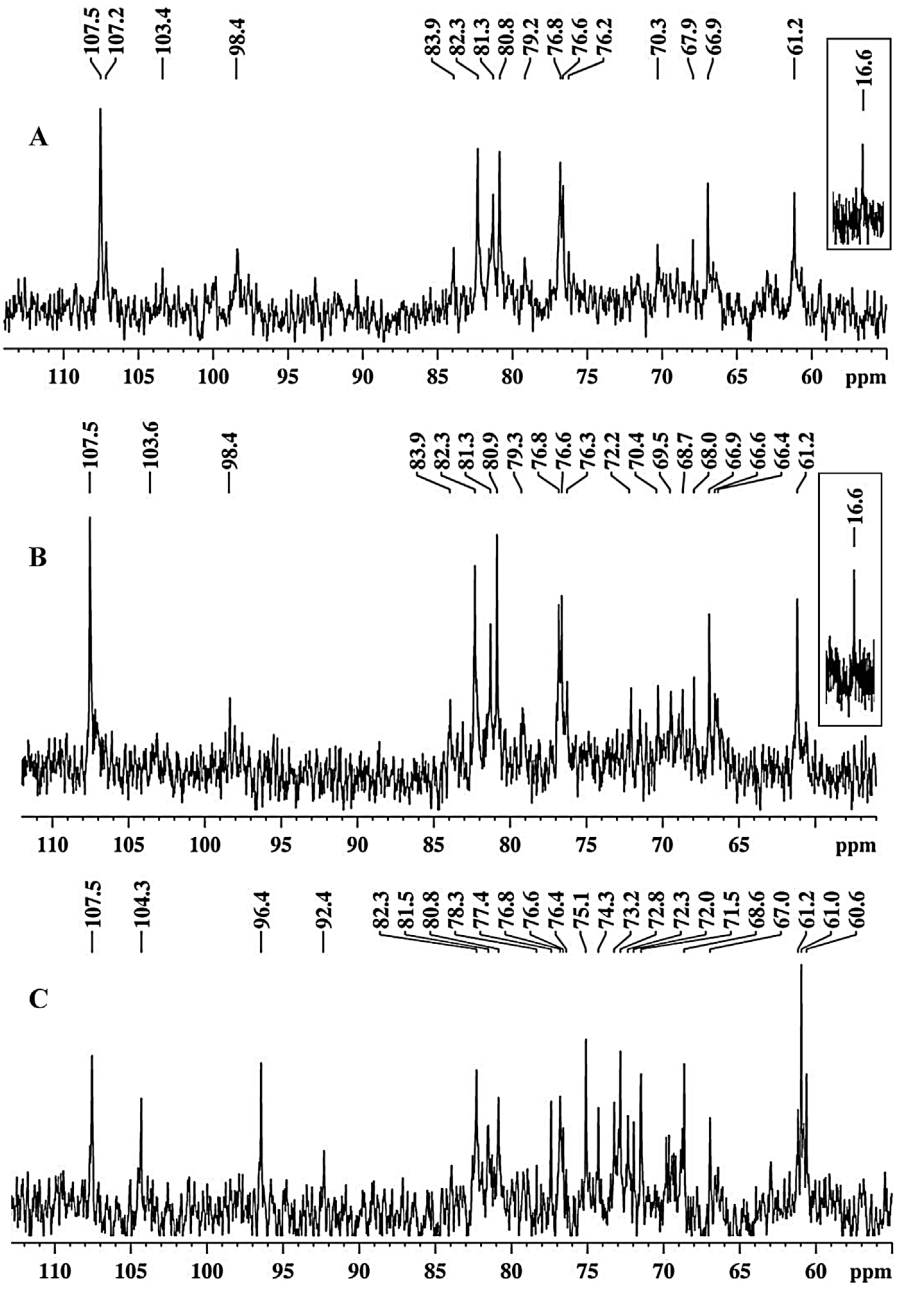

Fig. 4. ^{13}C NMR spectra of (A) fraction SFSCK-GAL5, (B) fraction SFSCK-GAL10 and (C) fraction SFSCK-GAL5S, in D_2O at 50 °C.

in relation to native sample, indicating that (1 → 4)-linked Gal*p*A units were also present. The presence of these units together with (1 → 2)- and (1 → 2,4)-linked Rha*p* confirms the presence of type I rhamnogalacturonan.

In order to estimate the relative importance of the neutral side chains to the rhamnogalacturonan backbone, the ratio of (Ara + Gal) to Rha was employed. The lower this ratio, the shorter the side chains attached to 4-*O*-Rha in RG-I backbone (Renard & Ginies, 2009). The calculated ratio was 16.9, demonstrating that SFSCK contained large neutral side chains. Among these neutral side chains, the arabinose to galactose ratio is an estimation of the proportions of Ara versus Gal-rich side chains (Renard & Ginies, 2009). This ratio was 6.0, indicating large amounts of arabinose. To verify the hypothesis that this high arabinose content could arise from a simultaneous presence of an arabinan and a type I arabinogalactan (or galactan) as neutral side chains of the rhamnogalacturonan, an enzymatic degradation was employed. Thus, fraction SFSCK was hydrolyzed with an endo-β-(1 → 4)-D-galactanase, at two incubation times (5 and 10 h), followed by ethanol precipitation. The precipitates (fractions SFSCK-GAL5 and SFSCK-GAL10) showed identical GPC elution profiles and that of fraction SFSCK-GAL5 is shown in Fig. 3B. In comparison with native SFSCK, it demonstrated the presence of a second peak eluted around 60 min. This peak was very intense (Fig. 3B) in the ethanol supernatants (fractions SFSCK-GAL5S and SFSCK-GAL10S) and corresponding to the fragments released by the galactanase treatment.

Monosaccharide analysis of the enzyme resistant polymers (SFSCK-GAL5 and SFSCK-GAL10) also showed similar composition (Table 1), indicating that 5 h of incubation was enough for a complete hydrolysis of the (1 → 4)-galactan moiety. When compared with SFSCK, both SFSCK-GAL5 and SFSCK-GAL10 fractions demonstrated an increase in their arabinose as well as a decrease

in their galactose content. The presence of the small amount of galactose in the enzyme resistant polymers could be due to the presence of (1 → 6)-linked Gal*p* units and/or resistant short (1 → 4)-galactooligomers that were not recognized by the enzyme and remained linked to the rhamnogalacturonan backbone, as could be seen in the methylation analysis of SFSCK-GAL5 (Table 2). In agreement with monosaccharide composition, this analysis was predominated by the arabinose derivatives and indicated an (1 → 5)-linked arabinan branched only at O-3. The side chains were formed by (1 → 2)- and (1 → 3)-linked Ara*f* units. Rhamnose (1 → 2)- and (1 → 2,4)-linked were also present and arise from the rhamnogalacturonan I backbone. GalA derivatives were not observed, once the sample was not carboxy-reduced prior to the methylation experiment. ^{13}C NMR spectra (Fig. 4A and B) also demonstrated intense α-L-arabinofuranosyl signals: at δ 107.5 and 107.2 corresponding to anomeric carbons, at δ 83.9–76.2 corresponding to ring carbons and at δ 66.9 and 61.2 corresponding to substituted and free C-5 carbons, respectively. Signals at δ 98.4 and δ 16.6 were also present and arise from C-1 and C-6 of α-L-Rha*p* units, respectively, while small signal at δ 103.4 arise from C-1 of residual β-D-Gal*p* units (Cipriani et al., 2004, 2009; Colquhoun et al., 1990; Cordeiro et al., 2012; Renard et al., 1998; Thude & Classen, 2005; Xu et al., 2010). Thus, the above results indicated the presence of an arabinan-rhamnogalacturonan complex in the galactanase-resistant polymers.

Monosaccharide analysis was performed in the fragments released by the enzymatic hydrolysis at 5 h (SFSCK-GAL5S). It showed the presence of arabinose and galactose (Table 1), indicating the existence of the type I arabinogalactan. Derivatives corresponding to AG-I backbone were observed in the methylation analysis (Table 2), being (1 → 4)- and (1 → 4,6)-linked Gal*p* units. The side chains were formed by (1 → 5)-, (1 → 3,5)- and (1 → 3)-linked Ara*f* units. Interestingly, (1 → 2)-linked Ara*f* units were not observed, being present only in the arabinan moiety of SFSCK-GAL5 fraction. The ^{13}C NMR spectrum of SFSCK-GAL5S (Fig. 4C) showed anomeric signals at δ 107.5 and δ 104.3 corresponding to α-L-Ara*f* and β-D-Gal*p* units, respectively. The signals at δ 96.4 and 92.4 corresponding to the β- and α- anomeric carbons, respectively, of galactose units in the reducing-end (Gorin & Mazurek, 1975).

Finally, the enzymatic degradation results demonstrated that fraction SFSCK contains a pectin, formed by a rhamnogalacturonan I to which a branched arabinan and a type I arabinogalactan are attached. The proportion of arabinan side chain is higher than arabinogalactan. In fruits, RG-I with side chains richer in arabinans than in galactans were already found in albedo cell walls of orange (*Citrus sinensis*) (Prabasari et al., 2011) and in *Argania spinosa* fruit pulp (Aboughe-Angone et al., 2008). Moreover, RG-I with similar amounts of Ara and Gal were extracted from citrus peel (Yapo et al., 2007), while RG-I branched with type I arabinogalactans was purified from caja fruits and prunes (Iacomini et al., 2005, Cantu-Jungles et al., 2014). A highly ramified RG-I associated with cellulose was characterized in ripe apples (Oechslin et al., 2003). Attached to its backbone was a xylogalacturonan, a highly ramified arabinan, a (1 → 4)-linked galactan with few (1 → 3)-and (1 → 6)-linked short chains and a short strictly linear (1 → 4)-galactooligomers.

4. Conclusions

A pectin, formed by a rhamnogalacturonan I to which a branched arabinan and a type I arabinogalactan are attached was purified and characterized from starfruit. It was composed of rhamnose, arabinose, galactose and uronic acid in the 5.0:72.5:12.1:10.4 molar ratios, respectively. After enzymatic degradation procedure with an endo-β-(1 → 4)-D-galactanase, an arabinan-rhamnogalacturonan remained as the resistant polymer, while the arabinogalactan fragments were released.

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