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Structure of an arabinogalactan from the edible tropical fruit tamarillo (*Solanum betaceum*) and its antinociceptive activity

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ABSTRACT

A structural characterization of polysaccharides obtained by aqueous extraction of ripe pulp of the edible exotic tropical fruit named tamarillo (*Solanum betaceum*) was carried out. After fractionation by freeze–thaw and α-amylase treatments, a fraction containing a mixture of highly-methoxylated homogalacturonan and of arabinogalactan was obtained. A degree of methylesterification (DE) of 71% and a degree of acetylation (DA) of 1.3% was determined by ^{1}H NMR spectroscopy and spectrophotometric quantification, respectively. A type I arabinogalactan was purified via Fehling precipitation and ultrafiltration through 50 kDa (cut-off) membrane. Its chemical structure was performed by sugar composition, HPSEC, methylation, carboxy-reduction and ^{13}C NMR spectroscopy analysis. Intraperitoneal administration of the arabinogalactan did not reduce the nociception induced by intraplantar injection of 2.5% formalin in mice, but significantly reduced the number of abdominal constrictions induced by 0.6% acetic acid, indicating that fraction has an antinociceptive effect on the visceral inflammatory pain model.

1. Introduction

Tamarillo (*Solanum betaceum* Cav. syn *Cyphomandra betacea* Sendt.), also known by popular name as tomato tree is a small tree native from the Andes, and belongs to the Solanaceae family. This specie is cultivated in subtropical or warm temperate regions and, in countries like Colombia and New Zealand, it is a commercial crop for international export.

This exotic fruit is low in fat and calories and has high nutritional value providing significant amounts of micronutrients such as vitamins, minerals and bioactive components, like anthocyanins, carotenoids and flavonoids (Bobbio, Bobbio, & Rodriguez-Amaya, 1983; Mertz et al., 2009; Osorio et al., 2012). The levels of vitamins B_6, C and E and the levels of trace elements as iron, magnesium, copper and potassium present in one tamarillo supply over 5% of the RDI (recommended daily intake) of these nutrients (Lister, Morrison, Kerkhofs, & Wright, 2005; Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin, 2009).

In folk medicine, leaves and fruits of tamarillo are used in the treatment of sore throat, inflamed tonsils and gums (Bohs, 1989). Until recently, there were no studies dealing with the identification of components of tamarillo responsible for these anti-inflammatory and analgesic actions. Nascimento et al. (2013) demonstrated that a polysaccharide (galactoarabinoglucuronoxylan) isolated from tamarillo pulp showed antinociceptive effect on inflammatory pain models in mice. They also described the presence of a liner (1 → 5)-linked α-L-arabinan in the pulp.

We now investigate the structural characterization of pectic polysaccharides obtained by aqueous extraction from ripe pulp of tamarillo fruits and evaluated the antinociceptive effect of a purified type I arabinogalactan.

2. Materials and methods

2.1. Plant material

Ripe fruits of *S. betaceum*, 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.

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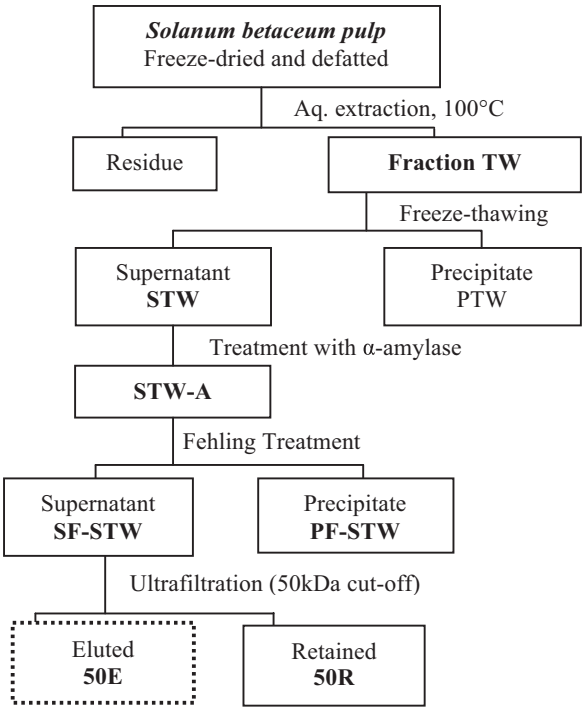

Fig. 1. Scheme of extraction and fractionation of pectic polysaccharides from pulp of the Tamarillo (*Solanum betaceum*).

2.2. Extraction and purification of polysaccharides

The fruits were peeled, the seeds and mucilage were manually removed and the pulp was freeze-dried and milled (235 g). Dried pulp powder was defatted with chloroform–methanol (1:1). Polysaccharides were extracted from the residue with water at 100 °C for 2 h (×7, 1 l each). The aqueous extracts were obtained by centrifugation (12,000 × *g*, 20 min at 25 °C), 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), and freeze-dried, giving fraction TW.

A freeze–thaw treatment was applied in fraction TW (Fig. 1), to give cold-water soluble and insoluble fractions STW and PTW, respectively. In this procedure, the sample was frozen and then thaw at room temperature followed by centrifugation.

In order to remove starch, fraction STW was extensively treated with α-amylase (from *Bacillus licheniformis*, Sigma A3403, 100 units/ml, 24 h at 37 °C) and dialyzed, yielding the fraction STW-A (Fig. 1). This was dissolved in distilled water and then treated with Fehling's solutions, according Jones and Stoodley (1965), resulting in a homogalacturonan-copper complex (PF-STW) and a soluble fraction (SF-STW), 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. After, fraction SF-STW was purified by ultrafiltration through membrane with cut-off of 50 kDa (14650-47D, Sartorius), yielding fractions eluted (50E) and retained (50R) on the membrane (Fig. 1).

The yields of polysaccharides were expressed as % based on weight of dried tamarillo pulp that was submitted to extraction (235 g).

2.3. General analytical methods

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

Fraction 50E 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 $-CH_2OH$.

The degree of acetylation (DA) was determined after spectrophotometric quantification of acetyl following the method of Hestrin (1949), employing galactose pentaacetate as a standard.

2.4. Sugar composition

Neutral monosaccharide components of the polysaccharides and their ratio were determined by hydrolysis with 2 M trifluoroacetic acid (TFA) for 8 h at 100 °C, followed by conversion to alditol acetates by successive $NaBH_4$ reduction, and acetylation with acetic anhydride-pyridine (1:1, v/v, 1 ml) 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.

Uronic acid contents were determined spectrophotometrically using the m-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991).

2.5. Determination of homogeneity and molecular weight of polysaccharides

The homogeneity and molecular weight (M_w) of soluble polysaccharides were determined by high performance size exclusion chromatography (HPSEC), using a Waters 2410 differential refractometer and a Pharmacia LKB Uvicord VW 2251 ultraviolet detector at 280 nm (UV) 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 sample, previously filtered through a membrane (0.22 μm, Millipore), was injected (250 μl loop) at a concentration of 1 mg/ml. The specific refractive index increment (dn/dc) was determined and the results were processed with software ASTRA provided by the manufacturer (Wyatt Technologies).

2.6. Methylation analysis of polysaccharide

Prior to glycosyl linkage analysis, uronic acids were reduced using carboxyl reduction method of Taylor and Conrad (1972). Carboxyl-reduced samples were *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 (80 °C, 2 h) followed by hydrolysis with H_2SO_4 (0.5 M, 22 h, at 100 °C) and neutralization with $BaCO_3$. The material was then submitted to reduction and acetylation as described above for sugar composition, except that the reduction was performed using $NaBD_4$. 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).

Table 1
Monosaccharide composition of fractions obtained from the pulp of tamarillo fruits (*S. betaceum*).

Fractions	Monosaccharide composition (%)[a]						
	Rha	Ara	Xyl	Man	Gal	Glc	Uronic acid[b]
STW	2.9	22.0	6.1	3.4	19.8	16.4	29.4
STW-A	1.9	29.6	3.8	5.8	28.5	3.8	30.4
SF-STW	3.5	16.7	1.5	4.6	54.7	–	19.0
50E	2.1	14.9	–	7.6	70.4	–	5.0

[a] % of peak area relative to total peak areas, determined by GC–MS.
[b] Determined spectrophotometrically using the m-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991).

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.

The degree of methylesterification (DE) was determined by ^1H NMR spectroscopy according Grasdalen, Bakoy, and Larsen (1988). Briefly, the fraction was deuterium-exchanged three times by freeze-drying with D_2O solutions, finally dissolved in D_2O, and transferred into 5-mm NMR tube. The ^1H NMR spectra were acquired at 80 °C, with 256 scans, at pD 5.0, on a Bruker AVANCE III 400 NMR spectrometer, operating at 9.5 T, observing ^1H at 400.13 MHz. Chemical shifts were expressed as δ ppm, using the resonances of HDO at 4.20 as internal reference. All pulse programs were supplied by Bruker.

2.8. Animals

Experiments were conducted using female Swiss mice (25–35 g), housed at 22 ± 2 °C under a 12 h light/12 h dark cycle and with free access to food and water. The experimental procedures were performed after approval of the protocols by the local Ethics Committee of Animal Experimentation of Federal University of Paraná (CEUA/BIO – UFPR; approval number 613). The study was conducted in accordance with the "Principles of Laboratory Animal Care" (NIH Publication 85-23, revised 1985) and with the ethical guidelines for investigations of experimental pain in conscious animals. Animals were acclimatized to the laboratory for at least 12 h before testing and were used only once for experiments. The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments.

2.9. Nociception induced by formalin

The procedure used was similar to that described by Rodrigues et al. (2012). Briefly, after the environmental habituation period, mice were intraperitoneally pretreated with vehicle (saline, 10 ml/kg) or fraction 50E (1, 10 or 100 mg/kg), 30 min before intraplantarly administration of 20 μl of a 2.5% formalin solution (0.92% formaldehyde in saline/paw). Animals were observed from 0 to 5 min (first phase) and 15–30 min (second phase) and the time that they spent licking the injected paw was considered as indicative of nociception.

2.10. Visceral nociception induced by acetic acid

The abdominal constrictions were induced according to procedures previously described (Rodrigues et al., 2012) and resulted in the contraction of the abdominal muscle together with a stretching of the hind limbs in response to an intraperitoneal injection of aqueous acetic acid (0.6%, 0.45 ml/mouse) at the time of the test. Mice were pretreated with vehicle (saline, 10 ml/kg) or fraction 50E (0.1, 1 10 and 100 mg/kg), 30 min before acetic acid challenge. The abdominal constrictions were counted cumulatively over a period of 20 min.

2.11. Statistical analysis

Data were expressed as means ± standard error of mean (S.E.M.) with 4–6 animals per group. Comparisons between experimental and control groups were performed by one-way analysis of variance (ANOVA) followed by Newman–Keul's test. *P* values less than 0.05 were considered as indicative of significance.

3. Results and discussion

The pulp of tamarillo fruits was freeze-dried (235 g) and then deffated with chloroform–methanol (1:1) in a Sohxlet apparatus, yielding a moisture and nonpolar compounds content of approximately 80% and 5%, respectively. Polysaccharides were extracted from deffated residue with successive water extraction, at 100 °C, giving fraction TW (9% yield) recovered by EtOH precipitation. Monosaccharide analysis of the fraction indicated arabinose, galactose and glucose as main neutral sugars in the 31.1:7.9:42.6 molar ratios, respectively.

A freeze–thaw treatment was applied in fraction TW, giving rise to precipitate (PTW, 0.5% yield) and supernatant (STW, 6.0% yield). According to our previous results (Nascimento et al., 2013) PTW has a linear (1 → 5)-linked α-L-arabinan.

Monosaccharide analysis of fraction STW revealed arabinose, galactose, glucose, xylose, mannose and rhamnose as neutral sugars (Table 1), and 29.4% of uronic acids, indicating the presence of pectic polysaccharides. When analyzed by size exclusion chromatography (HPSEC), this fraction showed a heterogeneous elution profile, with the presence of three peaks (I, II, III) (Fig. 2A), with molecular mass of 1300 kDa, 134 kDa and 40 kDa, respectively.

Due to the glucose content, which according to our previous work is related to starch (Nascimento et al., 2013), fraction STW was also treated with α-amylase, reducing the glucose content to 3.8%. Moreover, there was the disappearance of the peak III in the HPSEC elution profile of STW-A (Fig. 2A). The remaining monosaccharides observed were arabinose, galactose, mannose, xylose, rhamnose and uronic acids (Table 1).

In order to confirm the identity of the uronic acid present in STW-A, 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 STW-A (55.0%) indicating that the uronic acid present is the galacturonic acid.

The ^{13}C NMR spectrum of STW-A can be seen in Fig. 3A and showed signals at δ 100.2 and δ 99.4, corresponding to anomeric carbons of esterified and unesterified units of α-D-Gal*p*A, respectively. The signals at δ 170.8 and δ 52.8 could be assigned to C-6

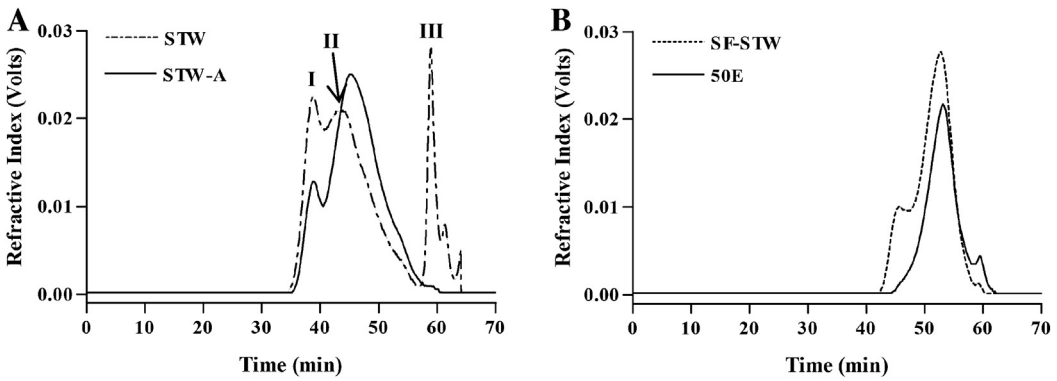

Fig. 2. HPSEC elution profile of (A) fractions STW and STW-A (STW after α-amylase treatment), (B) fractions SF-STW and 50E, obtained from pulp of tamarillo (*S. betaceum*) fruits (refractive index detector).

and methyl carbons of esterified Gal*p*A units, respectively. The remaining carbons of the α-D-Gal*p*A ring were seen at δ 78.8 (*O*-substituted C-4), δ 70.5 (C-5) and δ 67.9 (C-2 and C-3, overlapped). Moreover, the spectrum also showed the presence of signals at δ 107.5 corresponding to anomeric carbons of α-L-Ara*f* units and signals at δ 104.4, δ 77.6, δ 74.5, δ 73.4, δ 71.9 and δ 60.8, which can be attributed to C-1, C-4, C-5, C-3, C-2 and C-6 respectively, of (1 → 4)-linked-β-D-galactopyranosyl units. These assignments suggested the presence of a type I arabinogalactan (AG-I) and a methyl esterified homogalacturonan (HG). The assignments are in agreement with published literature data (Bock & Pedersen, 1983; Cipriani et al., 2009; Petersen, Meier, Duus, & Clausen, 2008; Xu, Dong, Qiu, Cong, & Ding, 2010).

Due to the presence of methylesterified α-D-Gal*p*A units, the degree of methylesterification (DE) was determined by ^{1}H NMR spectroscopy (Grasdalen, Bakoy, & Larsen, 1988). A value of 71% was

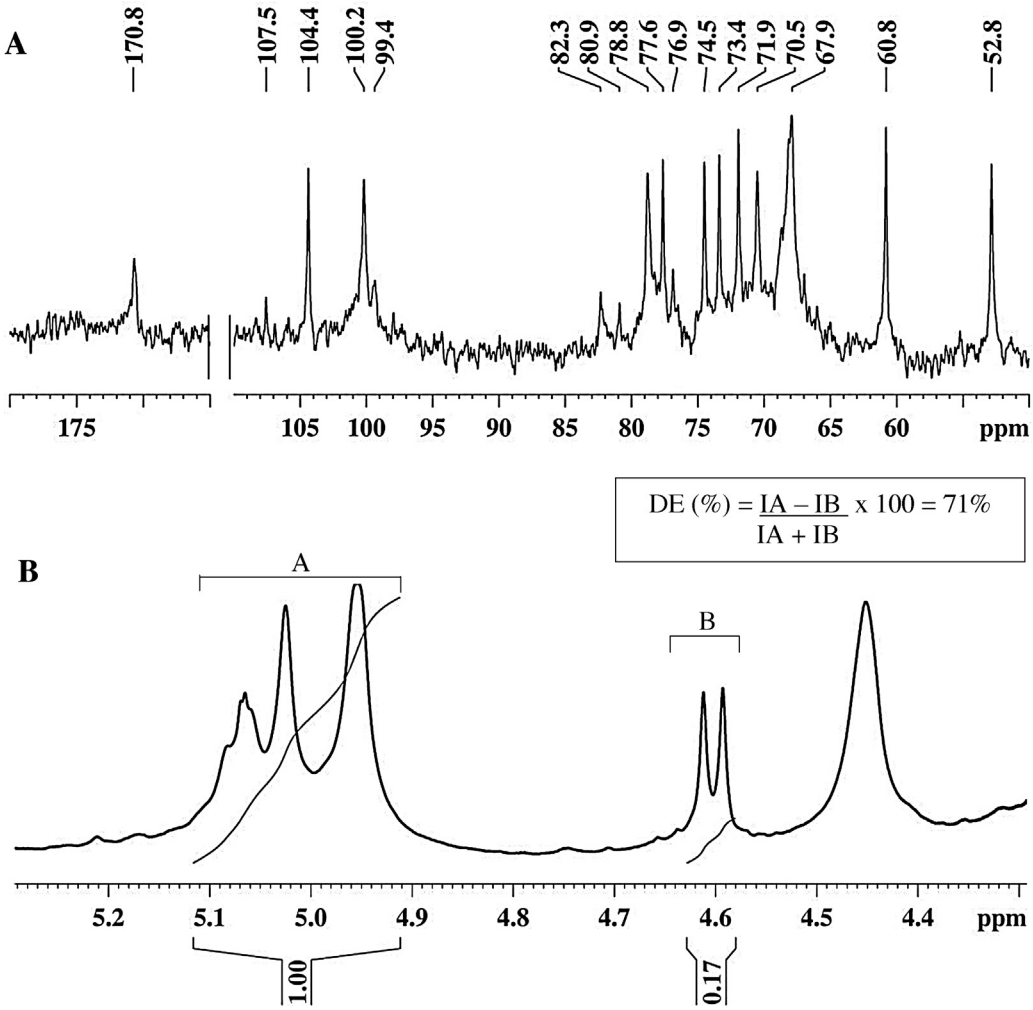

Fig. 3. (A) ^{13}C NMR spectrum of fraction STW-A, in D_2O at 50 °C and (B) ^{1}H NMR spectrum (low field region) of fraction STW-A used for determination of the degree of methylesterification (DE), in D_2O at 80 °C, 256 scans.

Table 2
Linkage types based on analysis of partially *O*-methylalditol acetates of fraction 50E purified from the pulp of tamarillo fruits (*S. betaceum*).

Partially *O*-methylalditol acetate[a]	50E[b]	Linkage type[c]
2,3,5-Me_3-Ara	5.0	Ara*f*-(1→
2,5-Me_2-Ara	1.5	→3)-Ara*f*-(1→
2,3-Me_2-Ara	5.0	→5)-Ara*f*-(1→
2-Me-Ara	2.5	→3,5)-Ara*f*-(1→
2,3,4,6-Me_4-Gal	14.0	Gal*p*-(1→
2,3,6-Me_3-Gal	51.0[d]	→4)-Gal*p*-(1→
2,3-Me_2-Gal	10.0	→4,6)-Gal*p*-(1→
3,4-Me_2-Rha	2.0	→2)-Rha*p*-(1→
3-Me-Rha	2.0	→2,4)-Rha*p*-(1→
2,3,6-Me_3-Man	7.0	→4)-Man*p*-(1→

[a] 2,3,5-Me_3-Ara = 2,3,5-tri-*O*-methylarabinitolacetate, etc.
[b] % 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.
[c] Based on derived *O*-methylalditol acetates.
[d] Also includes the percentage of carboxy-reduced Gal*p*A (5%).

found for STW-A (Fig. 3B), characterizing this as a high-methoxyl (HM) pectin (Rolin, 1993). Fruits considered good for producing jams and jellies have a content of methyl esterification similar to that found in fraction STW-A, as in the case of guava (71%), strawberry (72%) apple (75%) and blemish (71%) (Lima, Paiva, Andrade, & Paixão, 2010). The degree of acetylation (DA) was also measured, and showed low acetyl content (1.3%).

In order to separate the AG-I from the methylesterified homogalacturonan, fraction STW-A was treated with Fehling solution (Fig. 1). In this process, the homogalacturonan formed a complex with cooper and precipitated (fraction PF-STW), while the AG-I remained in the supernatant (fraction SF-STW).

Monosaccharide analysis of fraction SF-STW showed arabinose, galactose, mannose, rhamnose, xylose and uronic acids (Table 1). When analyzed by HPSEC, the fraction demonstrated the presence of two peaks (Fig. 2B), and thus was fractionated by ultrafiltration through membrane with cut-off of 50 kDa (Fig. 1). This strategy was highly efficient, since it produced a homogeneous fraction eluted in this membrane (50E), as could be seen by its elution profile on HPSEC analysis (Fig. 2B). Its molecular mass was 57 kDa.

Fraction 50E, on monosaccharide analysis, revealed the presence of arabinose, galactose, mannose, rhamnose and uronic acids (Table 1). Due to the presence of these uronic acids, the methylation analysis of 50E was performed in the carboxy-reduced sample and it is shown in Table 2. The main observed derivatives were the 2,3,6-Me_3- and 2,3-Me_2-Gal-ol acetate, which correspond to (1 → 4)- and (1 → 4,6)-linked Gal*p* units. These indicate a (1 → 4)-linked galactan as main chain, carrying branches exclusively at *O*-6, and confirm the presence of type I arabinogalactan. According to the ratio of 2,3,6-Me_3- and 2,3-Me_2-Gal-ol acetate, it can be suggested that the main chain has approximately 17% of branches. The side chains consisted of 5-*O*-, 3-*O*-, 3,5-di-*O*-substituted Ara*f* units, as well as terminal Ara*f* and Gal*p* units. The terminal Gal*p* units may be attached directly to the galactan core or via arabinofuranosyl residue. Due to the 3,4-Me_2- and 3-Me-Rha-ol acetate derivatives, it is also possible suggest the presence of a type I rhamnogalacturonan, in which the AG-I is anchored. The presence of (1 → 4)-linked Man*p* was also observed and further studies should be performed to verify if this residue belongs to the structure of the tamarillo pectic polysaccharides. However, Dong et al. (2012) have also recently shown the presence of mannose residues in a type I arabinogalactan-containing fraction obtained from the edible plant *Basella rubra*.

The ^{13}C NMR spectrum of fraction 50E (Fig. 4) is dominated by the six signals related to (1 → 4)-β-D-Gal*p* units of the AG-I main chain, at δ 104.3 (C-1), δ 77.6 (C-4), δ 74.5 (C-5), δ 73.3 (C-3), δ 71.9 (C-2) e δ 60.7 (C-6). Other signals were of weak intensity and corresponded to sugars units present in the AG-I side chains and in the rhamnogalacturonan backbone, such as the signals at δ 107.5, which can be assigned to C-1 of Ara*f* units, and the signals at δ 98.6 and δ 97.5, which can be attributed to C-1 of →4)-Gal*p*A-(1 → 2)-Rha*p* and →4)-Gal*p*A-(1 → 2)-Rha*p* units, respectively (Cipriani et al., 2009; Dourado, Cardoso, Silva, Gama, & Coimbra, 2006; Xu et al., 2010).

The main sources of AG-I are seeds, bulbs, leaves and conifers (Clarke, Anderson, & Stone, 1979). In fruits, the structure of AG-I has been characterized only in Malay apple (*Spondias cytherea*) (Iacomini et al., 2005), *Opuntia ficus-indica* (Habibi, Mahrouz, Marais, & Vignon, 2004) and prunes (*Prunus domestica* L.) (Cantu-Jungles et al., 2014). Moreover, the presence of AG-I is suggested in kiwifruit (*Actinidia* spp.) (Redgwell, Melton, & Brasch, 1988; Sauvageau, Hinkley, Carnachan, & Sims, 2010), noni (*Morinda citrifolia*) (Bui, Bacic, & Pettolino, 2006), sweet orange (*Citrus sinensis*) (Prabasari, Pettolino, Liao, & Bacic, 2011) and guava (*Psidium guajava* L.) (Marcelin, Williams, & Brillouet, 1993).

Regarding biological properties of type I arabinogalactans, studies have already showed an effect in stimulating the immune responses (Gronhaug et al., 2011; Iacomini et al., 2005; Khramova et al., 2011; Nergard et al., 2005; Mellinger et al., 2005; Wu, Gao, Tsim & Tu, 2005), anti-ulcer (Cantu-Jungles et al., 2014; Cipriani et al., 2009; Tanaka et al., 2010) and antiviral activities (Dong et al., 2012). Considering the use in folk medicine of tamarillo fruit and our previous work with its polysaccharides, herein we also evaluated the antinociceptive and anti-inflammatory effects of AG-I present in 50E fraction. Thus, the antinociceptive

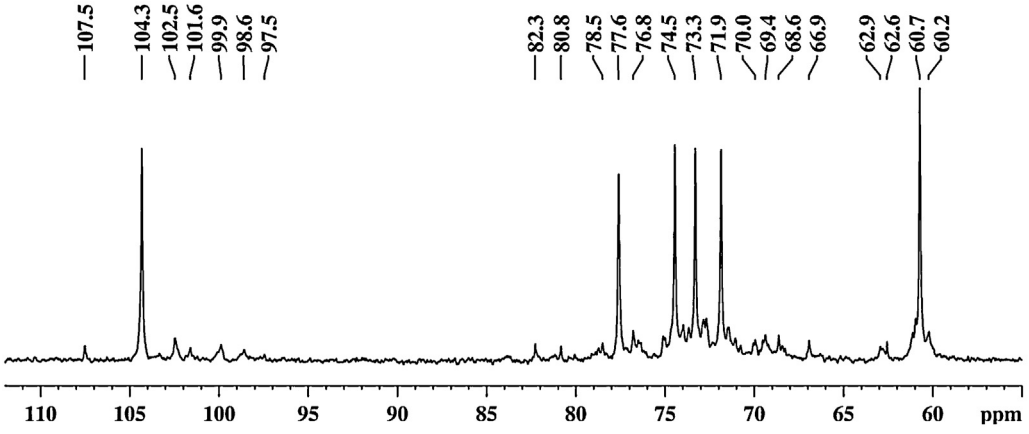

Fig. 4. ^{13}C NMR spectrum of fraction 50E, in D_2O at 50 °C.

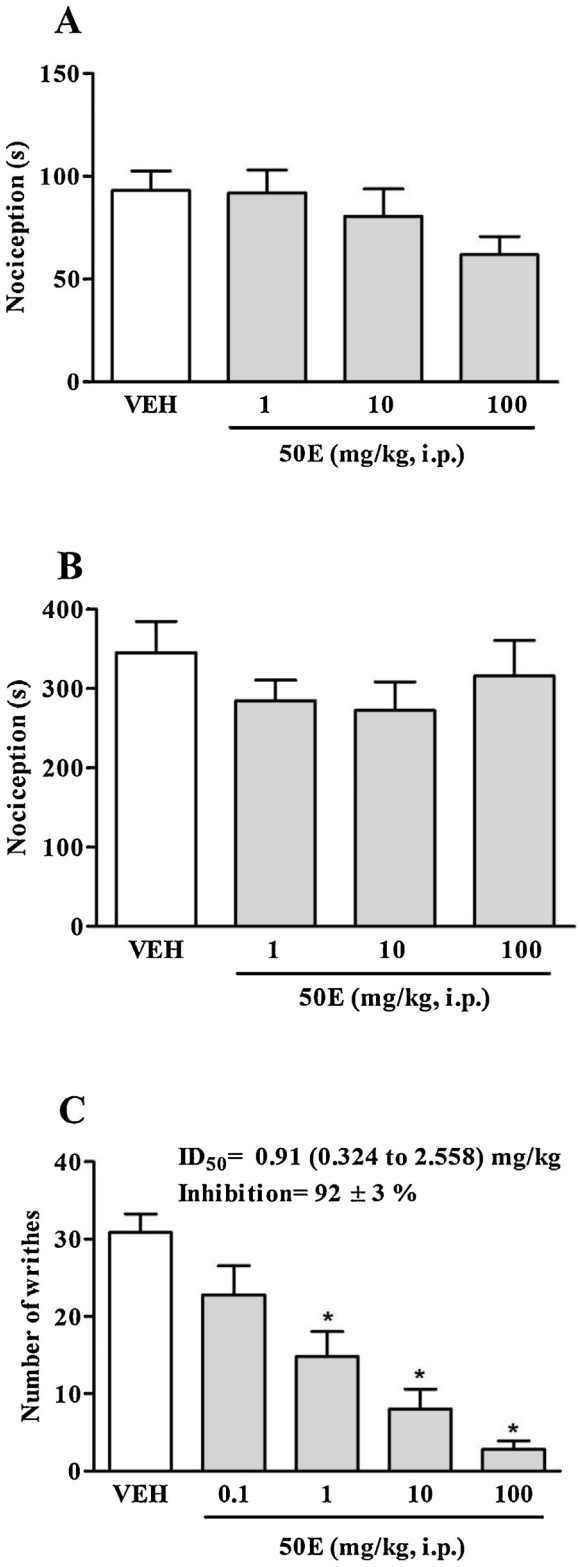

Fig. 5. Effect of intraperitoneal administration of fraction 50E on first (A) and second (B) phases of nociception induced by 2.5% formalin and on abdominal constriction (C) induced by 0.6% acetic acid in mice. The animals were treated with vehicle (VEH, 10 ml/kg, i.p.) or fraction 50E (0.1–100 mg/kg, i.p.). Data are expressed as means ± S.E.M. (n = 4–6) and statistical comparison was performed using one-way ANOVA followed by post hoc Newman–Keul's test. Differences from vehicle group ($^{*}P < 0.05$).

activity was initially evaluated by formalin-induced nociception that produces a biphasic behavioral response. The neurogenic or first phase is caused by the direct chemical stimulation of nociceptors and the second or inflammatory phase is related to the inflammation of the peripheral tissues. Different to the observed with the previously isolated galactoarabinoglucuronoxylan from tamarillo (Nascimento et al., 2013), intraperitoneal administration of fraction 50E (1–100 mg/kg) did not display antinociceptive effects on the formalin test (Fig. 5A and B). In addition, the acetic acid-induced abdominal constriction test is also a typical model of inflammatory pain that is widely used to screen for new agents with peripheral analgesic and anti-inflammatory properties. The treatment of animals with fraction 50E (0.1–10 mg/kg) reduced significantly the number of abdominal constriction induced by acetic acid. Here, 50E showed a dose related inhibition and displayed a mean ID_{50} of 0.91 (0.324–2.558) mg/kg (maximal inhibition of 92 ± 3% for 50E 100 mg/kg) (Fig. 5C). Comparing the results obtained herein with our previous report (Nascimento et al., 2013), we observed that the fraction 50E is less potent than the galactoarabinoglucuronoxylan, which at the same experimental conditions showed an ID_{50} on the writhing test of 0.47 mg/kg. The observed differences in the biological tests can possibly be attributed to the very different chemical structures of the tested polysaccharides. Fraction 50E is composed of a pectic type I arabinogalactan, while the galactoarabinoglucuronoxylan belongs to the hemicellulosic group of plant polysaccharides.

Concerning the antinociceptive activity, it was also reported for polysaccharides extracted from other sources. A heterogalactan and a mannogalactan isolated from the edible mushrooms *Lentinus edodes* (Carbonero et al., 2008) and *Pleurotus pulmonarius* (Smirdele et al., 2008) respectively, also showed significant inhibition of the acetic acid-induced nociceptive response. Moreover, a fucomannogalactan and a glucan isolated from *Amanita muscaria* (Ruthes, Carbonero, Córdova, Baggio, Sassaki, et al., 2013) and β-glucans with different branching degree and solubility isolated from *Lactarius rufus* (Ruthes, Carbonero, Córdova, Baggio, Santos, et al., 2013) also displayed an activity on the second phase of the formalin test. Recently, a sulfated polysaccharide extracted from the red seaweed *Agardhiella ramosissima* (Batista et al., 2014) also demonstrated antinociceptive activity in both (acetic acid-induced writhing and formalin) tests. A glucomannan, isolated from the lichenized fungus *Heterodermia obscurata* (Córdova et al., 2013), demonstrated significant antinociceptive effect when tested in models of acute and chronic pain.

Finally, the results of the present study seems to support the view that fraction 50E displayed an antinociceptive effect on visceral pain related model, that could be related to its anti-inflammatory property, but a more extensive study is necessary to determine the mechanism(s) underlying this effect. Moreover, the present study amplifies the rool of biological activities displayed by type I arabinogalactans and suggest that the polysaccharides could be responsible, at least in part, by the anti-inflammatory and antinociceptive activities observed in folk medicine for the tamarillo fruit.

4. Conclusions

The structure and biological activity of pectic polysaccharides extracted from the pulp of edible tamarillo fruit was performed in this study. According to the results, a fraction containing a mixture of highly-methoxylated homogalacturonan and of arabinogalactan was obtained from aqueous extraction. Furthermore, a type I arabinogalactan inserted probably in a type I rhamnogalacturonan was purified, which significantly reduced the number of abdominal constrictions induced by 0.6% acetic acid, indicating that fraction has an antinociceptive effect on the visceral inflammatory pain model.

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