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Sarah da Costa Amaral, Denis Roux, François Caton, Marguerite Rinaudo, Shayla Fernanda Barbieri, et al.. Extraction, characterization and gelling ability of pectins from Araçá (*Psidium cattleianum* Sabine) fruits. *Food Hydrocolloids*, 2021, 121, pp.106845. 10.1016/j.foodhyd.2021.106845 . hal-03434113

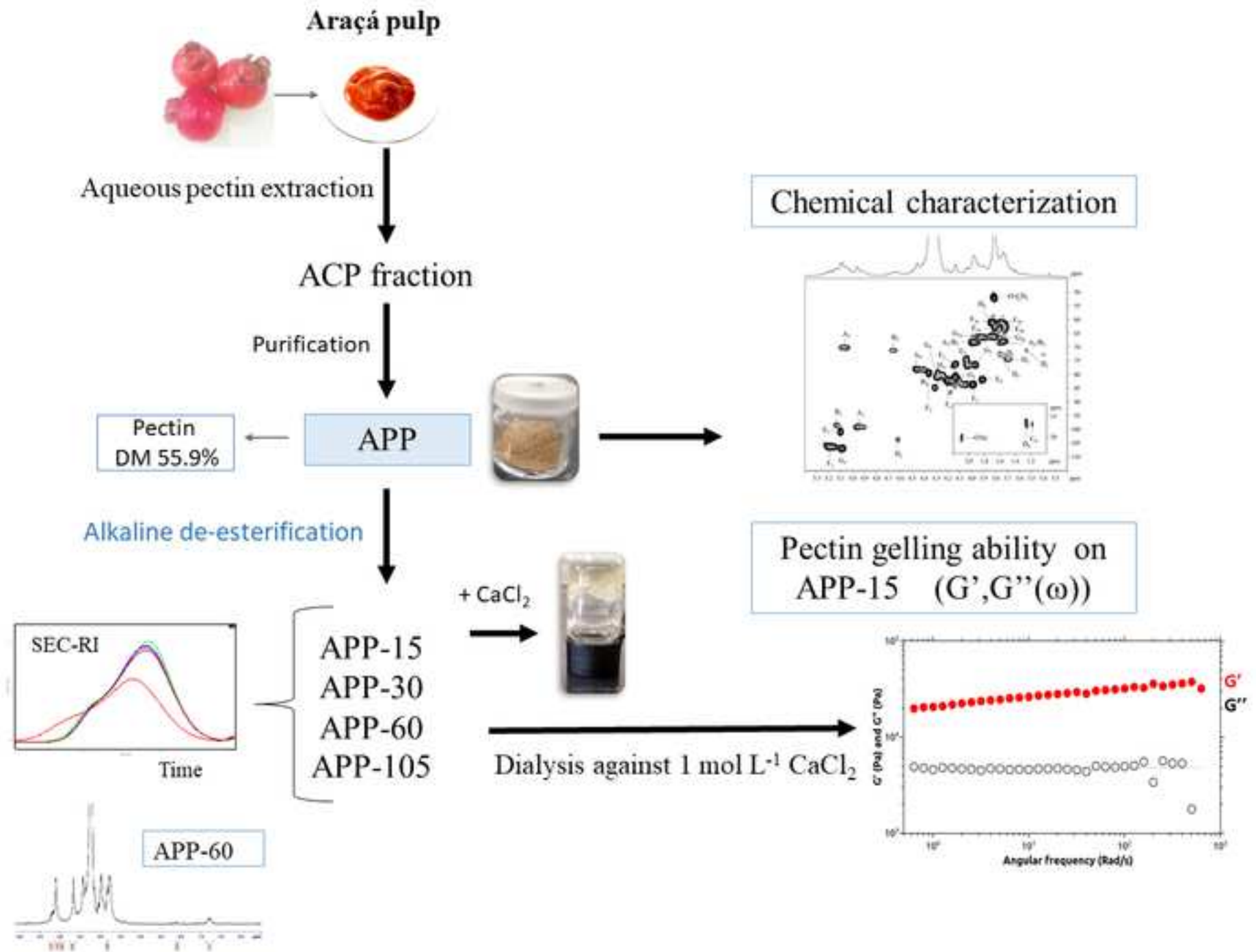
HAL Id: hal-03434113

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Submitted on 31 Aug 2022

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Extraction, characterization and gelling ability of pectins

from Araçá (*Psidium cattleianum* Sabine) fruits

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ABSTRACT

Pectins from Araçá (*Psidium cattleianum* Sabine) fruit pulp were extracted, purified and isolated under their sodium form. Yield was found 3.5% w/w of the initial dried material. From titration, monosaccharide analysis after total hydrolysis and ¹H and HSQC NMR analyses, it was established that the pectins are formed of long galacturonic acid blocks (HG) partially methylated (DM 55.9 mol %) and acetylated (2 mol %) and RG-I blocks with side chains containing mainly arabinose and galactose. No gelation was observed in presence of calcium for the unmodified Araçá Purified Pectin (APP). Then, pectins were treated in alkaline conditions to de-esterify progressively the initial sample down to DM = 0 after 60 minutes of treatment. Each sample obtained was characterized by SEC and NMR demonstrating the stability of the main chains, where Rha, GalA and M_w remained unchanged independently of the length of alkaline treatment. One of this sample (APP-15) was selected to test the gelling ability and rheological behavior. The gel was formed by dialysis against 1 mol L⁻¹ CaCl₂ and its rheological study allows to conclude that a homogeneous physical gel is formed stabilized by cooperative GalA⁻-Ca⁺² junctions.

28 **Keywords:** Araçá; pectin; molecular structure; de-esterification; gelation; rheology.

29

30 **1 INTRODUCTION**

31 The fruit by-products have been demonstrating potential for use in the development of
32 functional ingredients. In contrast, many fruits from native biodiversity remain an under-
33 explored source of bioactive compounds, unknown to the agri-food industry and to consumers
34 (Biazotto et al., 2019; Picot-Allain, Ramasawmy, & Emmambux, 2020). The *Psidium*
35 *cattleianum* Sabine (Myrtaceae family), also known as Araçá in Brazil and as strawberry guava
36 or cattley guava in other countries, is a Brazilian native species that has been studied mainly
37 regarding its biological activity (Franzon, Campos, Proença, & Sousa-Silva, 2009; Patel, 2012;
38 Pereira et al., 2018).

39 Studies report that Araçá fruit extracts have several pharmacological properties, such as
40 antimicrobial (Lima et al., 2020), antioxidant (Denardin et al., 2015) and antihyperglycemic
41 (Vinholes, Lemos, Barbieri, Franzon, & Vizzotto, 2017) effects. These bioactivities are mainly
42 attributed to the high content of phenolic compounds, vitamin C, carotenes, anthocyanins and
43 flavonoids (Cardoso et al., 2018; Medina et al., 2011; Pereira et al., 2018) which are well known
44 as secondary metabolites with high antioxidant capacity (Pereira et al., 2018). Nevertheless,
45 few studies have been dedicated to the investigation of polysaccharides present in this fruit.

46 Vriesmann, Petkowicz, Carneiro, Costa, & Beleski-Carneiro, (2009) studied the pectic
47 polysaccharides present in the Araçá pulp. However, only parameters such as monosaccharide
48 composition and extraction yield were investigated, while the fine chemical structure of the
49 pectins of this fruit remains unknown, as well as reports on the purification, molar mass, and
50 the gelling behavior.

51 Pectins are polysaccharides with a wide range of applications due to their functional properties,
52 being widely used in the food industry as a gelling, thickening, texturizing, emulsifying and

53 stabilizing agent (Cao, Lu, Mata, Nishinari, & Fang, 2020; Ciriminna, Chavarría-Hernández,
54 Hernández, & Pagliaro, 2015; Naqash, Masoodi, Rather, Wani, & Gani, 2017). In addition,
55 pectins are also low toxic, biocompatible and biodegradable, and can be applied in different
56 areas beyond the food appliance, such as the pharmaceutical industry and biomedical
57 applications, where they can be used in the composition of hydrogel formulation and as a
58 biomaterial applicable to drug delivery, gene delivery, wound healing, tissue engineering,
59 among others (Khotimchenko, 2020; Munarin, Tanzi, & Petrini, 2012; Noreen et al., 2017).

60 Structurally, pectins form a group of complex polysaccharides and, despite the wide variation
61 in structure depending on the type of source, the location on the plant and the extraction method,
62 they can be classified into three main types of blocks, according to common characteristics:
63 homogalacturonans (HG), rhamnogalacturonans type I (RG-I) and rhamnogalacturonans type
64 II (RG-II) (Gawkowska, Cybulska, & Zdunek, 2018; Kravtchenko, Voragen, & Pilnik, 1992;
65 Voragen, Coenen, Verhoef, & Schols, 2009).

66 Most of the structure of pectins (~65%) usually consists of HG blocks which are homopolymers
67 of (1→4)-linked α -D-galacturonic acid units that are partially methyl-esterified at C-6. The
68 degree of methyl-esterification (DM) is commonly used to classify pectins into: high methoxyl
69 pectin (HM), when DM is larger than 50% and low methoxyl pectin (LM) when DM is less
70 than 50% (Chan, Choo, Young, & Loh, 2017; Picot-Allain et al., 2020; Voragen et al., 2009).

71 The HG blocks are interrupted by RG-I, which usually represents about 20-35% of the structure,
72 formed by the repeating disaccharide $[\rightarrow 2)\text{-}\alpha\text{-L-Rhap}\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-GalAp}\text{-}(1\rightarrow)]_{n\geq 1}$, with neutral
73 oligosaccharide side chains mainly composed of arabinans, galactans, and arabinogalactans
74 (AG) (Khotimchenko, 2020; Voragen et al., 2009; Yapo, 2011), but the chemical structure is
75 related to the conditions of extraction as demonstrated in many papers (Dranca & Oroian, 2018;
76 Naqash et al., 2017; Zhang et al., 2018).

77 The chemical structure of pectins and the degree of methyl-esterification (DM) affects the
78 gelling properties of these polysaccharides. While the physical-chemical conditions for the
79 formation of gels with HM pectins usually are the presence of co-solutes (such as sucrose) in
80 high concentrations (60-65%) and in acidic pH (pH < 3.5), LM pectins gels are formed in the
81 presence of Ca²⁺ ions through associations between sequences of charged carboxylic groups of
82 two different chains (Rinaudo, 1996; Stephen, Phillips, & Williams, 2006).

83 Native pectins are commonly HM type. Thus, studies have been dedicated to producing LM
84 pectins and to investigate the calcium binding properties with different levels and patterns of
85 controlled de-esterification (Chan et al., 2017; Ngouémazong et al, 2012; Ralet, Dronnet,
86 Buchholt, & Thibault, 2001; Ventura, Jammal, & Bianco-Peled, 2013), given that Ca²⁺-
87 dependent gelation is one of the important functional properties of pectins to enable widespread
88 use in the industry (Cao et al., 2020). Indeed, anionic hydrogels with hydrophilic properties
89 and lower sensitivity towards pH variation are widely used in various fields of biomedicine
90 including bondages, highly effective absorbents, and targeted drug delivery systems
91 (Khotimchenko, 2020). In addition, LM pectins are important ingredients in the food industry
92 to form low sugar jams, since it does not require the addition of sucrose for gelation (Noreen et
93 al., 2017).

94 However, most studies have been carried out with commercial citrus pectins and until now, the
95 chemical structure of Araçá pectins, molar mass distribution, as well as their interaction process
96 with calcium ions, remains unknown.

97 Therefore, the present work aims to elucidate the chemical and molecular structure of pectic
98 polysaccharides extracted and purified from Araçá fruits. In addition, Araçá pectins with
99 different degrees of methyl-esterification are prepared and investigated in relation to the role of
100 DM in the pectin gelation process in the presence of acid or calcium excess.

101

102 2 MATERIALS AND METHODS

103 2-1. Materials

104 Ripe Araçá (*Psidium cattleianum* Sabine) fruits were collected from a conservation area in Irati-
105 Paraná/Brazil, located at geographic coordinates of 25° 25' South latitude, 50° 36' West
106 longitude, and 25° 17' South latitude, 50° 30' West longitude. The fruits were selected, washed,
107 and the seeds were removed manually. Then, the seedless fruits were homogenized using a
108 mixer (Black & Decker - SB60-BR, 300 W), giving rise to Araçá pulp (AP).

109 Ethanol (99.5%) was purchased from Alphatec. Methanol ($\geq 99.8\%$), Sulfuric acid (95.0-
110 98.0%), 3,5-dinitrosalicilic acid (DNS), and Sodium Tetraborate decahydrate ($\geq 99.5\%$) were
111 obtained from VETEC (Duque de Caxias, Brazil). NaN_3 ($>99\%$), Ethanol (P.A., $\geq 99.9\%$), and
112 HCl 1 mol L⁻¹ (1 N) Titripur[®] were purchased from Merck (Darmstadt, Germany). Inositol
113 (myo-Inositol, $\geq 99\%$), m-hydroxydiphenyl (phenyl phenol, $\geq 90\%$), NaBH_4 ($\geq 98.0\%$), NaCl
114 ($\geq 99\%$), NaOAc ($\geq 99\%$), NaOH ($\geq 97\%$), NaNO_2 ($\geq 99\%$), Pyridine ($\geq 99\%$), %, the standards
115 glucose (Glc - purity $\geq 99\%$), galacturonic acid (GalA - purity $\geq 97\%$), glucuronic acid (GlcA -
116 purity $\geq 99\%$), arabinose (Ara - purity $\geq 99\%$), rhamnose (Rha - purity $\geq 99\%$), xylose (Xyl -
117 purity $\geq 99\%$), mannose (Man - purity $\geq 99\%$), and Trifluoroacetic acid (TFA, $\geq 99.0\%$), were
118 purchased from Sigma-Aldrich (St. Louis, USA).

119 Cellulose MWCO 3500 D cut off membrane from acquired Spectrum Labs[™] and ultrapure
120 water (18 M Ω , pH 6.9) was obtained through the Master system-MS2000/Gehaka.

121

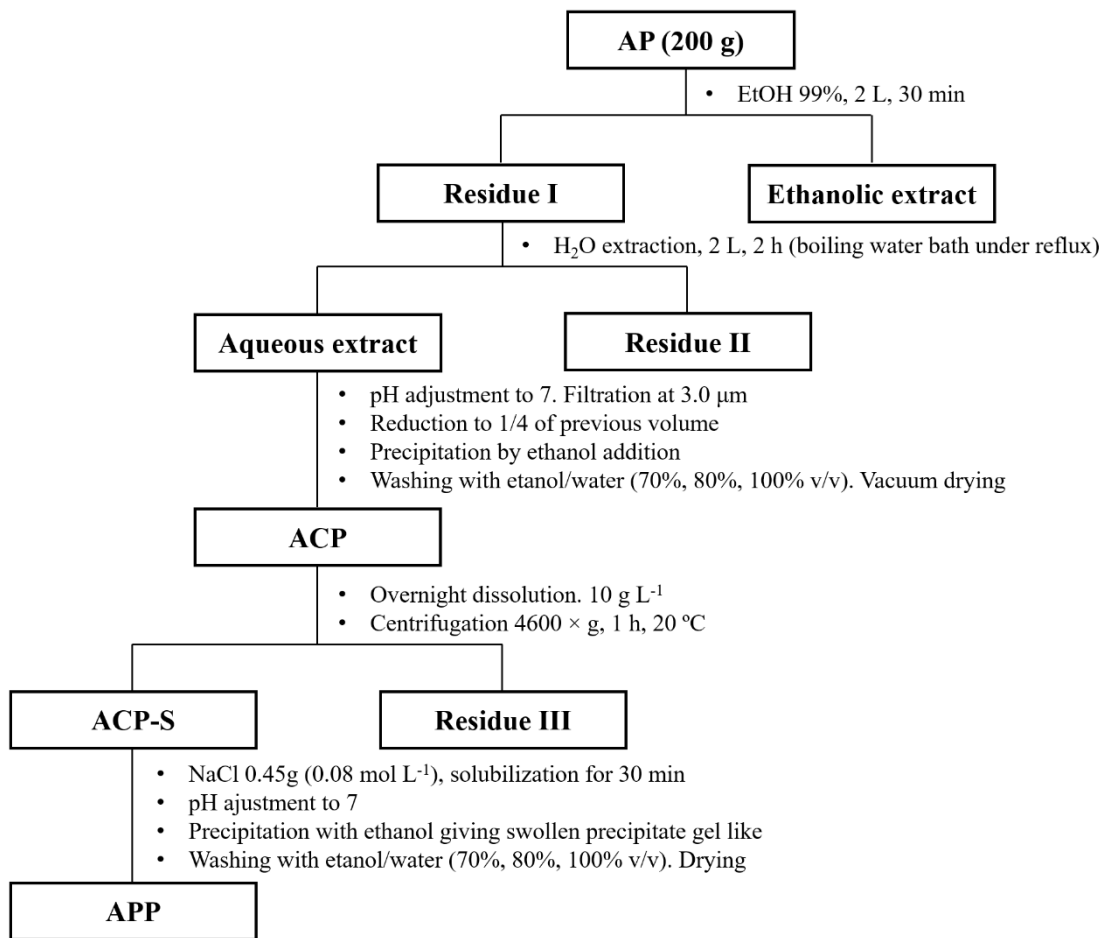
122 2-2. Extraction, fractionation, and purification of polysaccharides

123 The Araçá pulp (AP, 200 g) was submitted to an enzymatic inactivation and depigmentation
124 treatment with 99.5% ethanol in a boiling water bath under reflux for 30 minutes, giving the
125 alcohol insoluble residues (Residue I). The aqueous extract from the Residue I in mild
126 conditions was obtained by extraction with hot ultrapure water in a boiling water bath under

127 reflux for 2 h. Then, the pH of the solution was adjusted to 7 with 1 mol L⁻¹ NaOH solution.
128 The volume was reduced to a quarter of the initial volume under vacuum, followed by
129 precipitation of the water soluble components with ethanol/water (up to around 1:1, v/v),
130 washing the precipitate with increasing concentrations of ethanol /water (70%, 80% v/v) and at
131 end with ethanol P.A. and then dried under vacuum. The isolated polymers are identified as
132 Araçá Crude Polysaccharide (ACP) (Fig. 1).

133 The ACP fraction was further purified according to Rinaudo, 2005 with some adjustments: 1 g
134 of the obtained crude fraction ACP was solubilized in 100 mL of deionized water. This solution
135 was left under magnetic stirring overnight (16 h). Then, the solution was centrifuged (4600 x
136 g, 1 h, at 20 °C) to remove water-insoluble impurities and get ACP-S. After centrifugation, the
137 precipitate was discarded and 0.45 g of NaCl was added to the supernatant (0.08 mol L⁻¹), under
138 stirring and left to stir for 30 min to get the sodium form of charged polymers. This step allows
139 to exchange calcium counterions existing often in pectin extracts. The pH was adjusted to 7
140 with 0.1 mol L⁻¹ NaOH solution. Then, ethanol precipitation was carried out with increasing
141 concentrations of ethanol. The polysaccharide precipitation was observed for a final
142 ethanol/water mixture of approximately 60% (v/v). At relatively low ethanol content may avoid
143 the precipitation of neutral polysaccharides able to exist in aqueous extract of pulp or peel.
144 After, the precipitate was washed with increasing concentrations of ethanol/water (70%, 80%
145 v/v) and at end with ethanol P.A. The sample was dried at atmospheric conditions until constant
146 weight, obtaining the Araçá Purified Pectin (APP) (Fig. 1). As the precipitate is obtained at a
147 relatively low yield in ethanol (60%) allows to assume that it is a charged polymer, mainly
148 pectic materials.

149



150

151 **Fig. 1.** Scheme of extraction and purification of pectins from the pulp of Araçá (*Psidium*
152 *cattleianum* Sabine) fruits.

153

154 2-3. Protein content

155 Protein content of the Araçá pectin samples was determined using the Bradford method
156 (Bradford, 1976). A calibration curve of bovine serum albumin (BSA) was built as a standard.

157 Absorbance was measured by spectrophotometry (Epoch, BioTek, USA) at 595 nm, and results
158 were expressed in g protein/100 g of sample.

159

160 2-4. Uronic acid content and degree of methyl-esterification (DM)

161 The uronic acid content and the degree of methyl-esterification (DM) were quantified using a
162 conductometric titration technique described previously (Cárdenas, Goycoolea, & Rinaudo,

163 2008; Thibault & Rinaudo, 1985). 20 mg of APP sample was dissolved in 50 mL of deionized
164 water and solubilized overnight. Then, to determine the uronic acid content in free carboxylic
165 acid, titration was performed with 0.02 mol L⁻¹ HCl solution followed by titration with 0.02
166 mol L⁻¹ NaOH, using a conductometer (Thermo Scientific, Orion Star A215). The same titration
167 was also used after total de-esterification (as described in the next section 2-5). The difference
168 between the uronic acid content after total de-esterification (total GalA) and the initial
169 carboxylic groups content (GalA⁻Na⁺) allowed to get the value of methyl-esterified uronic acid
170 yield (GalA-Met). The degree of methyl-esterification (DM) was calculated through the ratio
171 between GalA-Met and total GalA expressed in molarity x 100 as usually adopted.

172 Additionally, from those determinations, the fraction of neutral carbohydrates (corresponding
173 to the neutral side chains of the pectins in the sample) can be deduced by the difference between
174 the total mass of sample and total GalA content. And finally, to convert the percentages
175 obtained from g/g to mol/g, the values were normalized considering the molar mass of 198 g
176 mol⁻¹, 190 g mol⁻¹, and 162 g mol⁻¹ for GalA⁻Na⁺, GalA-Met and neutral monosaccharides,
177 respectively.

178 The content of total uronic acids of the Araçá pectin samples were also analysed by the
179 colorimetric *m*-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973).

180 The identity of the uronic acid was determined by anion exchange chromatography with pulse
181 amperometric detection (HPAEC-PAD). Samples were prepared as described by Amaral et al.,
182 2019 and injected into a Thermo Scientific Dionex ICS-5000 chromatograph (Thermo Fisher
183 Scientific, USA) with CarboPac PA20 column (3×150 mm), with a gradient of 0.5 mol L⁻¹
184 NaOH and 1 mol L⁻¹ NaOAc as eluent (Nagel, Sirisakulwat, Carle, & Neidhart, 2014) in an N₂
185 atmosphere in a flow of 0.2 ml min⁻¹ at 24 °C. The data was collected and analyzed using the
186 Chromeleon TM 7.2 Chromatography Data System software. The galacturonic acid was used
187 as the standard and analysis was carried out in triplicate.

188

189 **2-5. APP de-esterification**

190 To prepare pectins with different degrees of methyl-esterification (DM), the de-esterification
191 reaction was carried out with 0.02 mol L⁻¹ NaOH for 15 min (APP-15), 30 min (APP-30), 60
192 min (APP-60), and 105 min (APP-105). Briefly, 150 mg of sample (APP) was solubilized in 24
193 mL of deionized water under stirring for 1 h and kept refrigerated overnight (~ 16 h). After, 6
194 mL of 0.1 mol L⁻¹ NaOH was added under stirring. After 15, 30, 60 or 105 min of reaction in
195 alkaline conditions, 1 mol L⁻¹ HCl was added gradually until reaching pH ~ 7-7.5. Then, ethanol
196 precipitation and drying of APP de-esterified samples were carried out as described in the
197 section 2-2.

198

199 **2-6. Neutral monosaccharide composition**

200 Neutral monosaccharides involved in the polysaccharide structure may be determined after total
201 hydrolysis of the initial sample and fractionation. However, the kinetic and conditions of
202 hydrolysis are important (Nagel et al., 2014). Then, in order to determine the best hydrolysis
203 condition to obtain the neutral monosaccharide rate, a hydrolysis kinetics was performed with
204 the APP sample.

205 Briefly, 10 mg of APP were solubilized with 3.5 mL of 2 mol L⁻¹ TFA and this solution was
206 divided into 7 tubes for hydrolysis at 100 °C at different times: 0.5, 1, 2, 3, 4, 6, and 8 h. After
207 hydrolysis, the acid was removed by evaporation at room temperature under air current and
208 washed with methanol (1 mL x 3). Then, 1 mL of ultrapure water was added to each tube.

209 At each time (0.5, 1, 2, 3, 4, 6, and 8 h) of hydrolysis kinetics, the total carbohydrates (phenol-
210 sulfuric acid method, Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and reducing
211 carbohydrates (dinitrosalicylic acid - DNS method, Miller, 1959) were measured. Both

212 determinations were calibrated using a standard solution containing GalA and Ara standards
213 (70:30, w/w).

214 The neutral monosaccharides composition was determined using Gas–Liquid Chromatography
215 (GLC - Thermo Scientific Trace GC Ultra gas chromatograph) after acid hydrolysis. For that
216 purpose, 10 mg of samples, added of 50 μL of an inositol solution at 10 mg mL^{-1} used as internal
217 standard, were hydrolysed with 2 mol L^{-1} TFA for 1 or 8 h at 100 $^{\circ}\text{C}$, followed by conversion
218 to alditol acetates by NaBH_4 reduction (100 $^{\circ}\text{C}$ for 10 min) (Wolfrom & Thompson, 1963a)
219 and acetylation with acetic anhydride (Ac_2O)-pyridine (1:1, v/v, 1 mL) at 100 $^{\circ}\text{C}$ for 30 min
220 (Wolfrom & Thompson, 1963b). The resulting alditol acetates were extracted with CHCl_3 , and
221 identified by comparing their profiles and retention times with standards after GLC. A mixture
222 of He and N_2 with compressed air was applied as a carrier gas at 1 mL min^{-1} , and a DB-225-
223 MS column programmed from 100 $^{\circ}\text{C}$ to 230 $^{\circ}\text{C}$ at a heating rate of 60 $^{\circ}\text{C min}^{-1}$ was utilized.
224 To determine the total neutral monosaccharide (TNM) content, the peak areas of all the detected
225 monosaccharides were summed and quantified using inositol as internal standard.

226 **2-7. Size exclusion chromatography**

227 The weight-average molecular weights (M_w) of each sample were determined through Size
228 Exclusion Chromatography (SEC) measurements using two columns in series from Shodex SB
229 806 M HQ associated with a pre-column SB-G 6B. The solvent used was 0.1 mol L^{-1} NaNO_3
230 including 0.3 g L^{-1} NaN_3 . A laser light scattering Mini Dawn Treos detector from Wyatt was
231 associated with a Wyatt differential refractometer. The selected flow rate was 0.5 mL min^{-1} at
232 30 $^{\circ}\text{C}$, the dn/dc was equal to 0.155 and sample concentration injected was 4 mg mL^{-1} . The
233 samples were dissolved in water under stirring during 24 h and filtrated on a 0.2 μm porous
234 membrane before injection.

235 **2-8. Nuclear magnetic resonance (NMR) spectroscopy**

236 Mono-dimensional (^1H -) and bi-dimensional (HSQC) NMR spectra were acquired at 70 °C with
237 a Bruker Avance III 400 MHz or Bruker Avance III HD 600 MHz spectrometers, equipped with
238 a BBI 5 mm probe (400 MHz) or with a 5 mm CPP-TCI probe (600 MHz) (Bruker, Billerica,
239 Massachusetts, USA).

240 The samples were solubilized in D_2O and the chemical shifts of the polysaccharide were
241 expressed as δ (ppm), using the resonances of $-\text{CH}_3$ groups of acetone (^1H at δ 2.22; ^{13}C at δ
242 30.20) as internal references. The data were collected and processed using the Software
243 TOPSPIN, version 3.1 (Bruker Biospin, Rheinstetten, Germany).

244 Complementary ^1H NMR measurements were performed at 353 K on a Bruker AVANCE I
245 instrument equipped with a 5 mm probe operating at 400.2 MHz. An amount of 3 mg of
246 polymer was dissolved in 0.5 mL of D_2O , and 32 scans were recorded. Chemical shifts are
247 expressed using H_2O at 4.25 ppm at 80 °C as reference.

248 The values of degree of methyl-esterification (DM) were determined by ^1H - NMR
249 spectroscopy integrating the hydrogen areas corresponding to H-1, H-5 of unesterified α -D-
250 GalAp units and H-5 of esterified α -D-GalAp units as discussed by Grasdalen, Bakøy, & Larsen,
251 1988. The hydrogen from $-\text{CH}_3$ around 3.76 ppm gives DM using H-1 integral as reference
252 (Patova et al., 2019).

253

254 **2-9. Gelation ability and characterization**

255 Depending on the degree of methyl-esterification, pectins are able to form gel in acidic
256 conditions (excess of HCl) and/or in presence of calcium. Different amounts of the each
257 samples were solubilized in 1 mL of water. Then, 100 μL of 1 mol L^{-1} HCl or 100 μL of 1 mol
258 L^{-1} CaCl_2 was added to determine the critical concentration necessary for the formation of
259 homogeneous gel. Then, the critical concentration for gelation was determined at different
260 degree of methyl-esterifications.

261 To prepare the pectin gels, a 10 g L⁻¹ solution previously dissolved in deionized water were
262 placed into a dialysis cellulose bag (Spectrum™ Labs Spectra/Por™, MWCO 3500 D) and
263 placed to dialysis against a 1 mol L⁻¹ CaCl₂ solution for 24 h (Rahelivao, Andriamanantoanina,
264 Heyraud, & Rinaudo, 2013). The resulting formed gels was recovered by cutting off the dialysis
265 membrane with a scalpel and punched into 2 mm thickness disks for the rheological
266 measurements.

267

268 **2-10. Rheological characterization**

269 Rheological gel characterization was performed on an ARES-G2 rheometer from TA
270 Instruments Company. The APP-15 gel at 10 g L⁻¹ formed in 1 mol L⁻¹ CaCl₂ was loaded
271 between the two rough plates separated by a gap of 2 mm and of 10 mm diameter. All
272 experiments were performed at 28 °C, thanks to the forced convection oven. Three different
273 types of experiments were conducted: (1) a classical oscillatory shear frequency sweep, (2) an
274 orthogonal oscillatory frequency sweep and (3) one compression test from 2 to 1.5 mm gap at
275 constant velocity 0,05 mm s⁻¹. Experiments (1) and (2) were performed under respectively 0.1%
276 and 0.05% strain well under the upper limit of the linear regime determined at 0.5% strain.

277

278 **3 RESULTS AND DISCUSSION**

279 **3.1. Extraction, purification and structural characterization of the polysaccharide**

280 **extracted from Araçá pulp**

281 The Araçá Crude Polysaccharyde (ACP) was obtained after extraction with hot water and
282 precipitation with ethanol (Fig. 1). The extraction yield of ACP reached 4.7% (w/w) of the dry
283 fruit (AP). The ACP fraction was then purified by centrifugation in order to remove insoluble
284 particles, followed by addition of monovalent salt (Fig. 1) to exchange multivalent cations for
285 sodium and to screen the long-range electrostatic repulsions (Rinaudo, 2005). Then, after

286 ethanol precipitation, the Araçá Purified Pectin (APP) fraction was obtained, with the yield of
287 75% (w/w) in relation to the starting ACP fraction (i.e. 3.5% w/w of the initial material).

288 Firstly, the APP fraction was composed of 1.6 g/100g of proteins. This value is of the same
289 order than that reported for apple pectin given as 1.6% of proteins (Kravtchenko et al., 1992).

290 Considering that the ability of pectins to form hydrogels can be affected by intrinsic parameters,
291 as the degree of methyl-esterification (DM), the APP fraction was submitted to chemical
292 modification to obtain four pectins with different DM. After 15, 30, 60, and 105 min of
293 hydrolysis reaction of the ester groups, from conductometric titration, it was obtained the APP-
294 15, APP-30, APP-60, and APP-105 values of DM (Table 1).

295 By conductimetry, it was possible to determine the content of free uronic acid (on $-\text{COO}^-\text{Na}^+$
296 form) on each sample. Firstly, the titration is performed on APP-60 which is fully demethylated
297 as shown by ^1H -RMN (Table 1, Fig. 4D). It is found $363 \times 10^{-5} \text{ }-\text{COO}^-$ per g which means 0.363
298 mol GalA in 100g of dried sample. Taking into account the molar mass of the repeat unit $M_m =$
299 198 g mol^{-1} , the fraction of GalA in the polysaccharide equals 71.9 g/100g sample. Then, 28.1
300 g/100g should be represented by proteins and neutral saccharides (1.6 and supposed 26.5 g/100g
301 respectively).

302 Each sample was titrated to get the number of ionic unit in the dried weight which is expressed
303 in g/100g sample (Table 1). Taking into account the total number of GalA units, the esterified
304 sample is deduced and following the degree of methyl-esterification. The nearly constant value
305 of total GalA^-Na^+ content up to 105 min confirms that total de-esterification was achieved after
306 60 min of reaction. These values were compared with ^1H NMR experiments (Table 1). There
307 is a relatively good agreement excluding APP-15 value which seems to be too large when using
308 titration.

309 The content of uronic acid was confirmed by Blumenkrantz & Asboe-Hansen, 1973 method
310 and a similar value (76.9 g/100g) was obtained (Table 1), comparing to the value of 71.9 g/100g

311 obtained by titration method. Furthermore, the identification of the uronic acid type of the APP
 312 sample was determined as GalA by HPAEC-PAD.

313 The degree of methyl-esterification (DM) of the APP sample obtained from conductometric
 314 titration equals DM = 55.9% characterizing this polymer as a high methoxyl (HM) type
 315 (Munarin et al., 2012). A similar high DM (60%) was observed for the GW pectin extracted
 316 from the Gabiroba (*Campomanesia xanthocarpa* Berg) (Barbieri et al., 2019) and for the Murta
 317 (*Ugni molinae* Turcz) pectin (57%) (Taboada et al., 2010) both belonging to the Myrtaceae
 318 family.

319

320 **Table 1.** Uronic acid and DM of pectins obtained from the Araçá pulp.

Uronic acid							
Sample	GalA·Na ⁺ (x10 ⁻³) ^a	GalA·Na ⁺ ^b	GalA-Met ^b	Total GalA ^b	GalA ^c	DM (%) ^a	NMR DM (%)
APP	162.4	32.1	39.8	71.9	76.9	55.9	58
APP-15	208.0	41.2	30.7	71.9	70.2	43.5	18
APP-30	324.1	64.2	7.7	71.9	66.3	11.9	10
APP-60	368.1	71.9	0	71.9	66.9	0	0
APP-105	345.8	68.4	0	68.4	Nd	0	0

321 ^a Quantified by conductometric titration. GalA·Na⁺ expressed in -COO⁻ per 100 g. DM in molar ratio x100

322 ^b GalA-Met, free Gal-A and total GalA expressed in g/100 g of initial dried sample.

323 ^c (g/100g) Determined by Blumenkrantz & Asboe-Hansen, 1973 method.

324 Nd = not determined

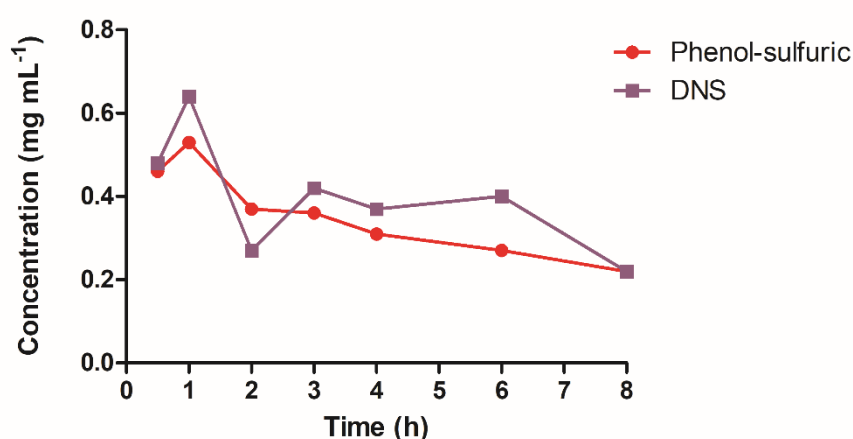
325

326 In order to determine the value of total neutral monosaccharides of the APP sample it was first
 327 selected the best hydrolysis conditions, taking into account the wide variation on the stability
 328 of glycosidic bonds to acid hydrolysis and that different monosaccharides are destroyed by acid
 329 at different rates (Mankarios, Jones, Jarvis, Threfall, & Friend, 1979).

330 Therefore, considering the balance between the total hydrolysis time and the degradation of
331 free monosaccharides, a kinetics of hydrolysis was obtained by dosage of the total
332 carbohydrates (Dubois et al., 1956) and the reducing carbohydrates (Miller, 1959). Through
333 Fig. 2 it is possible to observe that 1 hour of acid hydrolysis gives the maximum value of
334 carbohydrates, which we adopt as the best condition for the determination of total neutral
335 monosaccharides for the APP sample.

336

337



338

339 **Fig. 2.** Hydrolysis kinetics of the APP sample. Concentration of total carbohydrates (phenol-sulfuric) and reducing
340 carbohydrates (DNS) at different hydrolysis times (0.5 h – 8 h) on 1.43 mg in 1 mL.

341

342 From the hydrolysis kinetics, the times of 1 hour (best condition) and 8 hours of hydrolysis,
343 condition usually used (Sasaki et al., 2008), were selected for the GLC analysis. The APP
344 sample showed a total reducing carbohydrates of 0.64 mg/mL after 1 hour of hydrolysis
345 corresponding to 50.4 % as the maximum yield hydrolysis of the initial dried sample. After 8
346 hour of hydrolysis, 0.22 mg/mL of monosaccharide are produced i.e. the fraction of sample
347 equals 17.3%. This value is lower than after 1 hour confirming the occurrence of neutral
348 monosaccharide degradation in longer hydrolysis times with 2 mol L⁻¹ TFA at 100 °C. The
349 difference between the degrees of acid hydrolysis with time may be due to the difficulty of

350 finding a compromise between destruction of the less stable monosaccharide (Ara) and
 351 incomplete hydrolysis of the more resistant glycosidic bonds in polyuronates, such as pectins
 352 (Mankarios et al., 1979). The neutral carbohydrates analysis on these hydrolyses allows to
 353 determine Rha, Ara and Gal contents with inositol as reference giving a total neutral
 354 monosaccharide (TNM) content of 14.6 g and 10.6 g/100g of APP sample for 1 and 8 hours of
 355 hydrolysis, respectively (Table 2). Considering the maximum degree of hydrolysis, it is
 356 concluded that the content in neutral carbohydrates may correspond to 28.9 g/100g dried sample
 357 ($(14.6/50.4) \times 100 = 28.9$ g/100g) completing the total galacturonic acid determined as
 358 71.9g/100g by titration. In addition, from Table 2, it may be concluded that rhamnose involved
 359 in RG-I zone is progressively released due to the neighbor galacturonic acid preventing
 360 hydrolysis when arabinose is labile.

361

362 **Table 2.** Monosaccharide composition of pectins obtained from the Araçá fruit.

Monosaccharide composition (g/100g)						
Sample	GalA ^e	Rha	Ara	Gal	TNM ^f	Protein ^g
APP ^{a,b}	71.9	0.2	14.1	0.4	14.6	1.6
APP ^{a,c}	71.9	0.6	9.2	0.8	10.6	1.6
APP ^d	-	2.6	29	Nd	Nd	Nd

363 ^a Monosaccharide composition, determined by GLC using inositol as standard.

364 ^b Monosaccharide composition and TNM after 1 h of hydrolysis

365 ^c Monosaccharide composition and TNM after 8 h of hydrolysis

366 ^d Monosaccharide composition by ¹H NMR

367 ^e g/100g of total uronic acid determined by titration. GalA was identified by HPAEC-PAD.

368 ^f TNM: Total neutral monosaccharides after hydrolysis

369 ^g g/100g determined by Bradford method

370 Nd = Not determined

371

372 The GalA/Rha molar ratio of the monosaccharide composition obtained for APP equals 27.6
373 considering the rhamnose content obtained by NMR. The large difference between rhamnose
374 obtained by acid hydrolysis and by NMR may be attributed mainly to rhamnose involved in the
375 RG-I blocks resistant to hydrolysis. The large ratio GalA/ Rha indicates predominance of long
376 HG regions (Zhang et al, 2018).

377 Despite the decrease in the TNM value, the relative proportion between monosaccharides was
378 maintained even after 8 h of hydrolysis, with only a small decrease in Ara and an increase in
379 Rha and Gal contents. In fact, arabinose in the pectin side chains is the monosaccharide to be
380 released more rapidly and which tends to be more liable to breakdown following their release
381 during acid hydrolysis (Mankarios et al., 1979).

382

383 **3-2. NMR structure characterization.**

384

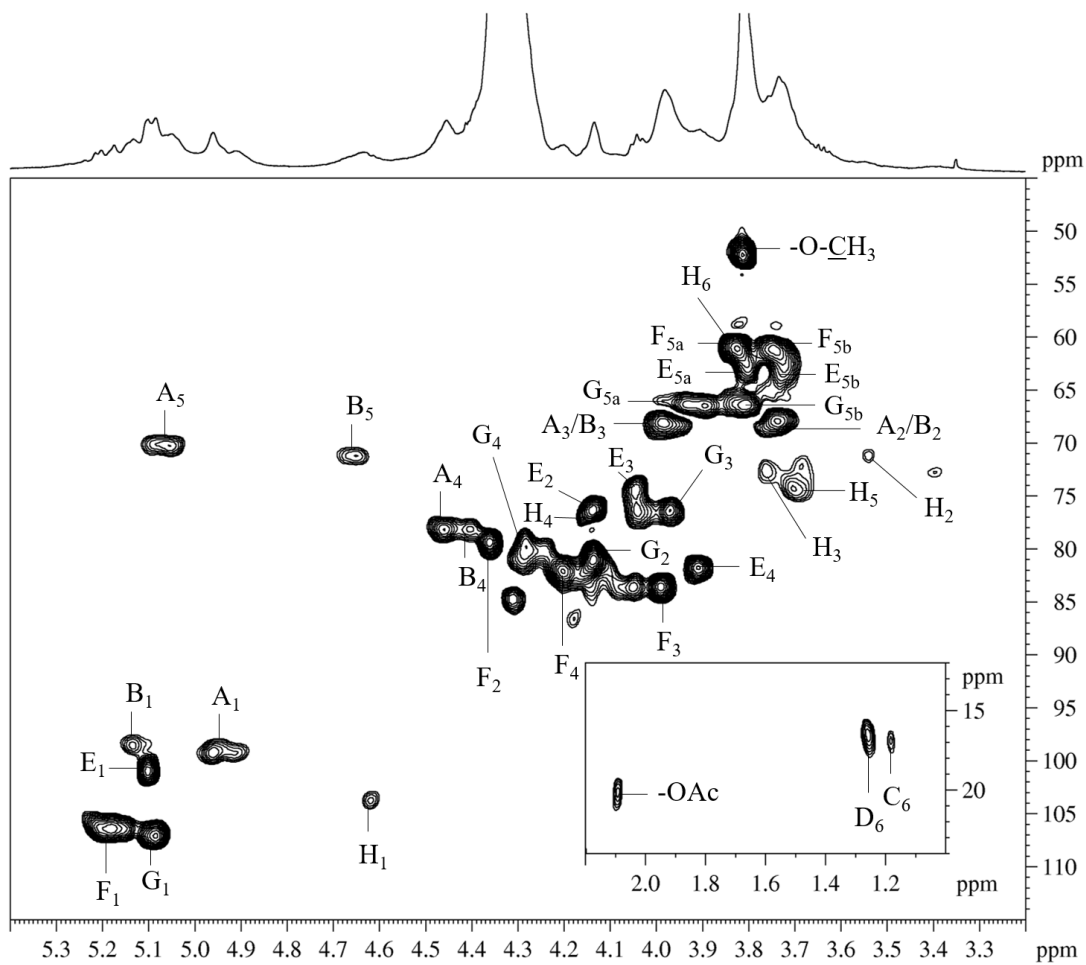
385 $^1\text{H}/^{13}\text{C}$ heterocorrelated HSQC NMR spectrum was employed to provide detailed structural
386 information about the APP sample isolated Araçá pulp. In Fig. 3 the HSQC NMR spectrum
387 revealed $^1\text{H}/^{13}\text{C}$ correlations from HG assigned to the anomeric carbon of $\rightarrow 4$)- α -D-6MeGalAp-
388 (1 \rightarrow at δ 4.96/99.6 (unit A₁) and of $\rightarrow 4$)- α -D-GalAp-(1 \rightarrow at δ 5.13/99.0 (unit B₁) (Dias,
389 Barbieri, Fetzer, Corazza, & Silveira, 2020). The remaining unesterified and methyl-esterified
390 units of α -D-GalAp ring correlations were showed in Table 3. The signal at δ 3.81/52.8
391 corresponding to methyl group (O-CH₃) of α -D-6Me-GalAp (1 \rightarrow 4) units, whereas the acetyl
392 group (O-Ac) was observed at δ 2.09/20.09 ppm (Colodel, Vriesmann, Teófilo, & Petkowicz,
393 2018; Patova et al., 2019).

394 Characteristic correlations attributed to the presence of RG-I blocks was also observed
395 at δ 1.17/16.5 (H-6/C-6) to $\rightarrow 2$)- α -L-Rhap-(1 \rightarrow (units C₆) and at δ 1.25/16.5 ppm to substituted

396 \rightarrow 2,4)- α -L-Rhap-(1 \rightarrow (unit D₆) (Fig. 3, Table 3), in agreement with the presence of Rha
 397 determined by GLC (Table 2).

398 In addition, the $^1\text{H}/^{13}\text{C}$ correlations at δ 5.10/101.6 (H-1/C-1), δ 4.13/76.6 (H-2/C-2), δ
 399 4.04/74.4 (H-3/C-3), δ 3.90/81.9 (H-4/C-4) were assigned to terminal β -L-Araf-(1 \rightarrow (unit E).

400 Despite few reports of the presence of the t- β -L-Araf-(1 \rightarrow units, this signal was recently
 401 observed for pectins obtained from Myrtaceae family fruits as Guavira pomace (Schneider,
 402 Iacomini, & Cordeiro, 2019) and Gabiroba pulp (Dias et al., 2020).



403
 404 **Fig. 3.** $^1\text{H}/^{13}\text{C}$ HSQC spectrum of the APP sample from the Araça pulp. The sample was dissolved in D_2O and
 405 data were collected at a probe temperature of 70 $^\circ\text{C}$ and measured relative to acetone at ^1H (δ 2.22) and ^{13}C (δ
 406 30.2).

407
 408
 409 **Table 3.** $^1\text{H}/^{13}\text{C}$ HSQC chemical shifts (δ , ppm) of the APP sample from the Araça pulp.

Glycosil units		Chemical shifts, δ (ppm)								-OCH ₃
		1	2	3	4	5 5a	5b	6		
A	\rightarrow 4)- α -D-6MeGalAp-(1 \rightarrow	¹³ C	99.6	68.1	68.1	78.2	70.4	-		52.8
		¹ H	4.96	3.73	3.98	4.45	5.05	-	nd	3.81
B	\rightarrow 4)- α -D-GalAp-(1 \rightarrow	¹³ C	99.0	68.1	68.1	78.2	71.5	-		-
		¹ H	5.13	3.73	3.98	4.40	4.64	-	nd	-
C	\rightarrow 2)- α -L-Rhap-(1 \rightarrow	¹³ C						-	16.5	-
		¹ H	nd	nd	nd	nd	nd	-	1.17	-
D	\rightarrow 2,4)- α -L-Rhap-(1 \rightarrow	¹³ C						-	16.5	-
		¹ H	nd	nd	nd	nd	nd	-	1.25	-
E	<i>t</i> - β -L-Araf-(1 \rightarrow	¹³ C	101.6	76.6	74.4	81.9	63.0			-
		¹ H	5.10	4.13	4.04	3.90	3.80	3.72		-
F	\rightarrow 3)- α -L-Araf-(1 \rightarrow	¹³ C	107.0	79.4	83.6	82.2	61.3	-		-
		¹ H	5.17	4.36	3.99	4.19	3.81	3.73	-	-
G	\rightarrow 5)- α -L-Araf-(1 \rightarrow	¹³ C	107.6	81.2	76.6	80.2	66.4			-
		¹ H	5.08	4.13	4.04	4.28	3.89	3.81	-	-
H	\rightarrow 4)- β -D-Galp-(1 \rightarrow	¹³ C	104.3	71.3	72.5	77.6	74.6	-	61.0	-
		¹ H	4.61	3.54	3.75	4.13	3.70	-	3.81	-

410 nd: not determined.

411

412 The other anomeric signals (H-1/C-1) at δ 5.17/107.0 and δ 5.08/107.6 ppm were attributed to

413 \rightarrow 3)- α -L-Araf-(1 \rightarrow (unit F) and \rightarrow 5)- α -L-Araf-(1 \rightarrow (unit G), respectively. The correlations

414 corresponding to \rightarrow 4)- β -D-Galp-(1 \rightarrow (unit H), were also shown at δ 4.61/104.3, 3.54/71.3,

415 3.75/72.5, 4.13/77.6, 3.70/74.6, 3.81/61.0 assigned as H-1/C-1, H-2/C-2, H-3/C-3, H-4/C-4, H-

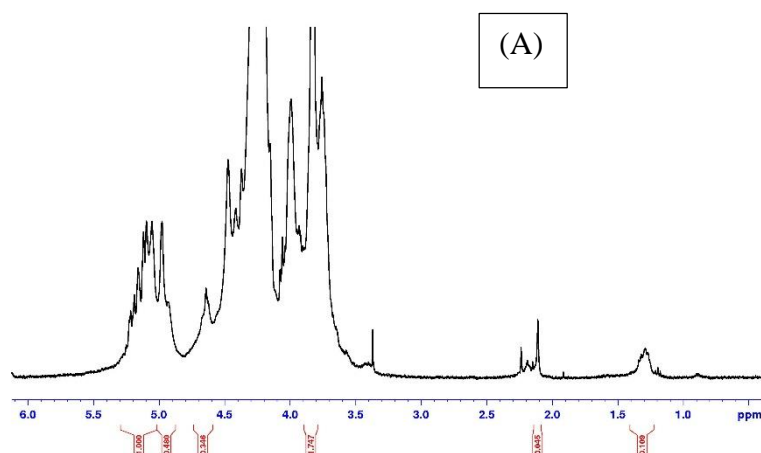
416 5/C-5 and H-6/C-6 of \rightarrow 4)- β -D-Galp-(1 \rightarrow (unit H), respectively (Fig. 3, table 3), suggesting

417 the presence of arabinans, galactans or type I arabinogalactans (AG-I), which can be linked as

418 a side chain in RG-I (Dias et al., 2020; Schneider et al., 2019). Thus, according to the

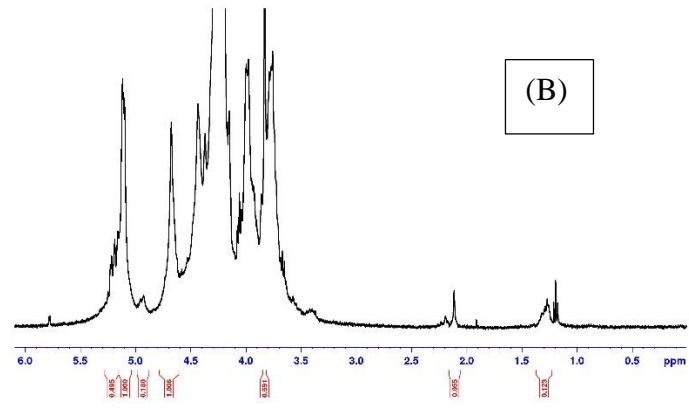
419 monosaccharide composition together with the NMR analyzes it is possible to suggest that the

420 APP sample has a structure formed mostly by HG blocks with a lower proportion of RG-I
421 blocks with a side chain consisting of arabinans, galactans or AG-I.
422 The bidimensional analysis was completed with ^1H NMR studied after different times of
423 hydrolysis as shown in Fig. 4. A large modification occurs as soon as hydrolysis starts allowing
424 to follow the demethylation rate. The spectra allows to quantify Rha, acetyl and methyl
425 substituents, and Ara relatively easily from the integrals around 1.3 ppm, 2.1 ppm, 3.8 ppm and
426 around 5.11 ppm, respectively. In addition, around 5-5.1 ppm are H-1 and 4.98 ppm is the H-
427 5 of Me-GalA and around 4.67 ppm is the H-5 of free GalA units. (as proposed by Grasdalen
428 et al., 1988). Interestingly, the ratio between the signal of H-5 of free GalA and total of H-5
429 also approach the DM determination avoiding some overlap of the 3.8 ppm signal.
430

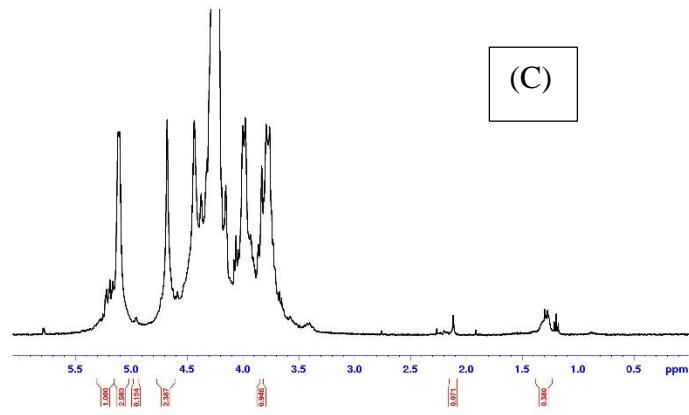


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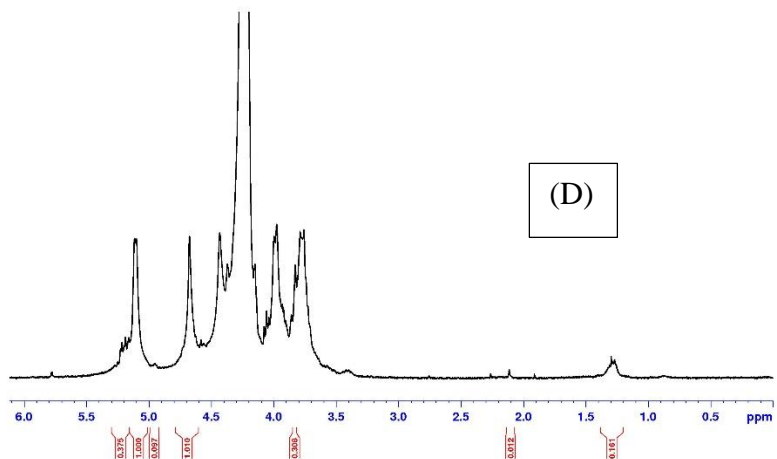
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434



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436



437 **Fig. 4.** ^1H NMR spectrum of the APP samples isolated from the Araçá fruit obtained at 80 °C in D_2O (chemical
438 shifts are expressed in δ , ppm). (A) Initial sample APP, (B) sample obtained after 15 minutes (APP-15) of de-
439 esterification reaction; (C) sample obtained after 30 minutes (APP-30) of de-esterification reaction ; (D) sample
440 obtained after 60 minutes (APP-60) of de-esterification reaction

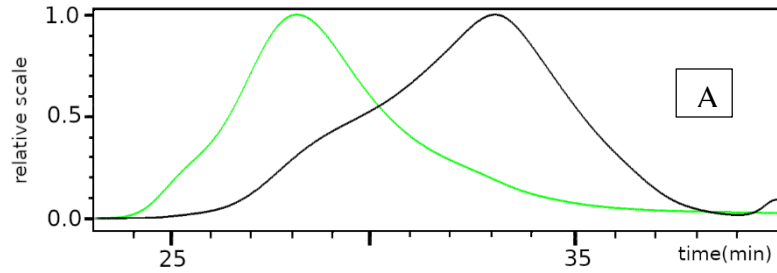
441
442 The evolution of the spectra allows to draw few conclusions concerning the molecular structure
443 of the different samples as a function of the alkaline hydrolysis time. Firstly, it is shown that the
444 acetyl as well as the methyl content obtained by ^1H NMR decreases progressively compared to
445 H-1 or H-5 signals. Concerning the degree of acetylation, it is obtained a ratio Acetyl
446 content/GalA starting as 0.018 (i.e.~2% of GalA units being acetylated) and decreasing to
447 0.004.

448 The analysis allows also to determine the DM values given in Table 1. and compared with the
449 values obtained by titration with a good agreement, excluding APP-15. It is also shown that
450 rhamnose content remains constant around 2.6 g/100g in the samples. Arabinose decreases
451 slightly from 29 g to 21 g/100g in agreement with its low stability also observed in acid
452 hydrolysis. Clearly, the monosaccharide contents (arabinose, rhamnose) are larger than the
453 values found after acid hydrolysis (1h or 8h) especially for rhamnose involved in the resistant
454 RG-I block. Those yields in neutral monosaccharides obtained by NMR look slightly
455 overestimated to complete the GalA content.

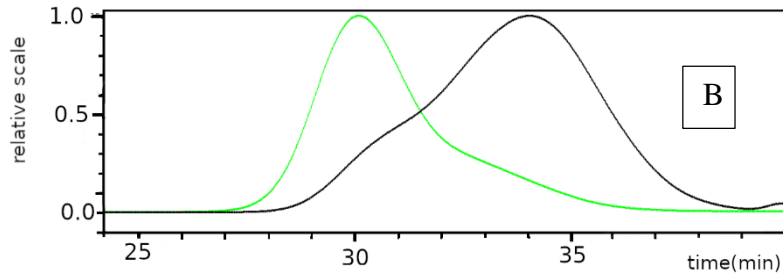
456 457 **3-3. Molecular weight characterization.**

458 SEC results are given in Fig. 5 and 6. A clear modification in the molecular weight distribution
459 occurs as soon as hydrolysis starts.

460



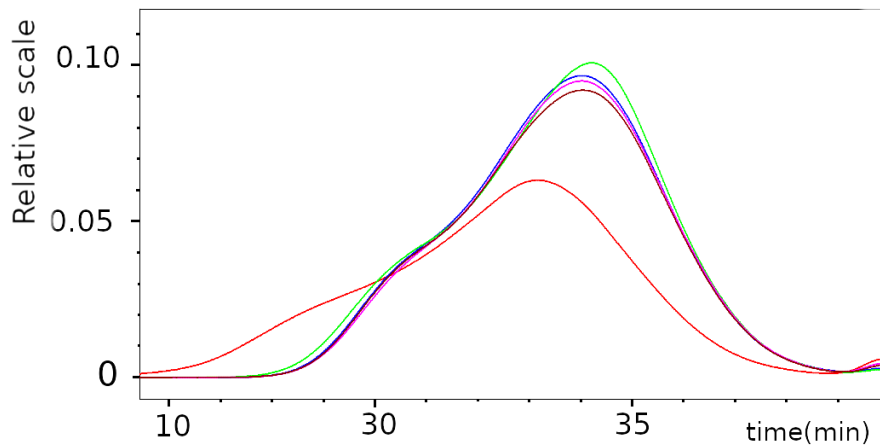
461
462



463

464 **Fig. 5.** SEC-MALLS-RI elution profile of APP samples: (A) unmodified APP; (B) APP-60. Light scattering (LS-
465 90° - green), and refractive index (RI - black) detectors.

466



467

468

469 **Fig. 6.** SEC-RI elution profile of APP samples after different times of hydrolysis: unmodified APP (Red); APP-
470 15 (Green); APP-30 (Pink); APP-60 (Brown); APP-105 (Blue)

471

472 In the initial sample (Fig. 5A), the chromatogram shows that the unmodified sample APP
473 demonstrates 3 different series of molecules. A shoulder in the light scattering signal at lower
474 elution volume at ~ 25 min, may be attributed to a small amount of aggregates of weight-
475 average molar mass in the range of 10^7 (lower than 1 weight %). Then, around 20 weight % of

476 molecules with $M_w \sim 1.5 \times 10^6$ are eluted at 30 min corresponding to the maximum of the light
477 scattering signal. At ~ 33 min, the maximum in the RI detector signal indicates that around 80
478 weight % of molecules have a $M_w \sim 160,000$. The same type of distribution was obtained
479 previously on different commercial pectins (Malovikova, Rinaudo, & Milas, 1993; Zhang et
480 al., 2018).

481 As soon as de-esterification reaction starts, the chromatogram is seriously modified with only
482 two fractions as shown in Fig. 5B. Around 20 weight % of molecules are eluted first with an
483 average $M_w \sim 800,000$ followed by 80 weight % with $M_w \sim 60,000$. The molecular weight
484 distributions of the de-esterified samples (APP-15, APP-30, APP-60, and APP-105) are
485 summarized in Fig. 6 where the refractive index detector signal indicates that all the samples
486 have nearly the same molecular weight distribution excluding the initial sample. In Table 4,
487 the weight-average molecular weights (M_w) of the different samples are listed showing the
488 molecular weight stability when hydrolysis time increases.

489 Table 4. M_w of the unmodified and de-esterified pectins samples obtained by SEC experiments.

Samples	APP	APP-15	APP-30	APP-60	APP-105
M_w	480,000	194,000	173,000	176,000	180,000

490

491 These data indicate that the molecular weight of de-esterified pectins samples (APP-15, APP-
492 30, APP-60, and APP-105) remained unchanged for the different alkaline treatments
493 performed. The only change compared with APP is related to the dissolution of pectins in
494 alkaline conditions which may dissociate association of molecules (forming aggregates) or/and
495 exchanging few remaining Ca counterions in excess of sodium even if the purification was
496 performed in excess of NaCl.

497

498 **3.4. Critical concentration for gelation**

499 In table 5, the observed gelation as a function of pectin concentrations are given in acidic and
 500 calcium excesses. For the tested conditions, the unmodified APP sample was not able to form
 501 a gel even at high polymer concentrations (10 mg mL^{-1}). This result was expected considering
 502 that this sample presented a high DM (DM = 55.9%). HM pectins does not contain sufficient
 503 blocks of free carboxyl groups to form gel or precipitate with calcium ions (Sriamornsak, 2003).
 504 In this case, the gelation of HM pectins are mainly imposed by hydrogen bonding and
 505 hydrophobic interactions in the presence of co-solutes, such as sucrose, which reduces the water
 506 activity, at pH lower than 3.5 that causes protonation of carboxylate residues (Picot-Allain et
 507 al., 2020; Rinaudo, 1996).

508 Different concentrations of the APP, APP-15, APP-30 and APP-60 samples were solubilized
 509 in 1 mL of water. Then, 100 μL of 1 mol L^{-1} HCl or 100 μL of 1 mol L^{-1} CaCl_2 was added to
 510 observe the gel formation (Table 5).

511

512 Table 5. Observed critical concentration for gelation of the Araçá pectin samples in excess of acid and calcium.

Samples	Concentration (mg mL^{-1})	Addition of	
		HCl	CaCl_2
APP	2-10	N	N
APP-15	1	D	D
	2 and 3	D	H
APP-30	1	D	D
	2 and 3	D	H
APP-60	1 and 2	D	D
	3	D	H

513 N: no gel; D: dispersed microgel; H: homogeneous gel

514

515 No gel was obtained with APP, but the de-esterified samples APP-15, APP-30 and APP-60
516 were able to start the gelation process at a concentration of 1 mg mL^{-1} after the addition of HCl
517 or calcium solutions, forming a dispersed microgel.

518 The formation of homogeneous gel was observed for all the de-esterified samples at a
519 concentration of 3 mg mL^{-1} after the addition of CaCl_2 . This ability to form gels in the presence
520 of calcium is in agreement with the chemical structure of Araçá pectin which is rich in GalA
521 blocks.

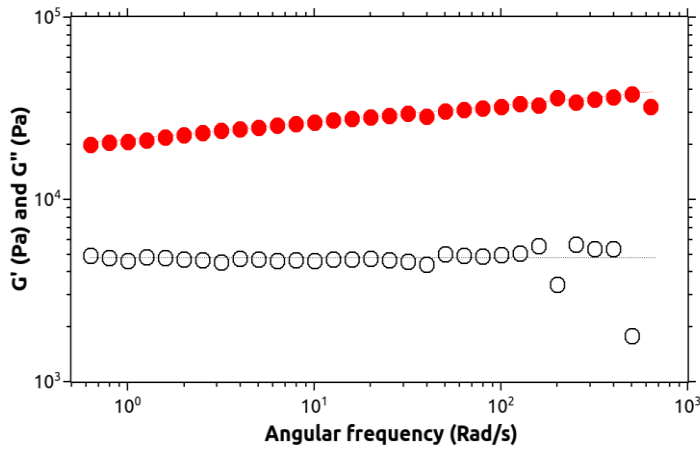
522 Therefore, to better understand the ability of the Araçá pectin to give good gels in presence of
523 calcium, the APP-15 sample was selected for rheological studies.

524

525 **3.5 Characterization of gelling properties**

526 In order to study the ability for gelation in presence of calcium ions in aqueous solution, a gel
527 was prepared with the obtained de-esterified sample APP-15 dialyzed against $1 \text{ mol L}^{-1} \text{ CaCl}_2$
528 solution. Thus, the rheological behaviour of the formed gels was investigated in oscillatory
529 mode.

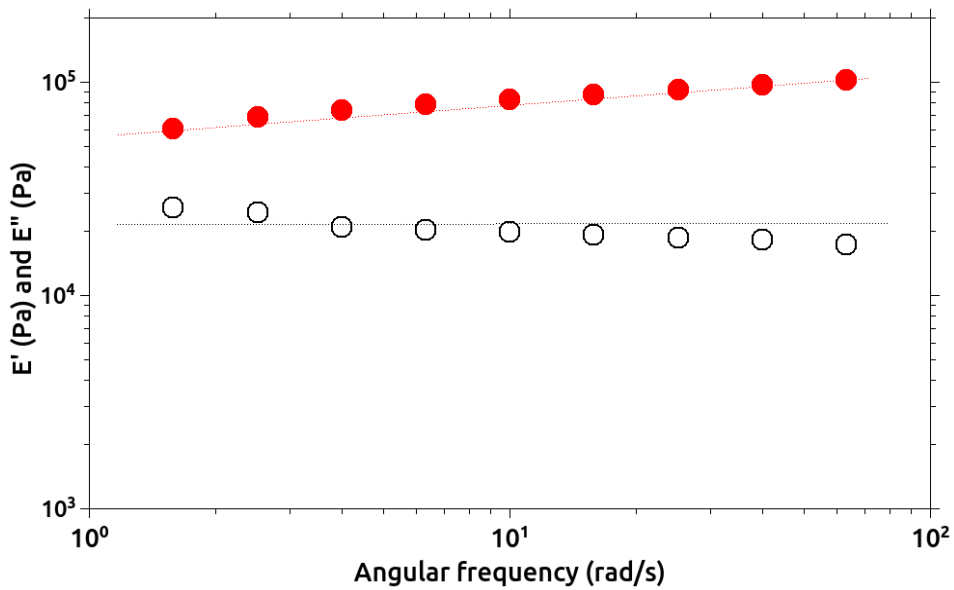
530 Fig. 7 shows the dependence of the elastic (G') and viscous (G'') moduli as a function of
531 angular frequency for an applied oscillatory strain of 0.1%. On the all range of investigated
532 angular frequency, the elastic modulus is almost 10 times higher than the viscous modulus. G''
533 is constant and approximately equal to $5 \times 10^3 \text{ Pa}$ and, at 1 rad/s , G' is equal 2×10^4 and
534 continuously increases with a power law $\omega^{0.17}$. This result is in agreement with the signature of
535 a strong gel for which the network was a consequence of calcium-mediated junctions in the gel
536 structure (Löfgren, Walkenström, & Hermansson, 2002; Vincent, & Williams, 2009).



537

538 Fig. 7. Viscoelasticity moduli versus angular frequency of APP-15 at 10 g L^{-1} in CaCl_2 at $28 \text{ }^\circ\text{C}$ for an applied
 539 oscillatory strain $\gamma = 0.1\%$. Open points correspond to the viscous modulus G'' (Pa) and full dots correspond to
 540 the elastic modulus G' (Pa).

541



542

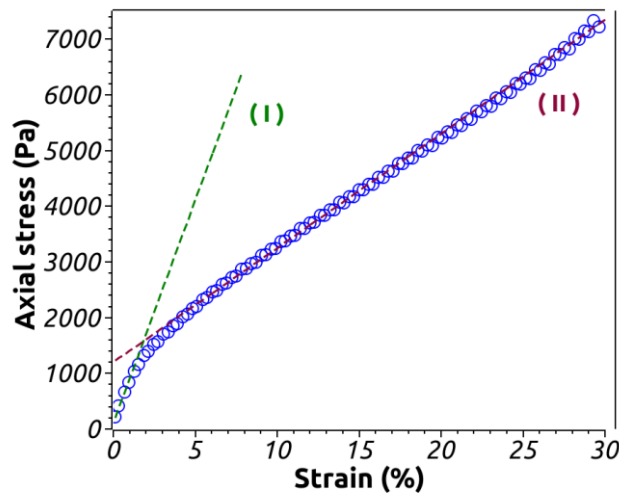
543 Fig. 8. Storage (E' , full dots) and loss (E'' , open dots) moduli for an orthogonal oscillatory frequency sweep at
 544 constant compression strain $\epsilon = 0.05\%$.

545

546 The loss modulus is quite constant and equal to $E''(\text{Pa}) \approx 2 \times 10^4 \text{ Pa}$ (Figure 8) whereas the
547 storage modulus $E'(\text{Pa})$ evolves slowly following a power law $\omega^{0.17}$ with a value $E' \approx 5.5 \times 10^4$
548 Pa at $\omega = 1 \text{ rad/s}$.

549 Fig. 9 shows the axial stress evolution as a function of the compression strain. Initially, the axial
550 stress evolves linearly with the strain with a slope $E_I = 8 \times 10^4 \text{ Pa}$ which is identified as the
551 Young modulus of the gel. The first linear behavior is followed by a second linear behavior
552 showing a proportional evolution of the axial stress versus strain with a slope $E_{II} = 2.05 \times 10^4$
553 Pa.

554



555

556 Fig. 9. Axial stress versus strain obtained by a compression test between a rough plate-plate geometry. Open points
557 were measured on APP-15 dialysed against $1 \text{ mol L}^{-1} \text{ CaCl}_2$ at $T = 28 \text{ }^\circ\text{C}$. Dotted lines correspond to best fits
558 obtained onto linear part of stress versus strain. (I) corresponds to a Young modulus $E_I = 8 \times 10^4 \text{ Pa}$ and (II) to a
559 Young modulus $E_{II} = 2.05 \times 10^4 \text{ Pa}$.

560

561

562

563 It is found that viscous terms named in compression loss modulus E'' is quite constant and equal
564 to $2 \times 10^4 \text{ Pa}$. In shear experiment, the viscous term is named shear modulus G'' and is quite
565 constant and equal to $5 \times 10^3 \text{ Pa}$ (Fig. 7 and 8). There is a direct link between those two
566 parameters moduli given by the classical Trouton ratio (Tr) which equals the ratio between the
567 extensional viscosity E'' to the shear viscosity G'' moduli. For isotropic solutions or soft

568 polymers in solutions $Tr = 3$ or 4 at low extension and shear rates depending of experimental
569 conditions (Amin, & Wang, 2020; Ng, Mun, Boger, & James, 1996). Nevertheless, it has been
570 shown that Trouton ratio drastically increases when molecular rigidity increases, or if the
571 polymer concentration increases or if the extensional strain rate is increased (Tirtaatmadja, &
572 Sridhar, 1993). In the present case, the Trouton ratio is equal 4 which is in agreement with
573 classical observations. The exact cause of this value is out of scope of the present article.
574 Storage moduli E' and G' increase slightly with a power law $\omega^{0.17}$. At $\omega = 1$ rad/s $E' \approx 5.5 \times 10^4$
575 Pa and $G' = 2 \times 10^4$ Pa. There is a direct connection between those elastic moduli given by the
576 following equation $E' = 2G'(1+\nu)$, in which ν is the Poisson coefficient equal to 0.375 , which
577 is in good agreement with Poisson coefficient for polymeric material. Those main
578 characteristics are the signature of a physical gel in which pectin gel is stabilized by cooperative
579 linkage with calcium counterions. This was also confirmed by the fact that before and after the
580 compression test (Fig. 9) evolution of viscoelastic moduli G' and G'' versus angular frequency
581 were identicals.

582

583 **4.CONCLUSION**

584 Pectins from Araçá (*Psidium cattleianum* Sabine) fruit pulp were obtained under sodium form
585 of GalA forming long blocks (HG blocks) interrupted by rhamnogalacturonan blocks (RG-I).
586 Different techniques were used to characterize the chemical structure based on titration of the
587 GalA units before and after de-methylation in alkaline conditions, and total GalA units by
588 Blumenkrantz & Asboe-Hansen technique with good agreement. In addition, different pectins
589 were prepared with different degrees of methylations using alkaline treatment and characterized
590 for their degree of methylation. In the initial sample (APP), total GalA is found around 72
591 weight %, a small protein amount lefted is detected (1.6 weight %) completed by neutral
592 saccharidic chains around 30 weight % as obtained from 1H NMR but slightly overestimated.

593 The conditions for total hydrolysis was investigated due to resistance of uronic acid block to
594 hydrolysis and low stability of some monosaccharides. The kinetic of hydrolysis was studied
595 up to 8 h in usual conditions. Maximum amount of monosaccharides was produced after 1 h,
596 indicating instability and resistance to hydrolysis of some part of pectins. From these data, it is
597 clear that other techniques must be used for accurate carbohydrate analysis. Then, NMR
598 analysis was performed to complete the structure analysis and to control the evolution of the
599 chemical structure during alkaline treatment. It is shown that, in the initial sample, few GalA
600 units are partially acetylated. The degree of methylation is confirmed and the composition of
601 the side chains are established with mainly galactose and high yield of arabinose.
602 From SEC experiments, the molecular weight distributions indicate the presence of two ranges
603 of chain length after alkaline treatment which remain unchanged independently of the time of
604 alkaline treatment. By bi-dimensional and H^1 NMR study, it is confirmed that the main chain
605 composition is stable too, with just the decrease of methylation and acetylation degrees with
606 time of treatment going to zero after 60 minutes of treatment. The fraction of arabinose is
607 slightly decreases also during this treatment.
608 At end, it is demonstrated that, even if the initial sample does not gel in presence of acid or
609 calcium excess, as soon as DM decreases over a critical polymer concentration around 2 mg
610 mL^{-1} a pectin gel is stabilized. Rheological tests were performed by classical oscillatory shear
611 frequency sweep, orthogonal oscillatory frequency sweep and a compression test on a gel
612 obtained by dialysis of a 10 g L^{-1} solution against 1 mol L^{-1} $CaCl_2$. It is concluded that in such
613 conditions, a homogeneous strong physical gel is obtained. The role of the degree of
614 methylation on physical and biological properties (gelation and interaction with proteins) of
615 Araçá pectins will be developed in our following work.

616

617 **ACKNOWLEDGEMENTS**

618 The authors gratefully acknowledge the following Brazilian agencies for financial
619 support: the Coordination for the Improvement of Higher Education Personnel – CAPES, the
620 National Council for Scientific and Technological Development - CNPq, and the Federal
621 University of Paraná - Brazil. J.L.M.S. is a research member of the CNPq Foundation and UFPR
622 (n° 476950/2013-9; 308296/2015-0; 309225/2018-3); S.C.A is the beneficiary of a post-
623 graduation scholarship (n° 141692/2018-9) provided by CNPQ and a Sandwich scholarship at
624 the University Grenoble Alpes provided by CAPES-PRINT - RIBBBA-UFPR (n°
625 88887.467969/2019-00); and S.F.B. is the beneficiary of a post-doctoral scholarship from
626 Coordination of Superior Level Staff Improvement – CAPES (n° 88887.335103/2019-00). The
627 authors would like to thank E. Bayma from PCANS technical platform at CERMAV (CNRS)
628 for SEC experiments, I. Jeacomine from the NMR Centers of RMN-ICMG (FR2607) -
629 Grenoble-France, A. Santana Filho and G. Sasaki from UFPR-Curitiba-Brazil for recording
630 the NMR spectra, K. Mischiatti and R. Bagatin for HPAEC-PAD and GLC analysis,
631 respectively, the Brazilian Agricultural Research Corporation/Embrapa Forests, and M. Mazza
632 for providing the Araçá fruits.

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There is no conflict of interest