



**UNIVERSIDADE TECNOLÓGICA
FEDERAL DO PARANÁ**

**Programa de Pós-Graduação em Tecnologia de
Alimentos**

**Physical and chemical properties and antioxidant activity
of modified and unmodified pectins extracted from orange
bagasse.**

Simoni Spohr Venzon

Campo Mourão

2013

Simoni Spohr Venzon

**Physical and chemical properties and antioxidant activity
of modified and unmodified pectins extracted from orange
bagasse.**

Dissertação apresentada ao programa de Pós
Graduação em Tecnologia de Alimentos da
Universidade Tecnológica Federal do Paraná,
como parte dos requisitos para obtenção do
título de mestre em Tecnologia de Alimentos.

Campo Mourão

2013

Orientador

Prof. Dr. Charles Windson Isidoro Haminiuk

Coorientadora

Prof. Dra. Maria Helene Giovanetti Canteri

BIOGRAFIA

Simoni Spohr Venzon, no ano de 2005, ingressou na Universidade Estadual do Oeste do Paraná - UNIOESTE, no curso de Engenharia química. Após um ano de curso começou o primeiro estágio nos laboratórios de Engenharia Química de Fenômenos de Transporte, Operações Unitárias e Bioquímica, com duração de 3 anos, contratada primeiramente pela UNIOESTE e depois pela Fundação Universitária de Toledo. Participou de projetos de iniciação científica na área de tratamento de efluentes, que resultaram algumas publicações, duas internacionais e duas nacionais:

- Borba, C.E., Silva, E.A., SPOHR, S., Santos, G.H.F., Guirardello, R. Application of the mass action law to describe ion exchange equilibrium in a fixed-bed column. **Chemical Engineering Journal**, v.172, p.312 - 320, 2011.

- Borba, C. E., Silva, E. A., SPOHR, S., Santos, G. H. F., Guirardello, R. Ion Exchange Equilibrium Prediction for the System Cu Zn Na. **Journal of Chemical and Engineering Data**, v.55, p.1333 - 1341, 2010.

- Santos, G. H. F., SPOHR, S., VAZ, L. G., Borba, C. E. Estudo do equilíbrio de troca iônica/ adsorção dos íons cobre (II) na resina de troca catiônica amberlite IR 120 em reator batelada. In: VII Congresso brasileiro de engenharia química em iniciação científica – COBEQ-IC, 2007, São Carlos. Anais do VII COBEQ-IC. , 2007.

- SPOHR, S., Santos, G. H. F., VAZ, L. G., Borba, C. E. Remoção dos íons cobre (II) de uma solução em coluna de leito fixo utilizando como adsorvente a resina de troca iônica Amberlite IR 120. In: VII Congresso brasileiro de engenharia química em iniciação científica – COBEQ-IC, 2007, São Carlos. Anais do VII COBEQ-IC. , 2007.

Ainda durante a graduação realizou alguns estágios, nos períodos de férias, na Frimesa – Cooperativa Central em Medianeira-PR nas áreas de pesquisa e desenvolvimento e controle de qualidade. Em 2009, tornou-se colaboradora desta empresa.

Entre 2010–2011 participou do projeto “Estudo da competição na adsorção/bioacumulação de macronutrientes e metal pesado em solução hidropônica por espécies de macrófitas aquáticas flutuantes”, com bolsa financiada pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq e como colaboradora do projeto “Avaliação da influência da Cidade de Toledo sobre a qualidade da água do Rio Toledo”.

Ainda em 2010, realizou outro estágio na empresa BV Tecnologia Industrial Ltda onde ministrou alguns mini-cursos sobre aços inoxidáveis e descarte e tratamento de resíduos aos colaboradores da empresa.

Trabalhou como professora contratada PSS pela Secretaria do Estado da Educação do Paraná, ministrando aulas de física e química para o Ensino Médio.

Atualmente é discente do Mestrado em Tecnologia de Alimentos da Universidade Tecnológica Federal do Paraná – UTFPR, bolsista do programa DS/CAPES.

APRESENTAÇÃO

Esta dissertação é composta por um artigo científico submetido ao periódico Food research international:

Simoni Spohr Venzon, Maria Helene Giovanetti Canteri, Jade Varaschin Link , Charles Windson Isidoro Haminiuk. Physical and chemical properties and antioxidant activity of modified and unmodified pectins extracted from orange bagasse.

1 Physical and chemical properties and antioxidant activity of modified and unmodified
2 pectins extracted from orange bagasse.

3
4 Simoni Spohr Venzon, Maria Helene Giovanetti Canteri, Jade Varaschin Link,
5 Charles Windson Isidoro Haminiuk*

6
7 S. Spohr-Venzon J. V. Link C.W.I. Haminiuk*
8 Program of Post-Graduation in Food Technology, Federal University of Technology- Paraná, Campus
9 Campo Mourão, Brazil

10
11 S. Spohr-Venzon
12 e-mail: simonispohr@yahoo.com.br

13
14 J. V. Link
15 e-mail: jadejvl@hotmail.com

16
17 C.W.I. Haminiuk
18 e-mail: haminiuk@utfpr.edu.br
19 Tel.: +55 44-35181477

20
21 M. H. G. Canteri
22 Federal University of Technology- Paraná, Campus Ponta Grossa, Brazil
23 e-mail: mhelene5@hotmail.com

24
25 **Abstract**

26
27 Modified pectin is a polysaccharide rich in galacturonic acid altered by pH adjustment and thermal
28 treatment used especially as an anti-cancer agent. The aim of this work was to study the physical and
29 chemical properties of modified and unmodified pectins extracted from orange bagasse by using citric
30 and nitric acids. The galacturonic acid content, degree of esterification, Fourier Transform Infrared
31 Spectroscopy profile, molar mass, intrinsic viscosity, rheological properties and antioxidant activity of
32 the pectins were evaluated. The modification process caused the de-esterification of pectins,
33 responsible for improving the intestinal absorption of modified pectin and a decrease of molecular
34 weight due to removal of neutral sugars, maintaining the linear chain of galacturonic acid. Such
35 changes also caused a significant increase in the *in vitro* antioxidant activity and influenced the
36 rheological properties of pectin, reducing its viscosity. This work showed that the modification of pectin
37 from orange bagasse with citric and nitric acids altered its structural and physical characteristics as
38 well as its biological activity toward a free-radical, suggesting that some functional properties related to
39 antioxidant activity activity and absorption of nutrients may be increased.

40

41 *Keywords:* Pectin, modified pectin, degree of esterification, rheological properties, DPPH[•], FTIR.

42

43 1. Introduction

44

45 Brazil is responsible for about 30% of the production of fresh orange and 60% of
46 the worldwide production of orange juice and, in 2010, Brazil produced 19,112,300
47 tons of oranges. Orange bagasse is a byproduct from the orange juice industry and
48 accounts for up to 50% (w/w) of the fruit. The bagasse is obtained after extraction of
49 juice after two pressings which restrict the moisture content to around 65 to 75%. The
50 bagasse is then subjected to drying to be pelletized and marketed (Calliari, 2009).
51 The bagasse can be used in the manufacture of animal feed, the production of
52 biscuits, flavorings or extraction of pectins, thus increasing its commercial value in
53 the market and decreasing the industrial wastes.

54 Pectins are complex heteropolysaccharides on the cell wall of plants that provide
55 consistence and mechanical resistance to vegetal tissues (Taboada et al., 2010).
56 Pectic polysaccharides are mainly composed of polymers rich in galacturonic acid,
57 frequently with significant amounts of rhamnose, arabinose, galactose and around
58 thirteen other different monosaccharides. Three major chains are recognized:
59 homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II
60 (RG-II) (Fissore et al., 2009). The main chain of pectin may or may not be esterified
61 with methyl-ester groups in the carboxylic acid units. Pectins are commonly classified
62 according to their degree of esterification (DE) as high (HM) or low (LM) methoxyl
63 pectin, respectively, with a DE > 50% and < 50%. HM may produce a gel under
64 acidic conditions with high sugar concentrations (Evageliou et al., 2000); whereas LM

65 forms gels by the interaction of divalent cations, especially Ca^{2+} , between free
66 carboxyl groups (Cardoso et al., 2003).

67 Pectin is mainly used as a gelling, thickening and stabilizing agent in different
68 types of foods and beverages (Fissore et al., 2012; Videcoq et al., 2011).
69 Additionally, it has many uses in the pharmaceutical industry, with great potential in
70 the treatment against many diseases, such as obesity, diabetes, vesicle calculus, in
71 addition to other health benefits associated with dietetic fibers (Liu et al., 2010).
72 Recently, modified pectins have also been used in cancer treatment, especially as an
73 anti-cancer agent (Maxwell et al., 2012; Jiang et al., 2012; Videcoq et al., 2011).

74 Commercial pectins are generally produced by hot acid extraction from orange
75 and apple bagasses due to their high pectin polysaccharide contents (Guo et al.,
76 2012; Videcoq et al., 2011; Fissore et al., 2009). The chemical structure of pectins
77 varies according to the source, environmental factors, conditions of extraction and
78 modification techniques, which affect pectin yield and molecular characteristics, such
79 as the degree of esterification, galacturonic acid content, molar mass and rheological
80 behavior (Yapo, 2009a, b; Round et al., 2010; Maxwell et al., 2012). Particularly, this
81 complexity and variability of structure makes their characterization a difficult and
82 important task.

83 Modified pectin is a polysaccharide altered by pH adjustment and thermal
84 treatment, which breaks its chain into smaller fragments that can theoretically be
85 absorbed in the gastrointestinal tract (Maxwell et al., 2012; Glinsky & Raz, 2009). The
86 mechanisms involved are only partially understood, although evidence suggests that
87 pectin fragments with a small molar mass, but rich in galactose, bind themselves to
88 the protein linked to galactine-3 (GAL3). This binding may block GAL3 interactions
89 with other proteins and peptides, inhibiting their capacity to promote cell adhesion

90 and migration and preventing tumor growth (Maxwell et al., 2012; Glinsky & Raz,
91 2009; Platt, 2009).

92 Although several studies have dealt with the importance of modified pectin
93 (Maxwell et al., 2012; Nangia-Makker et al., 2002; Wai et al., 2010; Jun Yan & Katz,
94 2010), there is a lack of studies that deal with its physical and chemical properties.
95 The knowledge of these properties facilitates and broadens the applicability of the
96 modified pectin in other industries, for example, in the food industry. Therefore, the
97 objective of this work was to compare the physical and chemical properties of
98 commercial and experimental citrus pectin obtained by different extraction methods,
99 before and after the modification process.

100

101 2. Material and methods

102

103 2.1. Raw material

104

105 Orange bagasse was used as the raw material for the pectin extraction, and it was
106 obtained from the pressing of the fruits obtained from the local market of Medianeira,
107 Paraná State, Brazil. Nitric and citric acids (Merck, Brazil) were used to extract the
108 pectin from the citrus bagasse, and samples were named “nitric experimental pectin”
109 (NEP) and “citric experimental pectin” (CEP), respectively. Commercial citrus pectin
110 (CCP) was kindly supplied by CPKelco[®] (LI04050, Limeira- SP, Brazil) to compare
111 the results. All reagents were of analytical grade.

112

113 2.2. Obtaining orange bagasse flour

114

115 Bagasse from approximately 15 kg of oranges was dried to obtain the flour for
116 pectin extraction. After extracting the juice, the oranges were cut and the enzymes
117 were inactivated by bleaching by immersion into boiling water for three minutes,
118 followed by cooling in an ice bath (Kulkarni et al., 2010). The sample was dried at 55
119 ± 5 °C for approximately 24 h in a drying cabinet with air-circulation until constant
120 mass, and ground in a knife mill.

121

122 2.3. Pectin extraction

123

124 Pectins were obtained by acid extraction where citric and nitric acids were used as
125 extraction solvents (Fig. 1). Extraction with citric acid was performed according to the
126 methodology proposed by Canteri-Schemin et al. (2005), where approximately 50 g
127 of flour was suspended in 1 L of acidified water (pH 2.5 ± 0.5), with maceration for 30
128 min. The pH was adjusted to 2.5 ± 0.5 using a 1 mol L^{-1} citric acid solution, before
129 and after maceration. After maceration, this acid suspension was carried out to
130 extraction at boiling temperature (97 °C), by vigorously stirring for 30 min and the
131 process was interrupted by immersion in a water-ice bath. Based on the methodology
132 of Canteri et al. (2012), approximately 50 g of flour was hydrated with distilled water
133 for 10 min by magnetic agitation. The suspension was then completed with a solution
134 of nitric acid, both at 80 °C to obtain a final concentration of 50 mM acid. The
135 extraction was performed in a condensation system at 80 °C for 20 min, and the
136 process was interrupted by immersion in a water-ice bath.

137 Citric and nitric suspensions were then vacuum-filtered in synthetic tissue (silk
138 cloth) and stored at 4 °C. Two volumes of commercial ethanol 96 °GL were added to
139 the filtered liquid to form a gel of pectin. The obtained gel was collected, conditioned

140 in small cloth bags and immersed in acetone for approximately 15 h for the partial
141 removal of the acid. The pectins were dried in a drying cabinet with air-circulation at
142 40 °C for approximately 5 h, until a constant weight was achieved. Samples were
143 ground, homogenized and sieved in order to obtain powdered pectin.

144

145 2.4. Modification of pectins

146

147 The pectins obtained by different extraction methods and the commercial citrus
148 pectin were chemically modified as described by Nangia-Makker et al. (2002) and
149 Platt, (2009) with some modifications. The powdered pectin was solubilized as a
150 1.5%-w/v- solution in distilled water, and its pH was adjusted to 10.0 by adding NaOH
151 (3 mol L⁻¹). The mixture was stirred mechanically for 1 h at 55 ± 3 °C. The solution
152 was cooled at room temperature and the pH was adjusted to 3.0 with 3 mol L⁻¹ HCl
153 and then stored overnight. Finally, the pectin samples were precipitated with 95%
154 ethanol, filtered in synthetic tissue (silk cloth), washed with acetone and dried at 50
155 °C.

156

157 2.5. Yield

158

159 The yield of pectin extraction was calculated as a function of the pectin mass
160 obtained from the raw material (dry basis) used, according to Equation 1:

161

$$\% \text{Yield} = \frac{M_{\text{pectin}}}{M_{\text{raw material}}} \times 100 \quad (1)$$

162

163 where, M_{pectin} is the pectin mass obtained and $M_{\text{raw material}}$ is the raw material
164 utilized for extraction.

165

166 2.6. Galacturonic acid content

167

168 The galacturonic acid content of the pectins was determined using a
169 spectrophotometer at 520 nm by the alkaline m-hydroxydiphenyl method, according
170 to a classical methodology outlined by Blumenkrantz & Asboe-Hansen, (1973) using
171 monohydrated D-galacturonic acid (Sigma, USA) as a standard.

172

173 2.7. Determination of degree of esterification

174

175 The degree of esterification was estimated by the methodology proposed by
176 Bocek et al. (2001). Samples of dried pectin (0.05 g) were dissolved in 50 mL of
177 distilled water for 12–15 h in a drying cabinet at 50 °C in closed flasks. The solution
178 was titrated with 0.05 mol L⁻¹ NaOH until a pH of 8.5 ± 0.2 was reached using a
179 digital pH meter (Hanna, pH 21 pHmeter, Brazil). The used volume was named V_1 .
180 The saponification process was carried out by adding 10 mL of 0.5 mol L⁻¹ NaOH for
181 30 min at 30 °C in a drying cabinet. The solution was then neutralized by the addition
182 of the same volume of 0.5 mol L⁻¹ HCl. The excess of HCl was titrated with 0.05 mol
183 L⁻¹ NaOH, and the result was expressed as the final volume (V_2). The reactions
184 involved are shown in Figure 2a. The degree of esterification was calculated by
185 Equation 2:

186

$$DE(\%) = \left(\frac{V_2}{V_1 + V_2} \right) \times 100 \quad (2)$$

187

188 2.8. Determination of molar mass

189

190 The average molar mass of unmodified and modified pectin samples was
191 estimated using the Mark Houwink-Sakurada equation (Equation 3) (Arslan, 1995).

192

$$\eta = K \times M^a \quad (3)$$

193

194 where, K ($L g^{-1}$) and a are constants; M ($g mol^{-1}$) is the molar mass and η ($L g^{-1}$) is
195 the intrinsic viscosity defined according to Equation 4:

196

$$[\eta] = \lim_{c \rightarrow 0} \left(\frac{\eta_r - 1}{C} \right) \quad (4)$$

197

198 where η_r is the relative viscosity (solution for solvent) and C ($g L^{-1}$) is the pectin
199 concentration. Both constants K and a depend on the temperature and
200 characteristics of the solvent and solute. In the case of the pectin solution in 0.1 M
201 NaCl at pH 7.0, we may assume the value of K as $4.36 \times 10^{-5} L g^{-1}$ and of a as 0.78
202 (Garnier et al., 1993). The kinematic viscosities of pectin solutions at different
203 concentrations (among 0.3 and 2.0 $g L^{-1}$) were measured by a capillary viscometer
204 Cannon Fenske ($n^\circ 100$) at 25 °C (Liang et al., 2012a). The intrinsic viscosity of
205 pectins was calculated by fitting the experimental data to Huggins

206 $(\eta_{red} = \eta + K_H \cdot \eta^2 \cdot C)$ and Kraemer $(\frac{\ln(\eta_{rel})}{C} = \eta + (K_H - \frac{1}{2}) \cdot \eta^2 \cdot C)$ mathematical
207 models (Table 1).

208

209 2.9. Rheological analysis

210

211 Non-oscillatory rheological analysis of the pectin solutions was performed in a
212 Rheometer Brookfield (DV-III+), with spindle SC4-18, (Brookfield Engineering
213 Laboratories, MA, USA), connected to a thermostatic bath for temperature control.
214 Shear stress (τ) and rate ($\dot{\gamma}$) values were obtained by Rheocalc V 3.1-1 software
215 (Brookfield Engineering Laboratories, MA, USA).

216 Pectins (1 g L^{-1}) were dissolved in 0.1 mol L^{-1} NaCl solution by mechanical stirring
217 for 6 h at room temperature (Liang et al., 2012a; Min, Lim, Ko, Lee, Lee & Lee,
218 2011). Flow curves of pectin samples were obtained at different temperatures of
219 processing (10, 30 and $50 \text{ }^\circ\text{C}$). Each analysis had a duration of 4 min, with 40 points;
220 whereas, 20 points were in the ascending curve ($0\text{--}20 \text{ s}^{-1}$) and 20 points were in the
221 descendent curve ($20\text{--}0 \text{ s}^{-1}$). All flow curves of pectins at different temperatures were
222 fitted to the Power Law model.

223

224 2.10. Fourier Transform Infrared Spectroscopy (FTIR)

225

226 The FTIR spectra of unmodified and modified pectins were recorded on a
227 Shimadzu, FTIR – 8300 spectrophotometer in the 4000 cm^{-1} region using potassium
228 bromide (KBr) pellets (Jiang et al., 2012).

229

230 2.11. Evaluation of the antioxidant activity of pectin samples

231 The free radical scavenging activity was assessed with the DPPH• method as
 232 previously described by Mensor et al. (2001). Five different concentrations (25, 50,
 233 125, 250 and 500 mg L⁻¹ in 0.1 mol L⁻¹ NaCl) of the extract were used to perform the
 234 DPPH assay. A 0.3 mmol L⁻¹ DPPH ethanolic solution (1 mL) was added to 2.5 mL of
 235 the sample and the mixture was vortexed at room temperature. After 30 min, the
 236 absorbance values were measured at 518 nm, and they were converted into the
 237 antioxidant activity percentage (AA%) using the following equation (Equation 5):

238

$$AA\% = 100 - \left[\frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{\text{Abs}_{\text{control}}} \right] \quad (5)$$

239

240 where, Abs_{sample} is the absorbance of the sample; Abs_{blank} is the absorbance of the
 241 ethanol (1.0 mL) mixed with the pectin solution (2.5 mL) and Abs_{control} is the
 242 absorbance of the 0.3 mmol L⁻¹ DPPH solution (1.0 mL) mixed with ethanol (2.5 mL).

243

244 2.12. Activation energy measurement

245

246 Pectin samples (1 g L⁻¹) were dissolved in 0.1 mol L⁻¹ NaCl solution. The apparent
 247 viscosity was calculated according to the methodology of Haminiuk et al., (2006). The
 248 effect of temperature on the apparent viscosity of fluids at constant shear rates may
 249 be described by the Arrhenius equation (Rao et al., 1984). The shear rate of 10.53 s⁻¹
 250 was chosen to calculate activation energy (Ea) of the pectin samples.

251

252 2.13. Statistical analysis

253

254 All of the experiments were done in triplicate except for the antioxidant activity
255 analysis, which was done in duplicate. The data was analyzed using analysis of
256 variance (ANOVA) by OriginPro 8.0 (OriginLab Corporation, Northampton, USA), and
257 expressed as mean value and standard deviation, compared using Tukey's test at a
258 5% confidence level.

259

260 3. Results and discussion

261

262 3.1. Yield

263

264 The pectin yield by different extraction methods varied according to the processing
265 conditions and the characteristics of the raw material used (Liang et al., 2012a). The
266 yield of extraction with citric acid was 17.75% and a mild condition of extraction with
267 nitric acid was 10.9%. The values found were close to those obtained by Guo et al.,
268 (2012) in the pectin extraction of orange bagasse with chlorohydric acid (15.47%).
269 Canteri-Schemin et al. (2005) extracted 20% of apple pectin using water acidified
270 with citric acid (pH 2.5) at 100 °C for 110 minutes. The obtained values are also
271 consistent with those of the extraction yield (11.88%) of blackberry pectin by Liu et al.
272 (2010), and by Rha et al. (2011) with 10% in the extraction of apple pectin with oxalic
273 acid. The pectin concentration in different materials vary quantitatively according to
274 the source of raw material, but usually is between 2.9 and 22% in apples; 9 -30% in
275 lemons; 17 and 25% in mangoes and 5 and 30% in oranges (Koubala et al., 2008;
276 Rha et al., 2011; Min et al., 2011).

277 The type and concentration of extraction solvents also affect significantly the yield
278 of pectin. According to Fertoni et al. (2006), pectin may be extracted with diluted

279 acids, however, it can degrade with concentrated acids. Canteri-Schemin et al.
280 (2005) stated that nitric acid is an excellent extraction agent, however, citric acid may
281 cause the formation of large and impure molecules, with the incorporation of
282 esterified acid radicals to the hydroxyls, which are present in the neutral sugars. High
283 rates of pectin extraction by hot diluted acid, HCl or HNO₃, is suggested as the best
284 approach for production on an industrial scale (Liang et al., 2012b; Canteri-Schemin
285 et al., 2005).

286

287 3.2. Galacturonic acid

288

289 The pectins without the chemical modification showed values of galacturonic acid
290 of $70.00 \pm 3.27\%$ for commercial citrus pectin (CCP), $54.86 \pm 1.13\%$ for citric
291 extraction (CEP) and $60.63 \pm 2.29\%$ for the nitric extraction (NEP). On the other
292 hand, for the chemically modified pectins, values were found for galacturonic acid of
293 $87.82 \pm 1.16\%$, $56.10 \pm 4.10\%$ and $62.03 \pm 0.19\%$ for modified commercial citrus
294 pectin (MCCP), modified citric experimental pectin (MCEP) and modified nitric
295 experimental pectin (MNEP), respectively.

296 Guo et al. (2012) extracted pectins from the orange rind with 60–75% galacturonic
297 acid. Jiang et al. (2012) found 71.43% galacturonic acid in pectins extracted from
298 apples by citric acid. Santos et al. (2009) extracted pectins with citric acid of gabioba
299 and found values of galacturonic acid of 40%. An increase in galacturonic acid
300 content ranging from 75.1 to 87.2% was also reported by Einhorn-Stoll et al. (2012)
301 with alkaline modification and 61.5 to 64.1 by Kurita et al. (2012) in citrus pectin
302 modification.

303 Commercial pectins yield galacturonic acid contents higher than 65%, which is the
304 limit of purity of pectins established by the Food Chemical Codex - FCC (Maxwell et
305 al., 2012; Liang et al., 2012b). This standard was found for commercial citrus pectin
306 (CCP) used in this work. Nitric (NEP) and modified nitric pectins (MNEP) may also be
307 considered of high purity, since a statistical difference was not found ($p \leq 0.05$) when
308 compared to the commercial citrus pectin. The content of galacturonic acid, which is
309 predominant in the primary structure of the pectin (Ovodov, 2009) was higher for
310 nitric acid than for citric acid extraction. This fact confirms that a higher yield of citric
311 pectin is due to the incorporation of other compounds to pectin, such as ash,
312 proteins, esterified acid radicals and neutral sugars, by extraction with weak acids
313 (Fertonani et al., 2006; Kowalonek & Kaczmarek, 2010; Min et al., 2011; Einhorn-
314 Stoll et al., 2012).

315 Pectin modification increases the galacturonic acid content by the removal of
316 impurities due to the treatment with hydrochloric acid, which enhances the
317 solubilization of the minerals in the sample (Kowalonek & Kaczmarek, 2010). The
318 galacturonic acid content of modified commercial citrus pectin (MCCP) was
319 significantly higher than that of unmodified pectin. This fact indicated the possible
320 presence of additives in the samples, such as antioxidants and sugar for
321 standardization of the SAG (gelling power). The galacturonic acid content of citric
322 (CEP) and nitric (NEP) pectins has not changed significantly with modification.

323

324 3.3. Degree of esterification (DE)

325

326 All pectin samples presented high methoxylation ($DE > 50\%$). Commercial citrus
327 pectin (CCP) had the highest degree of esterification ($70.00 \pm 0.65\%$). Citric ($63.11 \pm$

328 0.25%) and nitric ($59.92 \pm 3.22\%$) pectins were statistically different between them
329 and CCP ($p \leq 0.05$). De-esterification promoted by citric acid in the extraction process
330 was slightly lower than that promoted by nitric acid due the greatest strength of this
331 acid.

332 Fertoni et al. (2006) (2009) obtained apple pectins with nitric and citric acids
333 with degrees of esterification between 50 and 54%, whereas pectin from beetroot
334 showed a degree of esterification of 58% (Mesbahi et al., 2005). Santos et al. (2010)
335 used citric acid and obtained pectin with a DE of 62.41%.

336 The pectin industry generally requires the production of water-soluble pectins with
337 high molar mass and high DE for gelification (Stephen, 1995). High methoxylated
338 pectins (DE > 50%) require sugar (sucrose) at a concentration higher than 55% w/w
339 and an acid condition with a pH between 2.0 and 3.5 for gel formation. However, low
340 methoxylated pectins (DE < 50%) require Ca^{2+} ions for the formation of gels within a
341 pH range of 2.0 and 7.0, regardless the amount of sugar (Löfgren & Hermansson,
342 2007).

343 The modification applied in this work caused the de-esterification of pectins
344 according to Wai et al. (2010) and Einhorn-Stoll et al. (2012) studying the
345 modifications of citrus and durian (*Durio zibethinus*) pectins. NaOH treatment during
346 modification caused the de-esterification of pectins and replaced a methyl with a
347 hydroxyl group (Fajardo et al., 2012). Figure 2b shows the proposed structure of
348 modified pectin.

349

350 3.4. Molar mass

351

352 Intrinsic viscosity, the measurement of hydrodynamic volume occupied by the
353 macromolecule, is closely related to the size and molecule conformation derived from
354 a specific solvent (Lai & Chiang, 2002). The intrinsic viscosity of pectins calculated by
355 data fit to Huggins and Kraemer mathematical models are showed in Table 1. The
356 mathematical model of Huggins showed a better fit to the experimental data of
357 unmodified pectins, whereas the model of Kraemer showed higher values of
358 determination of coefficient (R^2) for modified pectins.

359 According to the Huggins equation, nitric experimental pectin (NEP) had the
360 highest intrinsic viscosity followed by that of commercial citrus pectin (CCP), and
361 citric experimental pectin (CEP). The chemical modification decreased the intrinsic
362 viscosity of pectins, caused by the lower degree of esterification. The values of
363 intrinsic viscosity of pectin modified with respect to unmodified pectins were
364 statistically different ($p \leq 0.05$), except for CEP and MCEP.

365 The intrinsic viscosity values were higher than those reported by Jiang et al.
366 (2012) for pectin samples of apples extracted with citric acid (109–212 mL g⁻¹);
367 similar to citrus pectin, 427.6 and 359.1 mL g⁻¹, extracted by traditional heating and
368 microwave (Guo et al., 2012). Einhorn-Stoll et al., (2012) reported an intrinsic
369 viscosity of 312 mL g⁻¹ for pectin with alkaline modification.

370 The molar masses of the samples were 93,937; 83,486; 138,787 g mol⁻¹ (Da),
371 respectively, for CCP, CEP and NEP. These values are comparable to the molecular
372 weight of 140,68 Da for carrot and 78,60 Da of citrus pectin (Ngouémazong et al.,
373 2012). The extraction conditions used in this work produced different types of pectins
374 with different molar masses and conformations. The severe systems are necessarily
375 the explanation for both the low viscosity and low molecular weight (Canteri et al.,
376 2012).

377 The chemical modification of pectins resulted in a decrease of their molar masses
378 to 63,485, 77,528, 58,686 Da, respectively, this decrease has been touted to improve
379 the intestinal absorption of nutrients (Courts, 2012). Galacturonic acid content was
380 not changed with the chemical modification; the decrease in molecular mass can
381 suggest that there was a partial removal of neutral sugars while the linear chain of
382 galacturonic acid was not altered as suggested by Platt., (2009).

383 Pectins are highly heterogeneous with regard to their molar mass and chemical
384 structure. The molar mass average of pectins from several fruit sources varies
385 between 10^4 – 10^5 Da (Cui, 2005), which is similar to the values in the current study. It
386 has been reported that pectin medicinal value is closely related to its structural
387 characteristics. For example, pectin with low esterification values and low molecular
388 weight is more efficient in decreasing the risk of cancer metastasis; whereas, pectin
389 with high methoxyl content and high molecular weight is a good cholesterol-reducing
390 agent (Liu et al., 2010).

391

392 3.5 Fourier Transform Infrared Spectroscopy (FTIR)

393

394 FTIR spectra analyses identified important functional groups of unmodified and
395 modified pectins extracted by the citric and nitric acids. These spectra were
396 compared with the spectrum of commercial citrus pectin (CCP), which is shown in
397 Figure 3. All pectin FTIR spectra showed intense absorption at 800 and 1200 cm^{-1}
398 wave intervals, which is considered as the finger print region for carbohydrates and
399 allows the identification of major chemical groups in polysaccharides as the position
400 and intensity of the bands are specific for every polysaccharide (Nesic et al., 2011;
401 Liang et al., 2012b; Sivam et al., 2012). Since the FTIR spectra of pectins showed

402 absorbance intensity standards similar to CCP, the polysaccharides extracted were
403 confirmed as pectins.

404 The wide band of approximately 3440 cm^{-1} is attributed to the distension of a $-\text{OH}$
405 group (Liang et al., 2012b), a good indicator of pectin quality (Nesic et al., 2011).
406 Absorbance at approximately 2900 cm^{-1} refers to distensions $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$,
407 methyl esters of galacturonic acid (Kowalonek & Kaczmarek, 2010; Liu et al., 2010).

408 Strong absorption reported at intervals of $1730\text{--}1760$ and $1600\text{--}1630$ is caused by
409 distension $\text{C}=\text{O}$ of esterified carboxylic groups ($-\text{COOCH}_3$) and free carboxylic
410 groups ($-\text{COOH}$), respectively (Nesic et al., 2011; Fajardo et al., 2012). The ratio
411 between the peak area of esterified carboxylic group and the sum of peaks of
412 esterified and non-esterified carboxylic groups co-related linearly with the degree of
413 methoxylation of pectin (Liang et al., 2012b; Sivam et al., 2012).

414 The FTIR spectrum of commercial citrus pectin (CCP) had a higher absorbance at
415 1753 cm^{-1} than at 1630 cm^{-1} , characteristic of the high degree of esterified pectin.
416 The modified commercial citrus pectin (MCCP) also revealed the same behavior,
417 unlike the other pectins with a higher absorbance at 1630 cm^{-1} than at 1745 cm^{-1} .

418 All pectins had high methoxylation ($\text{DE} > 50\%$). CCP had the highest degree of
419 esterification ($71.48 \pm 0.06\%$). Citric ($64.03 \pm 0.05\%$) and nitric ($62.72 \pm 1.06\%$)
420 pectins were statistically different from CCP ($p \leq 0.05$). The chemical modification
421 decreased the values of the degree of esterification to 66.79 ± 0.12 , 62.03 ± 1.62 and
422 58.95 ± 0.08 for CCP, CEP and NEP, respectively.

423 Absorptions between 1100 and 1200 cm^{-1} in FTIR spectra correspond to the ether
424 R-O-R and cyclic C-C ring links of the pectin structure (Liu et al., 2010).

425 Bands occur at 1012 and 1106 cm^{-1} indicating vibration of C-C and vibration C-
426 O-C of backbone, respectively (Liang et al., 2012a). Modified citrus commercial

427 pectin had an increase in peak 1106 cm^{-1} which is consistent with an increase in the
428 galacturonic acid unit, while for while for other modified pectins, this peak was not
429 altered.

430

431 3.6. Rheological analysis

432

433 In the Food Science and Technology field, aqueous solutions of polymers are a
434 source of important materials. The solution properties of these carbohydrates are
435 highly interesting for several applications, such as thickeners of suspension and
436 gelification agents in sweet and non-sweet foods (Fissore et al., 2012). All flow
437 curves of pectins at different temperatures are presented in Figure 4. The
438 mathematical fit showed higher values of R^2 , whereas, the parameters of the
439 rheological model are presented in Table 2.

440 All samples showed pseudoplastic behavior due to the fact that the values of the
441 flow behavior index (η) were lower than 1 for all temperatures, as reported by
442 Sengkhampan et al. (2010); Min et al. (2011); and Bélafi-Bakó et al. (2012).

443 The consistence coefficients values were statistically different ($p \leq 0.05$) for all
444 pectins with an increase in temperature, according to the one-factor analysis of
445 variance (ANOVA). The consistence coefficient values (K) decreased when the
446 temperature increased for all pectins, with almost no changes in the flow behavior
447 index. A similar behavior for citrus pectin was found by Masuelli, (2011).

448 The chemical modification significantly affected the rheological behavior of pectins.
449 Figure 4 shows that the flow curves of unmodified and modified pectins belong to
450 distinct groups. When compared to the group of pectins without modification, the
451 group of modified pectins had a fast shear-stress fall with an increase in the shear-

452 rate values. After modification, decreases in the values of consistence coefficient (K)
453 and flow behavior index were observed. This fact revealed changes in molecular
454 structures and the non-Newtonian behavior of the samples (Steffe, 1992). In the
455 modified pectins, the consistence coefficient did not show a statistically difference at
456 10 and 30 °C ($p > 0.05$) showing some independence with respect to the extraction
457 method and solvents employed.

458 A decrease in apparent viscosity of the samples with an increase in shear rate and
459 temperatures was observed (data not shown). The same behavior was reported by
460 Agoda-Tandjawa et al. (2012) and Sengkhampan et al. (2010). A distinction
461 between unmodified and modified pectin groups was again observed in which the
462 apparent viscosity was lower for modified pectins. The modified pectin used in the
463 pharmaceutical industry need not form gels, thus, a lower viscosity is a positive factor
464 meaning less energy expenditure during processing.

465 The viscosity of the samples decreased for all pectins when the temperature was
466 increased. The decrease in viscosity can be attributed to an increase in
467 intermolecular distances, because of the thermal expansion caused by the increase
468 in temperature (Constenla et al., 1989).

469

470 3.7. Activation energy

471

472 Table 4 shows the activation energy calculated for all pectins, whereas the
473 Arrhenius model properly described the relation of apparent viscosity and the inverse
474 of absolute temperature at 10.53 s^{-1} . The activation energy values of the pectin
475 samples were statistically similar ($p > 0.05$), except to the citrus pectin (modified and
476 unmodified). The modification did not alter the Ea of pectins.

477 Ea values found in this work are consistent with those of Bélafi-Bakó et al. (2012)
478 who found values of activation energies for citrus pectin of 35.4 KJ.mol⁻¹ and 39.1
479 KJ.mol⁻¹ for beetroots and 33.3 KJ.mol⁻¹ for apples.

480

481 3.8. Antioxidant activity

482

483 The antioxidant capacity of pectin samples was evaluated by the antioxidant
484 methodology of the DPPH[•]. Table 3 shows the values of AA for the concentration of
485 50 mg L⁻¹. The antioxidant activity (AA) of all samples increased with an increase in
486 the polymer concentration. The chemical modification caused a slight increase in the
487 antioxidant capacity of the pectins, which was also reported by Rha et al. (2011). This
488 fact corroborates the fact that the antioxidant activity of pectin follows the same
489 behavior of donating oxygen of polyphenols (Serrano-Cruz et al., 2013). Indeed, the
490 modification causes the de-esterification of the methyl-ester groups of the samples
491 with an increase in the number of hydroxyls and consequent increase of antioxidant
492 activity.

493

494 4. Conclusion

495

496 Comparing the modified and unmodified pectins we realize that the modification
497 process caused the de-esterification of pectins, responsible for improving the
498 intestinal absorption of modified pectin and causing the decrease in molecular weight
499 due to removal of neutral sugars, maintaining its linear chain of galacturonic acid.
500 Such changes caused a slight, however significant, increase in *in vitro* antioxidant
501 activity and influence the rheological properties of pectin, reducing its viscosity.

502 The unmodified pectin has greater applicability in the food industry due to its high
503 viscosity. The modified pectin has its physical and structural properties altered,
504 associated in other studies with the increase of their bioactive properties, which may
505 be being applied in the production of functional foods and still representing less
506 energy in processing.

507

508 References

- 509 Agoda-Tandjawa, G., Durand, S., Gaillard, C., Garnier, C., & Doublier, J. L. (2012). Rheological
510 behaviour and microstructure of microfibrillated cellulose suspensions/low-methoxyl pectin mixed
511 systems. Effect of calcium ions. *Carbohydrate Polymers*, 87(2), 1045-1057.
512
- 513 Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic
514 acids. *Analytical Biochemistry*, 54(2), 484-489.
515
- 516 Bochek, A. M., Zabivalova, N. M., & Petropavlovskii, G. A. (2001). Determination of the Esterification
517 Degree of Polygalacturonic Acid. *Russian Journal of Applied Chemistry*, 74(5), 796-799.
518
- 519 Bélafi-Bakó, K., Cserjési, P., Beszédes, S., Csanádi, Z., & Hodúr, C. (2012). Berry Pectins: Microwave-
520 Assisted Extraction and Rheological Properties. *Food and Bioprocess Technology*, 5(3), 1100-1105.
521
- 522 Calliari, C. M. Extração aquosa de pectina de bagaço de laranja. *Revista Eletrônica Múltiplo Saber*,
523 Londrina, 5(1), 2009.
524
- 525 Canteri-Schemin, M. H., Fertonani, H. C. R., Waszczynskyj, N., & Wosiacki, G. (2005). Extraction of
526 pectin from apple pomace. *Brazilian archives of biology & technology*, 48(2), 259-266.
527
- 528 Canteri, M. H. G., Scheer, A. P., Wosiacki, G., Ginies, C., Reich, M., & Renard, C. M. C. G. (2012).
529 Rheological and macromolecular quality of pectin extracted with nitric acid from passion fruit rind.
530 *Journal of Food Process Engineering*, 35(5), 800-809.
531
- 532 Cardoso, S. M., Coimbra, M. A., & Lopes da Silva, J. A. (2003). Temperature dependence of the
533 formation and melting of pectin–Ca²⁺ networks: a rheological study. *Food Hydrocolloids*, 17(6), 801-
534 807.
535
- 536 Constenla, D. T., Lozano, J. E., & Crapiste, G. H. (1989). Thermophysical Properties of Clarified Apple
537 Juice as a Function of Concentration and Temperature. *Journal of Food Science*, 54(3), 663-668.
538
- 539 Courts, F.L. (2012). Profiling of modified citrus pectin oligosaccharide transport across Caco-2 cell
540 monolayers. *Pharma Nutrition*, doi:10.1016/j.phanu.2012.12.001.
541
- 542 Cui, S. W. (2005). *Food carbohydrates : chemistry, physical properties, and applications*. Boca Raton:
543 Taylor & Francis.
544
- 545 Da Silva Santos, M., Carneiro, P. I. B., Carneiro, E. B. B., Wosiacki, G., & De Oliveira Petkowicz, C. L.
546 (2009). Caracterização físico-química, extração e análise de pectinas de frutos de *Campomanesia*
547 *Xanthocarpa* B. (Gabioba). *Seminário:Ciencias Agrarias*, 30(1), 101-106.
548
- 549 Einhorn-Stoll, U., Hatakeyama, H., & Hatakeyama, T. (2012). Influence of pectin modification on
550 water binding properties. *Food Hydrocolloids*, 27(2), 494-502.
551
- 552 Evageliou, V., Richardson, R. K., & Morris, E. R. (2000). Effect of pH, sugar type and thermal annealing
553 on high-methoxy pectin gels. *Carbohydrate Polymers*, 42(3), 245-259.
554
- 555 Fajardo, A. R., Lopes, L. C., Pereira, A. G. B., Rubira, A. F., & Muniz, E. C. (2012). Polyelectrolyte
556 complexes based on pectin–NH₂ and chondroitin sulfate. *Carbohydrate Polymers*, 87(3), 1950-1955.
557

- 558 Fertonani, H. C. R., Scabio, A., Nogueira, A., Wosiacki, G., Carneiro, E. B. B., & Schemim, M. H. C.
559 (2009). Extraction model of low methoxyl pectin from apple pomace effects of acid concentration
560 and time on the process and the product. *Brazilian Archives of Biology and Technology*, 52(1), 177-
561 185.
- 562
- 563 Fissore, E. N., Matkovic, L., Wider, E., Rojas, A. M., & Gerschenson, L. N. (2009). Rheological
564 properties of pectin-enriched products isolated from butternut (*Cucurbita moschata* Duch ex Poiret).
565 *LWT - Food Science and Technology*, 42(8), 1413-1421.
- 566
- 567 Fissore, E. N., Rojas, A. M., & Gerschenson, L. N. (2012). Rheological performance of pectin-enriched
568 products isolated from red beet (*Beta vulgaris* L. var. *conditiva*) through alkaline and enzymatic
569 treatments. *Food Hydrocolloids*, 26(1), 249-260.
- 570
- 571 Fertonani, H. C. R., Scabio, A., Schemin, M. H. C., Carneiro, E. B. B., Nogueira, A., & Wosiacki, G.
572 (2006). Influence of acid concentration on extraction and quality of apple pomace pectin/

573 Influência da concentração de ácidos no processo de extração e na qualidade de pectina de bagaço
574 de maçã. Seminário: Ciências Agrárias (Online), State University of Londrina, 27, 599-612.
- 575
- 576 Garnier, C., Axelos, M. A. V., & Thibault, J.-F. (1993). Phase diagrams of pectin-calcium systems:
577 Influence of pH, ionic strength, and temperature on the gelation of pectins with different degrees of
578 methylation. *Carbohydrate Research*, 240(0), 219-232.
- 579
- 580 Glinsky, V. V., & Raz, A. (2009). Modified citrus pectin anti-metastatic properties: one bullet, multiple
581 targets. *Carbohydrate Research*, 344(14), 1788-1791.
- 582
- 583 Guo, X., Han, D., Xi, H., Rao, L., Liao, X., Hu, X., & Wu, J. (2012). Extraction of pectin from navel orange
584 peel assisted by ultra-high pressure, microwave or traditional heating: A comparison. *Carbohydrate*
585 *Polymers*, 88(2), 441-448.
- 586
- 587 Haminiuk, C. W. I., Sierakowski, M. R., Vidal, J. R. M. B., & Masson, M. L. (2006). Influence of
588 temperature on the rheological behavior of whole araca pulp (*Psidium cattleianum* Sabine). *LWT -*
589 *Food Science and Technology*, 39(4), 427-431.
- 590
- 591 Jiang, Y., Du, Y., Zhu, X., Xiong, H., Woo, M. W., & Hu, J. (2012). Physicochemical and comparative
592 properties of pectins extracted from *Akebia trifoliata* var. *australis* peel. *Carbohydrate Polymers*,
593 87(2), 1663-1669.
- 594
- 595 Jolie, R. P., Duvetter, T., Van Loey, A. M., & Hendrickx, M. E. (2010). Pectin methylesterase and its
596 proteinaceous inhibitor: a review. *Carbohydrate Research*, 345(18), 2583-2595.
- 597
- 598 Jun Yan, A. E., & Katz, A. (2010). PectaSol-C Modified Citrus Pectin Induces Apoptosis and Inhibition
599 of Proliferation in Human and Mouse Androgen-Dependent and- Independent Prostate Cancer Cells.
600 *Integrative Cancer Therapies*, 9(2), 197-203.
- 601
- 602 Koubala, B. B., Kansci, G., Mbome, L. I., Crépeau, M. J., Thibault, J. F., & Ralet, M. C. (2008). Effect of
603 extraction conditions on some physicochemical characteristics of pectins from "Améliorée" and
604 "Mango" mango peels. *Food Hydrocolloids*, 22(7), 1345-1351.
- 605
- 606 Kowalonek, J., & Kaczmarek, H. (2010). Studies of pectin/polyvinylpyrrolidone blends exposed to
607 ultraviolet radiation. *European Polymer Journal*, 46(2), 345-353.
- 608

- 609 Kulkarni, S.G., Vijayanand, P. (2010). Effect of extraction conditions on the quality characteristics of
610 pectin from passion fruit peel (*Passiflora edulis f. flavicarpa* L.). *LWT - Food Science and Technology*,
611 43, 1026–1031.
- 612
- 613 Kurita, O., Miyake, Y., & Yamazaki, E. (2012). Chemical modification of citrus pectin to improve its
614 dissolution into water. *Carbohydrate Polymers*, 87(2), 1720-1727.
- 615
- 616 Lai, L. S., & Chiang, H. F. (2002). Rheology of decolorized hsian-tso leaf gum in the dilute domain.
617 *Food Hydrocolloids - OXFORD-*, 16(5), 427-440.
- 618
- 619 Liang, R.-h., Chen, J., Liu, W., Liu, C.-m., Yu, W., Yuan, M., & Zhou, X.-q. (2012a). Extraction,
620 characterization and spontaneous gel-forming property of pectin from creeping fig (*Ficus pumila*
621 Linn.) seeds. *Carbohydrate Polymers*, 87(1), 76-83.
- 622
- 623 Liang, R. h., Chen, J., Liu, W., Liu, C. m., Yu, W., Yuan, M., & Zhou, X. q. (2012b). Extraction,
624 characterization and spontaneous gel-forming property of pectin from creeping fig (*Ficus pumila*
625 Linn.) seeds. *Carbohydrate Polymers*, 87(1), 76-83.
- 626
- 627 Liu, L., Cao, J., Huang, J., Cai, Y., & Yao, J. (2010). Extraction of pectins with different degrees of
628 esterification from mulberry branch bark. *Bioresource Technology*, 101(9), 3268-3273.
- 629
- 630 Löfgren, C., & Hermansson, A.-M. (2007). Synergistic rheological behaviour of mixed HM/LM pectin
631 gels. *Food Hydrocolloids*, 21(3), 480-486.
- 632
- 633 Masuelli, M. A. (2011). Viscometric study of pectin. Effect of temperature on the hydrodynamic
634 properties. *International Journal of Biological Macromolecules*, 48(2), 286-291.
- 635
- 636 Maxwell, E. G., Belshaw, N. J., Waldron, K. W., & Morris, V. J. (2012). Pectin – An emerging new
637 bioactive food polysaccharide. *Trends in Food Science & Technology*, 24(2), 64-73.
- 638
- 639 Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., dos Santos, T. C., Coube, C. S., & Leitão, S. G.
640 (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical
641 method. *Phytotherapy research : PTR*, 15(2), 127-130.
- 642
- 643 Mesbahi, G., Jamalian, J., & Farahnaky, A. (2005). A comparative study on functional properties of
644 beet and citrus pectins in food systems. *Food Hydrocolloids*, 19(4), 731-738.
- 645
- 646 Min, B., Lim, J., Ko, S., Lee, K.-G., Lee, S. H., & Lee, S. (2011). Environmentally friendly preparation of
647 pectins from agricultural byproducts and their structural/rheological characterization. *Bioresource*
648 *Technology*, 102(4), 3855-3860.
- 649
- 650 Nangia-Makker, P., Hogan, V., Honjo, Y., Baccarini, S., Tait, L., Bresalier, R., & Raz, A. (2002). Inhibition
651 of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin.
652 *Journal of the National Cancer Institute*, 94(24), 1854-1862.
- 653
- 654 Nestic, A. R., Trifunovic, S. S., Grujic, A. S., Velickovic, S. J., & Antonovic, D. G. (2011). Complexation of
655 amidated pectin with poly(itaconic acid) as a polycarboxylic polymer model compound. *Carbohydrate*
656 *Research*, 346(15), 2463-2468.
- 657
- 658 Ngouémazong, D. E., Kabuye, G., Fraeye, I., Cardinaels, R., Van Loey, A., Moldenaers, P., & Hendrickx,
659 M. (2012). Effect of debranching on the rheological properties of Ca²⁺–pectin gels. *Food*
660 *Hydrocolloids*, 26(1), 44-53.

- 661 Ovodov, Y. S. (2009). Current views on pectin substances. *Russian Journal of Bioorganic Chemistry*,
662 35(3), 269-284.
663
- 664 Platt D, inventor; Modified pectin. United States patent US 7491708 B1. 2009 Feb 17.
665
- 666 Pristov, J. B., Mitrovic, A., & Spasojevic, I. (2011). A comparative study of antioxidative activities of
667 cell-wall polysaccharides. *Carbohydrate Research*, 346(14), 2255-2259.
668
- 669 Rha, H. J., Bae, I. Y., Lee, S., Yoo, S.-H., Chang, P.-S., & Lee, H. G. (2011). Enhancement of anti-radical
670 activity of pectin from apple pomace by hydroxamation. *Food Hydrocolloids*, 25(3), 545-548.
671
- 672 Round, A. N., Rigby, N. M., MacDougall, A. J., & Morris, V. J. (2010). A new view of pectin structure
673 revealed by acid hydrolysis and atomic force microscopy. *Carbohydrate Research*, 345(4), 487-497.
674
- 675 Santos, M. D. S., Petkowicz, C. L. O., Haminiuk, C. W. I., & Candido, L. M. B. (2010). Polissacarídeos
676 extraídos da gabioba (*Campomanesia xanthocarpa* Berg): Propriedades químicas e perfil reológico.
677 *Polímeros*, 20(5), 352-358.
678
- 679 Sengkhampan, N., Sagis, L. M. C., de Vries, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J.
680 (2010). Physicochemical properties of pectins from okra (*Abelmoschus esculentus* (L.) Moench). *Food*
681 *Hydrocolloids*, 24(1), 35-41.
682
- 683 Serrano-Cruz, /M.R., Villanueva-Carvajal, A., Rosales, E.J.M., Dávila, J.F.R., Dominguez-Lopez, A.
684 (2013) Controlled release and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) extract
685 encapsulated in mixtures of carboxymethyl cellulose, whey protein, and pectin. *LWT - Food Science*
686 *and Technology*. 50, 554-561.
687
- 688 Sivam, A. S., Sun-Waterhouse, D., Perera, C. O., & Waterhouse, G. I. N. (2012). Exploring the
689 interactions between blackcurrant polyphenols, pectin and wheat biopolymers in model breads; a
690 FTIR and HPLC investigation. *Food Chemistry*, 131(3), 802-810.
691
- 692 Steffe, J. F. (1992). *Rheological methods in food process engineering*. East Lansing, Mich. USA:
693 Freeman Press.
694
- 695 Stephen, A. M. (1995). *Food polysaccharides and their applications*. New York: Marcel Dekker.
696
- 697 Taboada, E., Fisher, P., Jara, R., Zuniga, E., Gidekel, M., Cabrera, J. C., Pereira, E., Gutierrez-Moraga,
698 A., Villalonga, R., & Cabrera, G. (2010). Isolation and characterisation of pectic substances from
699 murta (*Ugni molinae* Turcz) fruits. *Food Chemistry*, 123(3), 669-678.
700
- 701 Videcoq, P., Garnier, C., Robert, P., & Bonnin, E. (2011). Influence of calcium on pectin
702 methylesterase behaviour in the presence of medium methylated pectins. *Carbohydrate Polymers*,
703 86(4), 1657-1664.
704
- 705 Wai, W. W., Alkarkhi, A. F. M., & Easa, A. M. (2010). Comparing biosorbent ability of modified citrus
706 and durian rind pectin. *Carbohydrate Polymers*, 79(3), 584-589.
707
- 708 Yapo, B. M. (2009a). Biochemical characteristics and gelling capacity of pectin from yellow passion
709 fruit rind as affected by acid extractant nature. *Journal of Agricultural and Food Chemistry*, 57(4),
710 1572-1578.
711

712 Yapo, B. M. (2009b). Lemon juice improves the extractability and quality characteristics of pectin
713 from yellow passion fruit by-product as compared with commercial citric acid extractant. *Bioresource*
714 *technology : biomass, bioenergy, biowastes, conversion technologies, biotransformations, production*
715 *technologies.*, 100(12), 3147-3151.

716

717 List of Tables

718 Table 1 – Intrinsic viscosity and molar mass of citrus pectins.

719 Table 2 – Rheological parameters of pectins.

720 Table 3 – Antioxidant activity of the pectins at a concentration of 50 mg L⁻¹.

721 Table 4 – Activation energy values of unmodified and modified pectins.

722

723 Table 1 – Intrinsic viscosity and molecular weight of pectins.

		Pectin			Modified pectin		
		CCP	CEP	NEP	MCCP	MCEP	MNEP
Huggins	Intrinsic viscosity (mL g ⁻¹)	329.82 ^{b*} ±0.01	300.82 ^c ±0.01	447.21 ^a ±0.01	242.98 ^d ±0.14	283.96 ^c ±0.002	228.51 ^d ±0.01
	Molar mass (g mol ⁻¹)	93,937 ^b ±3,284	83,486 ^c ±3,434	138,787 ^a ±2,824	63,485 ^d ±757	77,528 ^c ±779	58,686 ^d ±2,325
	R ²	0.99	0.95	0.99	0.90	0.96	0.98
Kraemer	Intrinsic viscosity (mL g ⁻¹)	369.07 ^a ±0.01	308.55 ^b ±0.01	337.06 ^{ab} ±0.01	268.16 ^c ±0.01	267.26 ^c ±0.007	225.97 ^d ±0.01
	Molar mass (g mol ⁻¹)	108,499 ^a ±2,585	86,256 ^b ±5,242	96,589 ^{ab} ±3,057	72,043 ^c ±2,113	71,736 ^c ±2,450	57,855 ^d ±3,309
	R ²	0.97	0.76	0.93	0.98	0.95	0.99

724 * Each value is expressed as mean ± standard deviation of triplicate tests. Means within the same line with different letters are
725 significantly different ($p \leq 0.05$), according to Tukey's Test. CCP: Commercial citrus pectin;
726 CEP: Citric experimental pectin; NEP: Nitric experimental pectin; MCCP: Modified commercial citrus pectin; MCEP: Modified citric
727 experimental pectin; MNEP: Modified nitric experimental pectin;

728 Table 2 – Rheological parameters of pectins.

Samples	Temp. (°C)	Consistency	Flow Behavior	R ²
		coefficient K (Pas ⁿ)	Index n (ad)	
CCP	10	10.50 ^{a*} ±0.50	0.91 ^a ±0.02	0.99
	30	3.75 ^b ±0.40	0.92 ^a ±0.02	0.99
	50	1.53 ^c ±0.02	0.97 ^a ±0.01	0.99
CEP	10	24.33 ^a ±5.75	0.68 ^a ±0.06	0.99
	30	3.45 ^b ±0.08	0.78 ^a ±0.001	0.99
	50	1.73 ^b ±0.01	0.77 ^a ±0.003	0.99
NEP	10	11.93 ^a ±0.83	0.83 ^b ±0.02	0.99
	30	3.66 ^b ±0.14	0.89 ^a ±0.003	0.99
	50	1.85 ^b ±0.001	0.90 ^a ±0.006	0.99
MCCP	10	1.35 ^a ±0.04	0.89 ^{ab} ±0.005	0.99
	30	0.36 ^b ±0.005	0.86 ^b ±0.01	0.97
	50	0.17 ^c ±0.007	0.92 ^a ±0.01	0.93
MCEP	10	0.94 ^a ±0.11	0.81 ^a ±0.03	0.99
	30	0.69 ^{ab} ±0.12	0.64 ^a ±0.07	0.95
	50	0.38 ^b ±0.07	0.65 ^a ±0.07	0.87
MNEP	10	1.47 ^a ±0.36	0.71 ^a ±0.06	0.99
	30	0.58 ^b ±0.06	0.68 ^a ±0.04	0.92
	50	0.28 ^b ±0.05	0.73 ^a ±0.06	0.93

729 * Each value is expressed as mean ± standard deviation of triplicate tests (n = 3).

730 The mean values of consistency of pectins, related to temperature variation, with
 731 different letters are significantly different ($p \leq 0.05$) according to Tukey's Test.

732 CEP: Citric experimental pectin; NEP: Nitric experimental pectin; MCCP: Modified
 733 commercial citrus pectin; MCEP: Modified citric experimental pectin; MNEP: Modified
 734 nitric experimental pectin;

735

736

737

738 Table 3 – Antioxidant activity (% of inhibition of the free-radical) of pectins at a
739 concentration of 50 mg.L⁻¹.

Unmodified	AA (%)	Modified	AA (%)
CCP	11.30 ^b ± 0.29	MCCP	14.51 ^{ab} ± 0.94
CEP	13.44 ^{ab} ± 0.72	MCEP	14.92 ^a ± 1.08
NEP	13.14 ^{ab} ± 0.29	MNEP	15.17 ^a ± 1.29

740 * Each value is expressed as mean ± standard deviation of duplicate tests (n = 2).

741 Means with different letters are significantly different ($p \leq 0.05$) according to Tukey's
742 Test.

743 CEP: Citric experimental pectin; NEP: Nitric experimental pectin; MCCP: Modified
744 commercial citrus pectin; MCEP: Modified citric experimental pectin; MNEP: Modified
745 nitric experimental pectin;

746

747 Table 4 – Activation energy (Ea) values of unmodified and modified pectins.

Pectins	Unmodified			Modified		
	CCP	CEP	NEP	MCCP	MCEP	MNEP
Ea (KJ mol ⁻¹)	36.49 ^{b*} ± 1.07	50.37 ^a ± 4.73	35.49 ^b ± 1.32	38.75 ^b ± 1.28	16.76 ^c ± 1.12	31.10 ^b ± 1.60
R ²	0.99	0.90	0.97	0.97	0.91	0.95

748 * Each value is expressed as the mean ± standard deviation of triplicate tests (n = 3).

749 Means with different letters are significantly different ($p \leq 0.05$) according to Tukey's
750 Test.

751 CEP: Citric experimental pectin; NEP: Nitric experimental pectin; MCCP: Modified
752 commercial citrus pectin; MCEP: Modified citric experimental pectin; MNEP: Modified
753 nitric experimental pectin;

754

755 List of figures

756 Figure 1 – Flowcharts of acid extraction: ^anitric acid ^bcitric acids.

757 Figure 2 – Reactions: a) Involved in determining the degree of esterification. b)

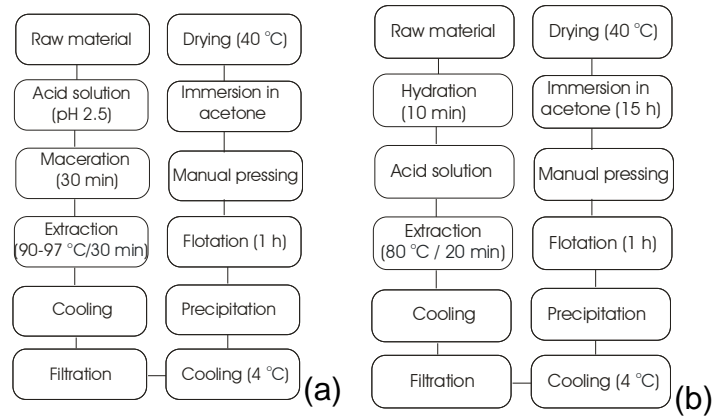
758 Modification of pectin.

759 Figure 3 – FTIR spectra of the pectins.

760 Figure 4 – Flow curves of unmodified and modified pectins. ■ 10 °C ● 30 °C ▲ 50 °C

761

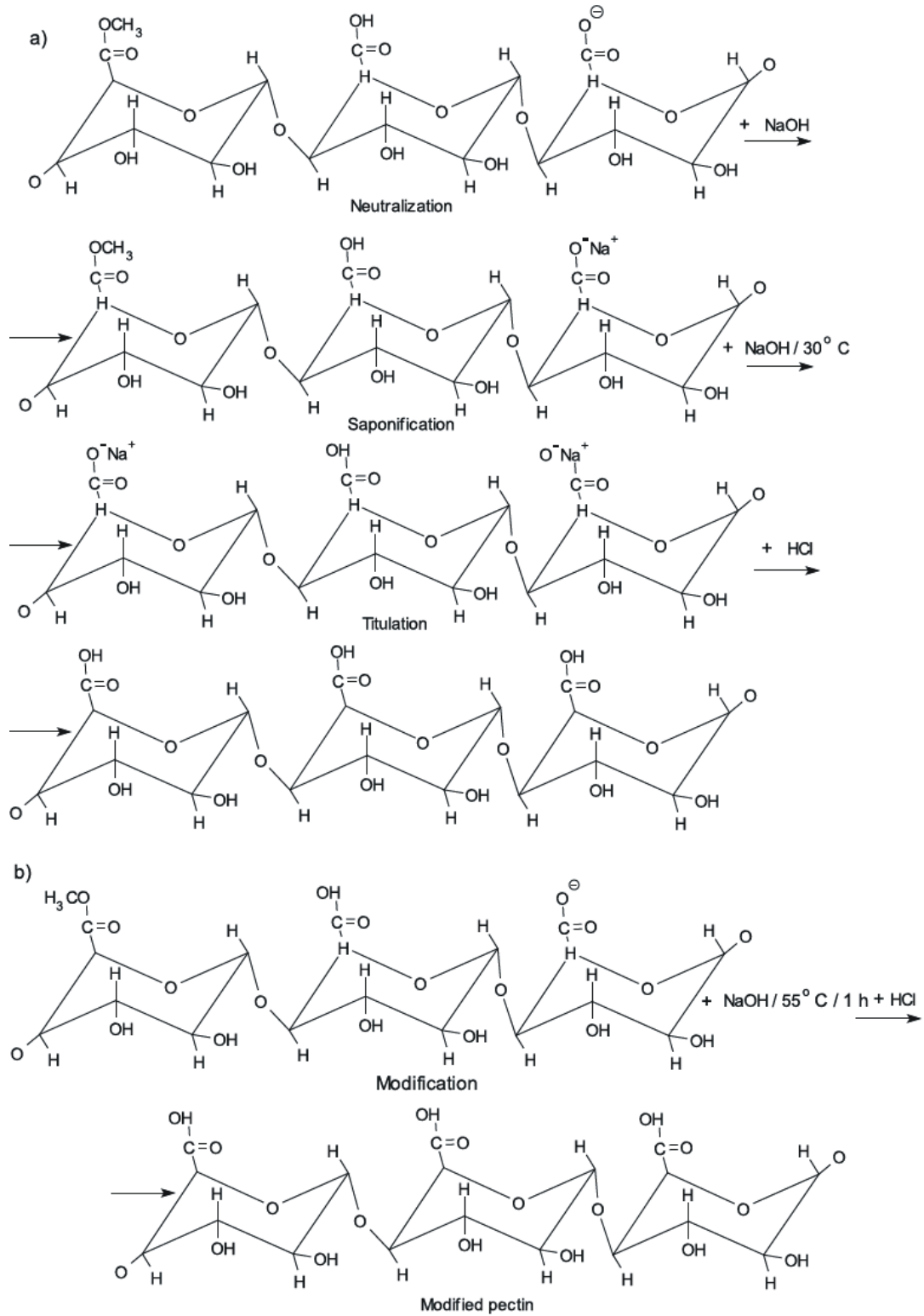
762 Fig.1



763

764

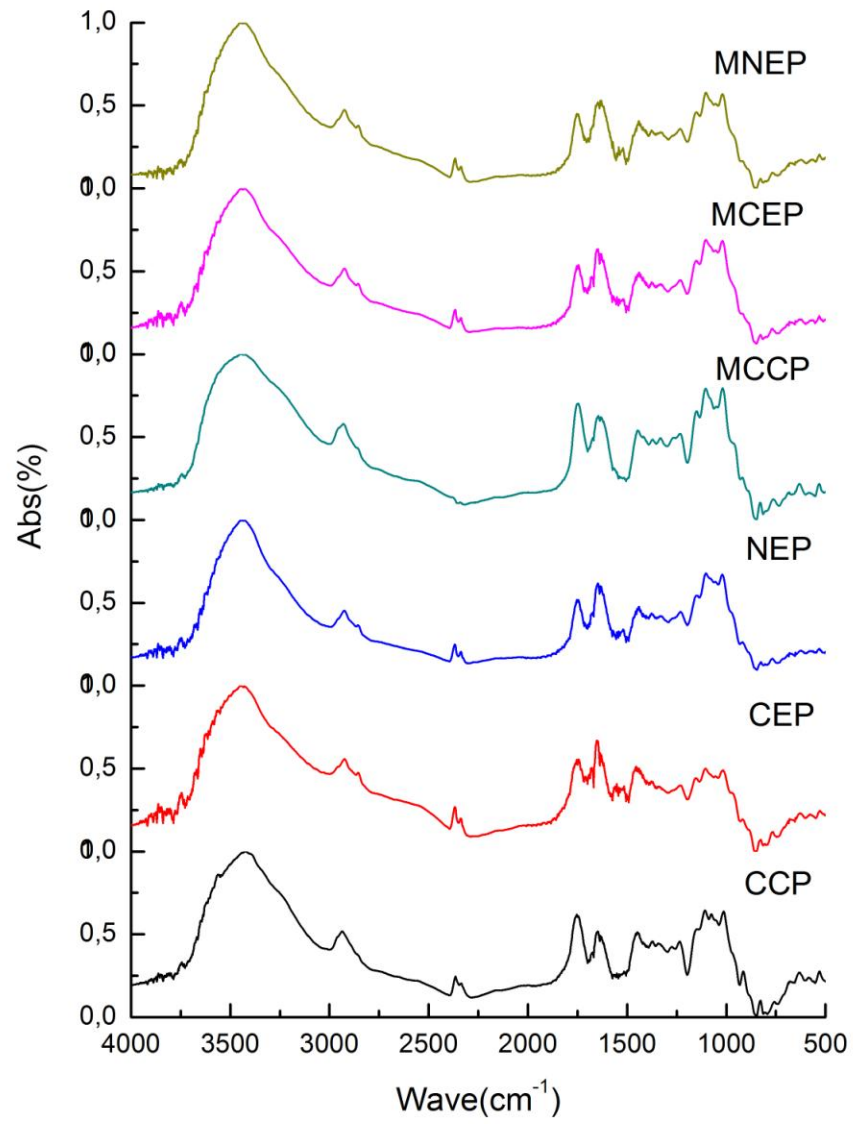
765 Fig.2



766

767

768 Fig.3



769

770

771 Fig.4

