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1 **Physicochemical characterization of pectin grafted with exogenous phenols**

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14

15 **Abstract**

16 Pectin is a natural polysaccharide, having valuable properties that enable its use in many
17 industrial fields. The aim of this work was to study the impact of pectin modification with
18 phenols, on the properties of this biopolymer. Results suggested that the enzymatic grafting of
19 ferulic acid (FA) oxidation products onto the pectin altered its morphological surface and its
20 thermal properties. Moreover, modified pectin showed a less hygroscopic behavior when
21 water activity is less than 0.50 and a higher ability to bound water above 0.5. Additionally,
22 modified pectin became less viscous than the native pectin and presented different calcium-
23 dependent gelation behavior. Finally, a significant improvement of the antioxidant properties
24 of pectin after functionalization was observed. As a conclusion, the modification of pectin
25 with phenolic compounds appeared as a promising way to produce a polysaccharide with new
26 properties that could enlarge the field of its potential applications.

27 **Keywords:** pectin; ferulic acid; functionalization; antioxidant; rheology; hygroscopy.

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32 **Introduction**

33 Pectin is an anionic high-molecular weight polysaccharide commercially extracted from the
34 cell wall of citrus peel, apple pomace and sugar beet (Qiu, Tian, Qiao, & Deng, 2009). It is
35 composed of a linearly α -1,4-linked D-galacturonic acid residues backbone interrupted
36 occasionally by (1,2)-linked rhamnose residues (Ridley, O'Neill, & Mohnen, 2001). **Pectin is**
37 **well known for its gelling and thickening properties that justify its extensive use as a food**
38 **ingredient. Many studies reported the existence of a correlation between the chemical**
39 **structure of pectin and its techno-functional properties (Chen et al., 2015; Sila et al., 2009).**
40 **The esterification and acetylation degrees of pectin appear as crucial structural characteristics**
41 **that are mostly exploited to produce biopolymers with modulated properties (Buchholt,**
42 **Christensen, Fallesen, Ralet, & Thibault, 2004; Cheng & Gu, 2012; van den Broek & Boeriu,**
43 **2013). Such modifications were performed aiming to overcome the major drawbacks of pectin**
44 **(formation of lumps, swelling behavior, hydrophilic nature) when used in some specific areas**
45 **(Kurita, Miyake, & Yamazaki, 2012). Another approach was described using a laccase-**
46 **catalyzed oxidation reaction of feruloylated sugar beet pectin. In this reaction, endogenous**
47 **ferulic acid units were oxidized leading to reactive semiquinones that can subsequently form**
48 **ferulic acid dimers allowing pectin crosslinking. The crosslinked structures have showed**
49 **improved rheological properties (Kuuva, Lantto, Reinikainen, Buchert, & Autio, 2003;**
50 **Zaidel, Chronakis, & Meyer, 2012), useful for the preparation of *in situ* hydrogels (Takei,**
51 **Sugihara, Ijima, & Kawakami, 2011) or for the formation of stable emulsions (Zaidel &**
52 **Meyer, 2013). This approach was also applied to conjugate sugar beet pectin with proteins. In**
53 **this case, the protein solubility in water was enhanced at its isoelectric point, avoiding its**
54 **precipitation (Jung & Wicker, 2012). As reported by several studies, laccases could also be**
55 **used to enrich polysaccharides with phenols. In this instance, semiquinones generated from**
56 **laccase-catalyzed oxidation of exogenous phenols reacted with nucleophilic functions present**

57 in the reaction medium leading to their grafting onto the polysaccharide chains (Elegir, Kindl,
58 Sadocco, & Orlandi, 2008; Y. Liu et al., 2014; Torres et al., 2012).

59 The main scientific issue related to this work was to compare the physical-chemical
60 properties of native pectin with those of pectin modified with exogenous phenolic species
61 issued from laccase-catalyzed oxidation of ferulic acid. The color, the thermal and
62 hygroscopic behavior, the morphological state, the rheological properties and the antioxidant
63 capacity were more specifically studied for a qualitative and/or quantitative examination of
64 the polysaccharide.

65 1. Materials and methods

66 1.1. Chemicals and enzyme

67 Citrus pectin with galacturonic acid $\geq 74.0\%$ (dried basis) and with a methoxylation
68 degree $\geq 6.7\%$, ferulic acid (FA), and 2,2'-azino-bis (3-éthylbenzothiazoline-6-acide
69 sulfonique) (ABTS) were purchased from Sigma–Aldrich (France). The following chemicals
70 1,1-Diphenyl-2-picrylhydrazyl (DPPH), methanol, ethanol and acetone were obtained from
71 Carlo Erba (Milwaukee, WI, USA).

72 An industrial laccase named Suberase[®] (Novo Nordisk A/S, Bagsvaerdt, Denmark) was
73 bought from the Society Novozymes under liquid form. The Suberase[®] is a fungal laccase
74 from *Myceliophthora thermophila* sp., which is considered as a member of family of
75 polyphenol oxidases, produced by submerged fermentation of a genetically modified
76 *Aspergillus oryzae*. The enzymatic preparation was supplied as a brown liquid with a density
77 of approximately 1.15 g.mL^{-1} . It was completely miscible with water. Laccase activity is 13.5
78 UI/mL.

79 The pectin was functionalized with laccase-mediated oxidation products of ferulic acid
80 according to the method described by Aljawish et al. (2012). Briefly, the modification
81 reaction was performed at 30 °C by the addition of 5 ml of methanol solution of ferulic acid

82 50 mM, 1 g of low methoxyl pectin and 45 ml of phosphate buffer (50 mM, pH 7.5) in the
83 reactor. The addition of 13.5 UI/mL of Suberase[®] (a fungal laccase from *Myceliophthora*
84 *thermophila*) trigger the reaction, which was carried out for four hours. The reaction medium
85 was then kept in a freezer for 24 h, then freeze-dried for 72 h. Next, the powder was washed
86 with organic solvents (methanol, ethanol and acetone) to remove unbounded phenols that
87 could be adsorbed onto pectin through electrostatic interactions. Finally, the powder was kept
88 in a desiccator until use.

89 1.2.Physicochemical properties

90 1.2.1.Thermal Analyses

91 Before analysis, all samples were dried at room temperature in a desiccator, containing
92 P₂O₅ as drying agent, for at least one week. The differential scanning calorimetry
93 measurement was carried out using a DSC (DSC 204 F1 Phoenix, Netzsch, Germany) under
94 dynamic inert nitrogen atmosphere, with a flow rate of 4 ml/min. Approximately 10 mg of
95 pectin powders were weighted in an aluminum capsule and placed in the DSC system in
96 parallel to an empty capsule used as reference. The program was fixed to perform a first round
97 of heating from 0 to 200 °C in a 5 K/min rate, followed by a cooling from 200 to 0 °C at the
98 same rate 5 K/min. Then a second heating from 0 to 300 °C at 5 K/min was performed. All
99 runs were performed at least in triplicate.

100 1.2.2.Surface analyses

101 The morphologies of native pectin and pectin grafted with phenols were observed using
102 a Hitachi scanning electron microscopy (SEM) S4800. Before testing, the samples were
103 evaporated with carbon and then coated with gold, to make the samples conductive. SEM was
104 performed under high vacuum at an accelerating voltage of 10 kV. The microphotographs
105 were taken using automatic image capture software.

106 1.2.3.Water sorption isotherms

107 The Dynamic Vapor Sorption was used to monitor the moisture sorption capacity of
108 pectin powders as a function of water activity (a_w). Measuring the water sorption, provided
109 information about the physical and chemical stability of the sample under given storage
110 conditions. Water sorption isotherms were determined gravimetrically using a DVS apparatus
111 (Surface Measurement Systems, London, UK) equipped with a Cahn microbalance. The
112 changes in sample weight over time at 25 °C and at any desired a_w (between 0 and 0.9) were
113 recorded. About 70 to 80 mg of sample were loaded onto the quartz sample pan. The program
114 was initially set to control the water activity at 0 for 12 h (drying phase). This step allowed the
115 sample water activity to decrease to zero and internally equilibrate. The sample was then
116 subjected to successive steps of 0.1 a_w increase, up to 0.9. For each step, the mass changes
117 (m) and the rate of mass changes (dm/dt) were plotted against time. The equilibrium was
118 considered to be reached when changes in mass with time were lower than 0.001% total
119 weight/min (i.e. 1 g water/100 g dry basis/day). All experiments were performed at 25 °C and
120 3 tests were carried out for each sample. The accuracy of the system was $\pm 0.01 a_w$ and ± 0.2
121 °C, respectively.

122 The rate at which the material equilibrated at each humidity level, as well as the overall
123 shape of the resulting adsorption profile, provided useful information about the structure
124 material and long-term stability.

125 1.2.4. Rheological measurements

126 1.2.4.1. Viscosity measurements

127 The viscosity of solutions prepared in deionized water (pH 6.5) with several
128 concentrations (from 1% to 4% w/v) of native and modified pectin were determined using a
129 Kinexus rotative rheometer (Malvern Instruments, KNX 2100, UK) with a cone-plate
130 geometry (50 mm of diameter, angle of 2°), at constant temperature (25 °C), just after the
131 pectin solutions were prepared. The shear rate was increased from 0.001 to 100 s⁻¹. The

132 Newtonian and power law models were used to analyze the rheological behavior of the
133 samples. Each viscosity measurement was performed in triplicate. The temperature was
134 controlled by a Peltier system and the sample was covered with paraffin oil to avoid
135 evaporation.

136 1.2.4.2. Oscillation measurements

137 Oscillatory measurements were used to determine the storage modulus (G') and loss
138 modulus (G'') of 2% pectin solutions using a 50 mm parallel plate at 25 °C. Strain sweep
139 (0.01–100 % at 1 Hz) was applied to test the linear viscoelastic region of the samples. The
140 frequency dependence of G' and G'' was determined by a frequency sweep (0.1–30 Hz at 1%
141 strain) (Zhang et al., 2013).

142 1.2.4.3. Gelation rate determination

143 2ml of solutions with 12.5 g of native and modified pectin were prepared in 0.1 M
144 NaCl, in order to screen electrostatic interactions. The pH was adjusted to 6.5 to obtain fully
145 charged chains (Capel, Nicolai, Durand, Boulenger, & Langendorff, 2006). Then, a certain
146 amount of CaCl_2 which corresponded to $R = (2 [\text{Ca}^{2+}] / ([\text{COO}^-]) = 0.58$ was added to the
147 pectin solutions (Fu & Rao, 2001). The solutions were immediately loaded onto the rheometer
148 cone-plane geometry and G' and G'' shear modulus at 1 Hz and 0.001% strain were
149 monitored. The strain was determined by a strain sweep test from 0.0001 to 10%. The
150 frequency was determined by a frequency sweep test from 0.01 to 100 Hz performed on the
151 gels after its formation to determine and compare the storage and loss moduli (G' and G'' ,
152 respectively). Rheological measurements were performed in three replicates.

153 1.3. Antiradical activity measurements

154 1.3.1. DPPH[•] free radical scavenging activity

155 Several amounts of pectin powder (from 0.5 to 40 mg) were added into the cuvette filled
156 with the solution containing DPPH[•] radicals (6.10^{-5} M) resulting in a final concentration of

157 0.5-40 mg/ml of pectin. After incubation for 30 min at room temperature in the dark, the
158 absorbance (A_i) at 517 nm was measured using an UV-Vis spectrometer (Shimadzu UV-1605)
159 against a blank without pectin, according to the slightly modified method of (Guo et al.,
160 2015). The scavenging ability was calculated by the Equation 1:

161 Equation 1: DPPH[•] Scavenging ability (%) = $(1 - \text{Abs sample} / \text{Abs control}) \times 100$

162 The EC₅₀ value, which expressed the antioxidant concentration required to reduce the radicals
163 by 50%, is a good indicator to quantify the antioxidant capacity. Each value represents the
164 average \pm standard deviation of three independent experiments.

165 1.3.2. ABTS^{•+} radical cation decolorization assay

166 The ABTS^{•+} radical scavenging activity method was based on the ability of one
167 compound to quench the ABTS^{•+} radical cation. ABTS^{•+} species were produced by the
168 reaction between ABTS (7 mM) and potassium persulfate (2.45 mM) in ratio 1:1 dissolved in
169 water. The mixture was kept in the dark overnight, and then diluted with ethanol until an absorbance
170 of 0.7 ± 0.25 at 734 nm was obtained. Several concentrations varying from 0.5 to 20 mg/ml of
171 pectin solubilized in distilled water were mixed with 1 ml of ABTS^{•+} solution. After 30 min in
172 the dark at room temperature, the absorbance at 734 nm was measured (Re et al., 1999) and
173 the scavenging capability was calculated using the Equation 2:

174 Equation 2: ABTS^{•+} radical scavenging activity (%) = $(1 - \text{Abs sample} / \text{Abs control}) \times 100$

175 ABTS^{•+} radical scavenging activity was expressed as EC₅₀ value, which corresponded to the
176 antioxidant concentration capable of reducing 50% of the ABTS^{•+} radicals. All analyses were
177 carried out in triplicate; results represented the mean values with standard deviation.

178 1.4. Statistical analysis

179 The experimental results were performed in triplicate. The data were recorded as means \pm
180 standard deviation (SD) and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc.).

181 One-way analysis of variance was performed by ANOVA procedures. Significant differences
182 between means were determined by Duncan's Multiple Range tests. Differences at $p < 0.05$
183 were considered significant.

184 2. Results and discussion

185 3.1. Physicochemical properties of pectin derivative

186 2.1.1. Thermal analyses

187 Thermal analysis of polymers provides characteristic data related to time and
188 temperature. The determination of temperature and heat flows associated with thermal
189 transitions give useful information about the properties and the end-use performances of
190 materials. In the present study, thermal analyses were performed to characterize pectin
191 powders, in order to evaluate the effect of functionalization on the molecular arrangement of
192 pectin chains and their interactions. Differential scanning calorimetry (DSC) was used to
193 study thermal transitions occurring in the course of heating of pectin powders. A first run
194 from 0 to 200 °C was made to remove all traces of water. **Indeed, as shown by several**
195 **authors, a peak around 100 °C was attributed to water evaporation, in citrus pectin samples**
196 (Einhorn-Stoll, Kunzek, & Dongowski, 2007). Similar results were obtained with sugar beet
197 pectin (Combo et al., 2013), apple peel pectin (Godeck, Kunzek, & Kabbert, 2001;
198 Monfregola, Bugatti, Amodeo, De Luca, & Vittoria, 2011) and products containing pectin
199 (Osorio, Carriazo, & Barbosa, 2011). Results obtained after the second run of heating are
200 shown in Table 1. **Differences at $p < 0.05$ were considered to be significant.**

201 For the native pectin, a glass transition was observed at 60 °C against 48 °C for the
202 modified pectin ($p < 0.05$). These results appeared somewhat higher than the glass transition
203 temperature of low or high methoxyl pectins (37 °C) reported by (Iijima, Nakamura,
204 Hatakeyama, & Hatakeyama, 2000). A weak exothermic peak corresponding to crystallization
205 was observed in the pectin grafted with phenols at 130 °C whereas no similar transition was

206 detected in native pectin. Such results suggested that pectin became under an amorphous form
207 once the crystalline pectin was melted (Iijima et al., 2000). The endothermic peak observed at
208 180 °C and 160 °C in native and modified pectin, respectively, was ascribed to the melting
209 transition phase. These results were consistent with those described by other authors who
210 found a melting temperature of citrus pectin around 180 °C (Pereira, Carmello-Guerreiro, &
211 Hubinger, 2009), at 200 °C (Osorio et al., 2011), or at 152 °C (Iijima et al., 2000). Moreover,
212 several exothermic peaks were found from 180 °C for the modified pectin and 225 °C for the
213 native one. These peaks were attributed to the degradation of the polymer followed by the
214 elimination of volatile products. At such temperatures, the degradation was primarily derived
215 from pyrolytic decomposition and decarboxylation pathways (Calce, Bugatti, Vittoria, & De
216 Luca, 2012).

217 In view of these results, the pectin grafted with phenols appeared as a less organized
218 polysaccharide compared with the native pectin. This was explained by the incorporation of
219 phenolic entities that might disorder, and then destabilize the overall structure of the polymer.
220 Phenolic entities were suspected to increase the free volume between the polysaccharide
221 chains, thus limiting their interactions. Depending on target applications, the thermal behavior
222 of the pectin grafted with phenols could be either an advantage or a limitation, when
223 compared to the native polysaccharide.

224

225

226 2.1.2. Morphology analyses

227 Morphological analysis is considered as an efficient method to study the shape of
228 particles, and then to explain their behavior and some of their physicochemical properties.
229 The morphology of pectin particles was shown in Figure 1.

230 According to the SEM images, the native pectin exhibited a rough surface contrary to
231 the pectin grafted with phenols that presented a smooth surface. These observations
232 highlighted the impact of the functionalization of pectin on the organization of the
233 polysaccharide chains.

234 Similar findings were reported in the case of starch granules functionalized through an
235 enzymatic esterification process (Lin, Li, Long, Su, & Huang, 2015). Grafting of rosin acid
236 occurred in the non-stereotyped area of starch and inside the crystalline regions making the
237 granules less organized. The morphology as well as the crystallinity of starch was affected by
238 the structural modifications brought to the polysaccharide.

239 2.1.3. Water sorption isotherms

240 Phenolic compounds are known to present a hydrophobic character. Their grafting onto
241 the pectin hydrophilic chains was expected to affect the affinity of the polysaccharide for
242 water as well as the overall structure of the polysaccharide. Moisture sorption isotherms of
243 native and modified pectin powders were determined (Figure 2).

244 The isotherm profile is conventionally divided into three zones. The first one
245 corresponding to the monolayer strongly bound water, this zone usually gives an idea of the
246 hygroscopic character of the substance. The second zone attributed to the linear region of
247 capillary adsorbed water which was more loosely bound, and in the third zone excess free
248 water was present in macrocapillaries (Mathlouthi, 2001; Saad et al., 2014). The profile
249 obtained with the native pectin was a slight sigmoid that reflected a Type II isotherm
250 suggesting a growing equilibrium moisture content with increasing water activity according to
251 the classification of (Brunauer, Deming, Deming, & Teller, 1940; Ricardo, Andrade, Lemus
252 M, Carmen, & Perez, 2011). The pectin grafted with phenols presented a J-shaped type III
253 isotherm, that accounted for a plasticizer effect of water at high a_w values (Andrade P., Lemus
254 M., & Pérez C., 2011). The monolayer moisture content (M_0) gave a good representation of

255 the hygroscopic behavior of polymers. It could be determined graphically as the tangent to the
256 adsorption curve when the first layer of water saturated all the sites of pectin ($0.0 < a_w < 0.1$).
257 M_0 of the modified pectin was lower (1.9 g/100 g dry bases) than that of the native pectin (5
258 g/100 g dry bases); suggesting the presence of less sorption sites accessible to water in modified
259 pectin and thus a less hygroscopic nature. These data were consistent with the fact that the
260 pectin grafted with phenols was somewhat more hydrophobic than the native one.
261 Furthermore, native pectin particles have a larger specific surface (rough surface) capable of
262 adsorbing more water than that of the modified pectin particles (smooth surface).

263 In addition, a transformation of the material of modified pectin seemed to occur above
264 the critical a_w value of 0.45. The isotherm profile suggested a change from the amorphous
265 glassy state to the amorphous rubbery state. At high a_w values, the growing capacity of the
266 modified pectin to absorb water could be explained by the open structure of the
267 polysaccharide. As a plasticizer, the water molecules can be inserted between the polymer
268 chains, thereby increasing the free volume and weakens the interchain interactions (Basu,
269 Shivhare, & Muley, 2013; Roos & Karel, 1990). On the contrary, the native pectin seemed to
270 maintain its structure when increasing water activity.

271 Some authors correlated the capacity of pectin to bind water with the degree of
272 esterification (DE). Wallingford & Labuza, 1983 found that low methoxyl pectin absorbed
273 more water than high methoxyl pectin for a given water activity. This result was inconsistent
274 with other studies reporting an opposite trend (Panchev, Slavov, Nikolova, & Kovacheva,
275 2010; Tsami, Vagenas, & Marinos-Kouris, 1992). These contradictory results suggested that
276 no direct correlation existed between the DE and the water adsorption capacity of pectin. In
277 our case, the DE of the modified pectin was higher than that of the native polysaccharide due
278 to the grafting of phenolic compounds. Considering the hydrophobic nature of phenols, the

279 less hygroscopic nature of the modified pectin appeared as a logical consequence of its higher
280 DE.

281 In the present study, the functionalization of pectin with phenols led to a less
282 hygroscopic polysaccharide at low water activities. This could constitute an advantage for
283 storage. For higher water activities, the less compact organization of the modified pectin
284 compared to the native one allowed water to act as a plasticizer, thus facilitating the
285 dispersion of the polysaccharide.

286 2.1.4. Rheological measurements

287 2.1.4.1. Viscosity of pectin solutions

288 The viscosity of the two pectin solutions decreased with increasing the shear rate. This
289 was caused by the random coil of pectin polysaccharide, demonstrating a shear-thinning
290 character, with a flow behavior index (n) lower than 1 (Figure 3). Hence, native and modified
291 pectin were considered as pseudoplastic fluids (L. Liu, Cao, Huang, Cai, & Yao, 2010; X.
292 Wang, Chen, & Lü, 2014; Zhang et al., 2013).

293 The two curves showed differences in their flow behavior patterns. Overall, the native
294 pectin had a slightly higher viscosities than the pectin grafted with phenols, whatever the
295 shear rate. Furthermore, the native pectin had a shorten power-law region. At shear rates
296 superior to 0.0325 s^{-1} , the native pectin started to behave like a Newtonian fluid where the
297 apparent viscosity was relatively constant. For the modified pectin, a slightly higher resistance
298 to shearing was observed (0.0511 s^{-1}). This behavior could be explained by the presence of
299 phenolic entities interacting within the pectin structure. Another assumption could be related
300 to the molecular weight of the two types of pectin (Yuan, Galloway, Hoffman, & Bhatt,
301 2011). The shear-thinning behavior of the two solutions was a consequence of the physical
302 disruption of chain entanglements which required time to be formed (S.-Q. Wang,
303 Ravindranath, Wang, & Boukany, 2007).

304 The viscosity was also determined depending on the polymer concentration. A shear
305 rate value of $2.78 \cdot 10^{-4} \text{ s}^{-1}$ was chosen so that both pectins adopted a shear-thinning behavior
306 (Figure 4). The slope in the shear thinning regions was correlated with the concentration.

307 At low concentration, the solutions prepared with the native and the modified pectins
308 presented similar viscosities. Whatever was the pectin type, the increase of concentration led
309 to an increased viscosity, indicating that the freedom of movement of individual chains
310 became restricted (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981; Sousa, Nielsen,
311 Armagan, Larsen, & Sørensen, 2015). **Moreover, the solution of pectin grafted with phenols**
312 **became less viscous than that of the native pectin.** This trend was even more pronounced as
313 the concentration increased.

314 2.1.4.2. Oscillation measurements of pectin solutions

315 At low frequency, both types of pectin exhibited the typical behavior of polysaccharide
316 in solution. The viscous modulus (G'') was higher than the elastic modulus (G'), indicating
317 the predominance of the viscous properties due to the dynamic balance between pectin
318 molecular entanglement and shearing (Figure 5).

319 When the frequency increased, G'' and G' crossed, indicating the viscoelastic behavior
320 of pectin. The crossover point indicates the exact frequency leading to elastic behavior or
321 approaching gel state (Zhang et al., 2013). These values were similar for the two types of
322 pectin (0.67 Hz and 0.69 Hz). The crossover point was correlated to the degree of elasticity
323 and more particularly to the relaxation time that corresponds to the time needed to regain the
324 original configuration (Choi, 2008). After the crossover point, G' became greater than G'' .
325 This trend was explained by the orientation of the polymeric chains leading to a gel
326 (Peressini, Bravin, Lapasin, Rizzotti, & Sensidoni, 2003; Piermaria, de la Canal, & Abraham,
327 2008; X. Wang et al., 2014).

328 2.1.4.3. Gelation rate determination

329 The influence of calcium addition on the gelation behavior of both types of pectin was
330 studied by monitoring the evolution of the storage modulus (G') with time. The gel set time
331 was determined as the time at which $G' > 1$ Pa.s (Urias-Orona et al., 2010).

332 The native pectin prepared in phosphate buffer form a gel immediately after the addition
333 of calcium, whereas the modified polysaccharide required more time (2871 s) (Figure 6). This
334 result could be explained by fewer interactions between the modified pectin and calcium ions
335 compared to the native polysaccharide, and then leading to a slow-set gelation (Löfgren,
336 Guillotin, Evenbratt, Schols, & Hermansson, 2005). The modified pectin is particularly
337 interesting in food industry a gelling agent for the production of clear jellies from clarified
338 fruit juices such as grape juice.

339 The difference observed in the kinetic of gel formation for the two types of pectin was
340 explained by the chemical modifications and the subsequent conformational changes brought
341 to the polysaccharide due to its functionalization. All these results suggested that the
342 enzymatically modified pectin has a higher degree of methyl esterification resulting in the
343 availability of fewer carboxyl groups to interact with calcium ions to form the egg box
344 architecture, and thus the gel.

345 2.2. *In vitro* antioxidant properties

346 The functionalization of pectin was expected to increase its antioxidant capacity due to
347 the incorporation of phenolic entities that can act as a hydrogen or electron donors (Li et al.,
348 2012; Molyneux, 2004). The ability of the pectin grafted with phenols to quench radical
349 species such as DPPH[•] and the ABTS^{•+} was studied and then compared to that of the native
350 pectin. The scavenging activity of the native and the modified pectin was shown in Table 2.
351 Differences at $p < 0.05$ were considered to be significant.

352 Whatever the radical species, the EC₅₀ values obtained with the modified pectin was
353 much lower than those observed for the native pectin, indicating a higher antioxidant capacity
354 ($p < 0.05$). This result could be explained by the grafting of phenolic entities produced from
355 the laccase-mediated oxidation of ferulic acid. Similar results were reported in the case of
356 other polymers grafted with ferulic acid oxidation products (Aljawish et al., 2012, 2014;
357 García-Zamora et al., 2015). The weak antioxidant activity observed for the native pectin was
358 attributed to few ferulic acid units naturally present in its structure.

359 In view of these results, the functionalization of pectin with phenols appeared as a
360 promising way to produce new polyfunctional derivatives with improved properties that are
361 expected to broaden the scope of polysaccharides.

362 **3. Conclusion**

363 In the present study, the physicochemical properties of pectin grafted with phenolic
364 compounds were investigated and compared to those of the native pectin. This work
365 demonstrated significant relationships between the structure of the polymer and its properties.
366 Experimental evidence demonstrated clearly that the modification of pectin with ferulic acid
367 oxidation products affected the organization of the polysaccharide chains and certainly also
368 their interactions. The introduction of phenol entities within the polymer structure increased
369 its hydrophobicity as well as its antioxidant activity and led to a looser organization. The
370 capacity to adsorb water and the rheological properties of pectin were also affected by the
371 functionalization, which could be either an advantageous or a drawback, depending on the
372 target applications. In any case, the grafting of phenols onto pectin polymeric chains allowed
373 modulating and even improving the techno-functional properties of the polysaccharide.
374 Thanks to its antioxidant activity and its physico-chemical properties, the modified pectin
375 appeared as a promising polyfunctional ingredient.

376 **4. References**

- 377 Aljawish, A., Chevalot, I., Jasniewski, J., Revol-Junelles, A.-M., Scher, J., & Muniglia, L.
378 (2014). Laccase-catalysed functionalisation of chitosan by ferulic acid and ethyl ferulate:
379 evaluation of physicochemical and biofunctional properties. *Food Chemistry*, 161, 279–287.
- 380 Aljawish, A., Chevalot, I., Piffaut, B., Rondeau-Mouro, C., Girardin, M., Jasniewski, J.,
381 Scher, J., Muniglia, L. (2012). Functionalization of chitosan by laccase-catalyzed oxidation of
382 ferulic acid and ethyl ferulate under heterogeneous reaction conditions. *Carbohydrate*
383 *Polymers*, 87(1), 537–544.
- 384 Andrade P., R. D., Lemus M., R., & Pérez C., C. E. (2011). Models of sorption isotherms for
385 food: uses and limitations. *Vitae*, 18(3), 325–334.
- 386 Basu, S., Shivhare, U. S., & Muley, S. (2013). Moisture adsorption isotherms and glass
387 transition temperature of pectin. *Journal of Food Science and Technology*, 50(3), 585–589.
- 388 Brunauer, S., Deming, L. S., Deming, W. E., & Teller, E. (1940). On a Theory of the van der
389 Waals Adsorption of Gases. *Journal of the American Chemical Society*, 62(7), 1723–1732.
- 390 Buchholt, H. C., Christensen, T. M. I. E., Fallesen, B., Ralet, M.-C., & Thibault, J.-F. (2004).
391 Preparation and properties of enzymatically and chemically modified sugar beet pectins.
392 *Carbohydrate Polymers*, 58(2), 149–161.
- 393 Calce, E., Bugatti, V., Vittoria, V., & De Luca, S. (2012). Solvent-Free Synthesis of Modified
394 Pectin Compounds Promoted by Microwave Irradiation. *Molecules*, 17(12), 12234–12242.
- 395 Capel, F., Nicolai, T., Durand, D., Boulenguer, P., & Langendorff, V. (2006). Calcium and
396 acid induced gelation of (amidated) low methoxyl pectin. *Food Hydrocolloids*, 20(6), 901–
397 907.
- 398 Cheng, H. N., & Gu, Q.-M. (2012). Enzyme-Catalyzed Modifications of Polysaccharides and
399 Poly(ethylene glycol). *Polymers*, 4(4), 1311–1330.
- 400 Chen, J., Liu, W., Liu, C.-M., Li, T., Liang, R.-H., & Luo, S.-J. (2015). Pectin modifications:
401 a review. *Critical Reviews in Food Science and Nutrition*, 55(12), 1684–1698.
- 402 Choi, S. K. (2008). PH Sensitive Polymers for Novel Conformance Control and Polymer
403 Flooding (PhD thesis). University of ProQuest.
- 404 Combo, A. M. M., Aguedo, M., Quiévy, N., Danthine, S., Goffin, D., Jacquet, N., Blecker, C.,
405 Devaux, J., Paquot, M. (2013). Characterization of sugar beet pectic-derived oligosaccharides
406 obtained by enzymatic hydrolysis. *International Journal of Biological Macromolecules*, 52,
407 148–156.
- 408 Einhorn-Stoll, U., Kunzek, H., & Dongowski, G. (2007). Thermal analysis of chemically and
409 mechanically modified pectins. *Food Hydrocolloids*, 21(7), 1101–1112.
- 410 Elegir, G., Kindl, A., Sadocco, P., & Orlandi, M. (2008). Development of antimicrobial
411 cellulose packaging through laccase-mediated grafting of phenolic compounds. *Enzyme and*
412 *Microbial Technology*, 43(2), 84–92.
- 413 Fu, J.-T., & Rao, M. A. (2001). Rheology and structure development during gelation of low-
414 methoxyl pectin gels: the effect of sucrose. *Food Hydrocolloids*, 15(1), 93–100.

415 García-Zamora, J. L., Sánchez-González, M., Lozano, J. A., Jáuregui, J., Zayas, T., Santacruz,
416 V., Hernández, F., Torres, E. (2015). Enzymatic treatment of wastewater from the corn tortilla
417 industry using chitosan as an adsorbent reduces the chemical oxygen demand and ferulic acid
418 content. *Process Biochemistry*, 50(1), 125–133.

419 Godeck, R., Kunzek, H., & Kabbert, R. (2001). Thermal analysis of plant cell wall materials
420 depending on the chemical structure and pre-treatment prior to drying. *European Food*
421 *Research and Technology*, 213(4-5), 395–404.

422 Guo, M., Ma, Y., Wang, C., Liu, H., Li, Q., & Fei, M. (2015). Synthesis, anti-oxidant activity,
423 and biodegradability of a novel recombinant polysaccharide derived from chitosan and
424 lactose. *Carbohydrate Polymers*, 118, 218–223.

425 Iijima, M., Nakamura, K., Hatakeyama, T., & Hatakeyama, H. (2000). Phase transition of
426 pectin with sorbed water. *Carbohydrate Polymers*, 41(1), 101–106.

427 Jung, J., & Wicker, L. (2012). Laccase mediated conjugation of sugar beet pectin and the
428 effect on emulsion stability. *Food Hydrocolloids*, 28(1), 168–173.

429 Kurita, O., Miyake, Y., & Yamazaki, E. (2012). Chemical modification of citrus pectin to
430 improve its dissolution into water. *Carbohydrate Polymers*, 87(2), 1720–1727.

431 Kuuva, T., Lantto, R., Reinikainen, T., Buchert, J., & Autio, K. (2003). Rheological
432 properties of laccase-induced sugar beet pectin gels. *Food Hydrocolloids*, 17(5), 679–684.

433 Lin, R., Li, H., Long, H., Su, J., & Huang, W. (2015). Structure and characteristics of lipase-
434 catalyzed rosin acid starch. *Food Hydrocolloids*, 43, 352–359.

435 Liu, L., Cao, J., Huang, J., Cai, Y., & Yao, J. (2010). Extraction of pectins with different
436 degrees of esterification from mulberry branch bark. *Bioresource Technology*, 101(9), 3268–
437 3273.

438 Liu, Y., Zhang, B., Javvaji, V., Kim, E., Lee, M. E., Raghavan, S. R., Wang, Q., Payne, G. F.
439 (2014). Tyrosinase-mediated grafting and crosslinking of natural phenols confers functional
440 properties to chitosan. *Biochemical Engineering Journal*, 89, 21–27.

441 Li, W. J., Cheng, X. L., Liu, J., Lin, R. C., Wang, G. L., Du, S. S., & Liu, Z. L. (2012).
442 Phenolic Compounds and Antioxidant Activities of *Liriope muscari*. *Molecules*, 17(2), 1797–
443 1808.

444 Löfgren, C., Guillotin, S., Evenbratt, H., Schols, H., & Hermansson, A.-M. (2005). Effects of
445 calcium, pH, and blockiness on kinetic rheological behavior and microstructure of HM pectin
446 gels. *Biomacromolecules*, 6(2), 646–652.

447 Mathlouthi, M. (2001). Water content, water activity, water structure and the stability of
448 foodstuffs. *Food Control*, 12(7), 409–417.

449 Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for
450 estimating antioxidant activity. *Songklanakarin J. Sci. Technol.*, 26(2), 211–219.

451 Monfregola, L., Bugatti, V., Amodeo, P., De Luca, S., & Vittoria, V. (2011). Physical and
452 water sorption properties of chemically modified pectin with an environmentally friendly
453 process. *Biomacromolecules*, 12(6), 2311–2318.

454 Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., & Price, J. (1981).
455 Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions.
456 *Carbohydrate Polymers*, 1(1), 5–21.

457 Osorio, C., Carriazo, J. G., & Barbosa, H. (2011). Thermal and structural study of guava
458 (*Psidium guajava* L) powders obtained by two dehydration methods. *Química Nova*, 34(4),
459 636–640.

460 Panchev, I. N., Slavov, A., Nikolova, K., & Kovacheva, D. (2010). On the water-sorption
461 properties of pectin. *Food Hydrocolloids*, 24(8), 763–769.

462 Pereira, L. M., Carmello-Guerreiro, S. M., & Hubinger, M. D. (2009). Microscopic features,
463 mechanical and thermal properties of osmotically dehydrated guavas. *LWT - Food Science*
464 *and Technology*, 42(1), 378–384.

465 Peressini, D., Bravin, B., Lapasin, R., Rizzotti, C., & Sensidoni, A. (2003). Starch–
466 methylcellulose based edible films: rheological properties of film-forming dispersions.
467 *Journal of Food Engineering*, 59(1), 25–32.

468 Piermaria, J. A., de la Canal, M. L., & Abraham, A. G. (2008). Gelling properties of kefiran, a
469 food-grade polysaccharide obtained from kefir grain. *Food Hydrocolloids*, 22(8), 1520–1527.

470 Qiu, N., Tian, Y., Qiao, S., & Deng, H. (2009). Apple Pectin Behavior Separated by
471 Ultrafiltration. *Agricultural Sciences in China*, 8(10), 1193–1202.

472 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999).
473 Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free*
474 *Radical Biology & Medicine*, 26(9-10), 1231–1237.

475 Ricardo, D., Andrade, P., Lemus M, R., Carmen, E., & Perez, C. (2011). Models of sorption
476 isotherms for food: uses and limitations. *Vitae*, 18(3), 325–334.

477 Ridley, B. L., O'Neill, M. A., & Mohnen, D. (2001). Pectins: structure, biosynthesis, and
478 oligogalacturonide-related signaling. *Phytochemistry*, 57(6), 929–967.

479 Roos, Y., & Karel, M. (1990). Differential Scanning Calorimetry Study of Phase Transitions
480 Affecting the Quality of Dehydrated Materials. *Biotechnology Progress*, 6(2), 159–163.

481 Saad, A., Touati, B., Draoui, B., Tabti, B., Abdenebi, A., Benaceur, S. (2014). Mathematical
482 Modeling of Moisture Sorption Isotherms and Determination of Isothermic Heats of Sorption of
483 *Ziziphus* Leaves. *Modelling and Simulation in Engineering*, *Modelling and Simulation in*
484 *Engineering*, 2014, e427842.

485 Sato, M. D. F., Rigoni, D. C., Canteri, M. H. G., Petkowicz, C. L. de O., Nogueira, A., &
486 Wosiacki, G. (2011). Chemical and instrumental characterization of pectin from dried pomace
487 of eleven apple cultivars. *Acta Scientiarum. Agronomy*, 33(3).

488 Sila, D. n., Van Buggenhout, S., Duvetter, T., Fraeye, I., De Roeck, A., Van Loey, A., &
489 Hendrickx, M. (2009). Pectins in Processed Fruits and Vegetables: Part II—Structure–
490 Function Relationships. *Comprehensive Reviews in Food Science and Food Safety*, 8(2), 86–
491 104.

492 Sousa, A. G., Nielsen, H. L., Armagan, I., Larsen, J., & Sørensen, S. O. (2015). The impact of
493 rhamnogalacturonan-I side chain monosaccharides on the rheological properties of citrus
494 pectin. *Food Hydrocolloids*, 47, 130–139.

495 Takei, T., Sugihara, K., Ijima, H., & Kawakami, K. (2011). In situ gellable sugar beet pectin
496 via enzyme-catalyzed coupling reaction of feruloyl groups for biomedical applications.
497 *Journal of Bioscience and Bioengineering*, 112(5), 491–494.

498 Torres, E., Marín, V., Aburto, J., Beltrán, H. I., Shirai, K., Villanueva, S., & Sandoval, G.
499 (2012). Enzymatic modification of chitosan with quercetin and its application as antioxidant
500 edible films. *Applied Biochemistry and Microbiology*, 48(2), 151–158.

501 Tsami, E., Vagenas, G. k., & Marinou-Kouris, D. (1992). Moisture sorption isotherms of
502 pectins. *Journal of Food Processing and Preservation*, 16(3), 151–161.

503 Urias-Orona, V., Rascón-Chu, A., Lizardi-Mendoza, J., Carvajal-Millán, E., Gardea, A. A., &
504 Ramírez-Wong, B. (2010). A Novel Pectin Material: Extraction, Characterization and Gelling
505 Properties. *International Journal of Molecular Sciences*, 11(10), 3686–3695.

506 Van den Broek, L. A. M., & Boeriu, C. G. (2013). Enzymatic synthesis of oligo- and
507 polysaccharide fatty acid esters. *Carbohydrate Polymers*, 93(1), 65–72.

508 Wallingford, L., & Labuza, T. P. (1983). Evaluation of the Water Binding Properties of Food
509 Hydrocolloids by Physical/Chemical Methods and in a Low Fat Meat Emulsion. *Journal of*
510 *Food Science*, 48(1), 1–5.

511 Wang, S.-Q., Ravindranath, S., Wang, Y., & Boukany, P. (2007). New theoretical
512 considerations in polymer rheology: Elastic breakdown of chain entanglement network. *The*
513 *Journal of Chemical Physics*, 127(6), 064903.

514 Wang, X., Chen, Q., & Lü, X. (2014). Pectin extracted from apple pomace and citrus peel by
515 subcritical water. *Food Hydrocolloids*, 38, 129–137.

516 Yuan, M., Galloway, J. A., Hoffman, R. J., & Bhatt, S. (2011). Influence of molecular weight
517 on rheological, thermal, and mechanical properties of PEEK. *Polymer Engineering & Science*,
518 51(1), 94–102.

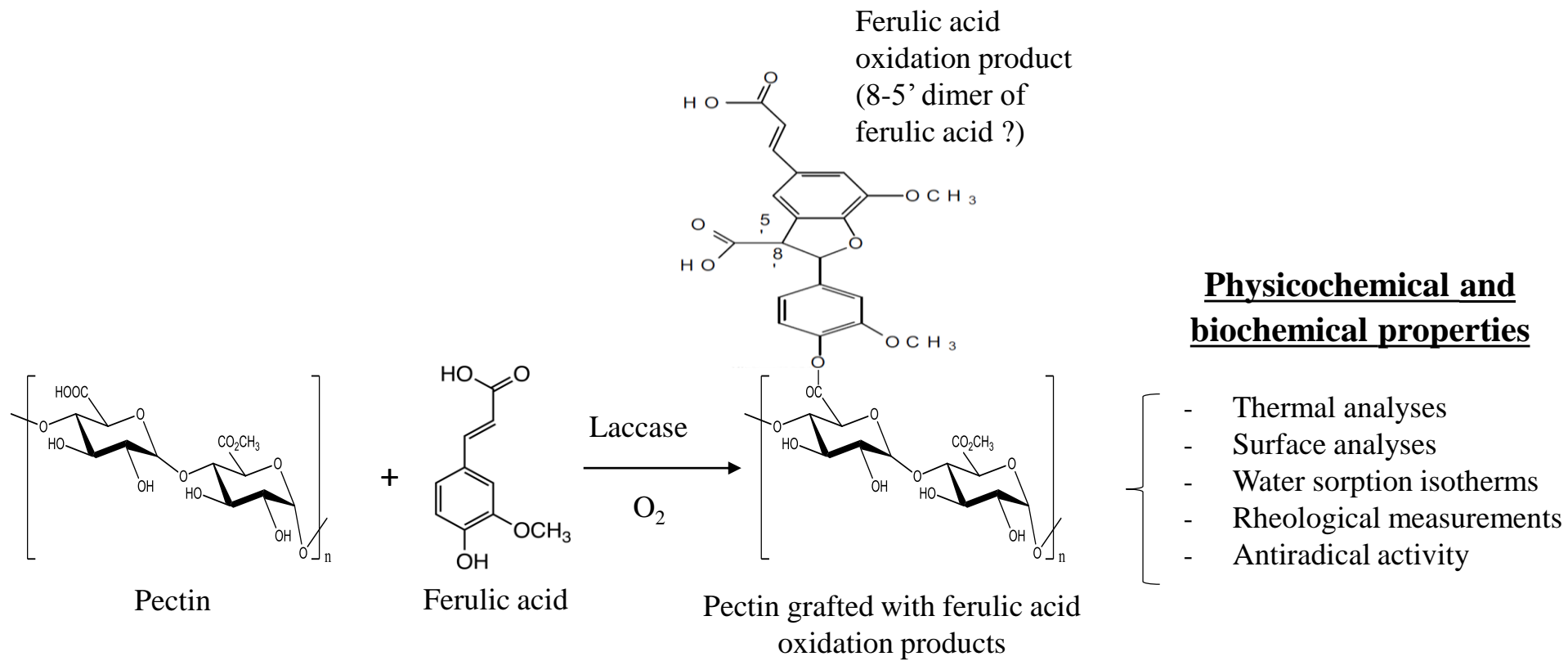
519 Zaidel, D. N. A., Chronakis, I. S., & Meyer, A. S. (2012). Enzyme catalyzed oxidative
520 gelation of sugar beet pectin: Kinetics and rheology. *Food Hydrocolloids*, 28(1), 130–140.

521 Zaidel, D. N. A., & Meyer, A.-S. (2013). Oxidative enzymatic gelation of sugar beet pectin
522 for emulsion stabilization. *Research Journal of Biotechnology*, 8(7), 81–86.

523 Zhang, L., Ye, X., Ding, T., Sun, X., Xu, Y., & Liu, D. (2013). Ultrasound effects on the
524 degradation kinetics, structure and rheological properties of apple pectin. *Ultrasonics*
525 *Sonochemistry*, 20(1), 222–231.

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Highlights:

- The physicochemical properties of pectin grafted with phenols were studied
- The morphological surface and the thermal properties of the pectin were altered
- Pectin grafted with phenols showed a slower gelation rate
- Grafting of phenols enhanced the antioxidant activity of pectin

Figures Captions

Figure 1: Scanning Electron Micrograph of native pectin (on the left) and pectin grafted with phenols (on the right)

Figure 2: Sorption isotherm profiles obtained for the native pectin (●) and the pectin grafted with phenols (○), estimated at 25 °C, from 0 to 0.9 of water activity

Figure 3: Variation of the shear viscosity depending on the shear stress for native (○) and modified pectins (●) (1% w/v in water). Viscosities were measured at 25 ± 1 °C with cone-plate geometry (50 mm, 2°), sd. was ≤ 4 %

Figure 4: Shear viscosity as a function of the concentration in native and modified pectin. Viscosities were measured at 25 ± 1 °C and a fixed rotational shear rate of $2.78 \cdot 10^{-4} \text{ s}^{-1}$

Figure 5: Variation of elastic modulus G' (● and ○) (Pa) and viscous modulus G'' (■ and □) (Pa) with the frequency (Hz) for the native (open symbols) or the modified pectin (filled symbols) at a concentration of 1% (w/v)

Figure 6: Variation of the storage (G') modulus versus time in a modified pectin solution (1.25% (w/v), in 0.1 N NaCl), with a volume of calcium corresponding to $R = (2 [\text{Ca}^{2+}] / ([\text{COO}^-]) = 0.58$ and pH 6.5. Measurements were performed at 25 °C, 1 Hz and 0.001% strain

Tables Captions

Table 1: Temperatures of thermal transitions in native pectin and in pectin grafted with phenolic compounds

Table 2: Capacity of native and modified pectin to scavenge DPPH[•] and ABTS^{•+} species, expressed as EC₅₀ values

Figure 1:

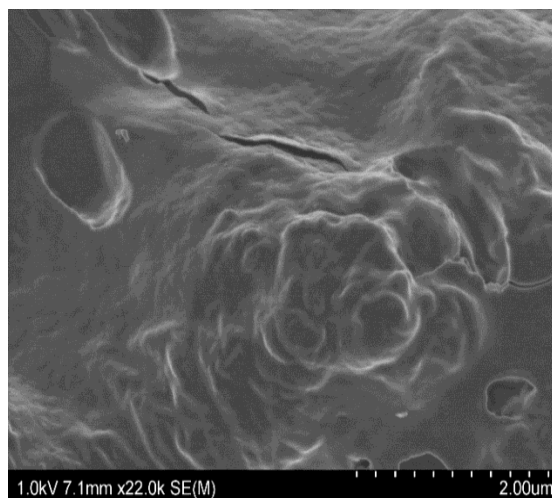
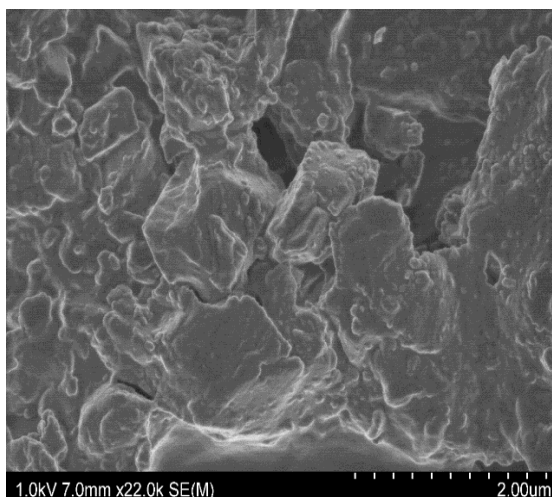


Figure 2:

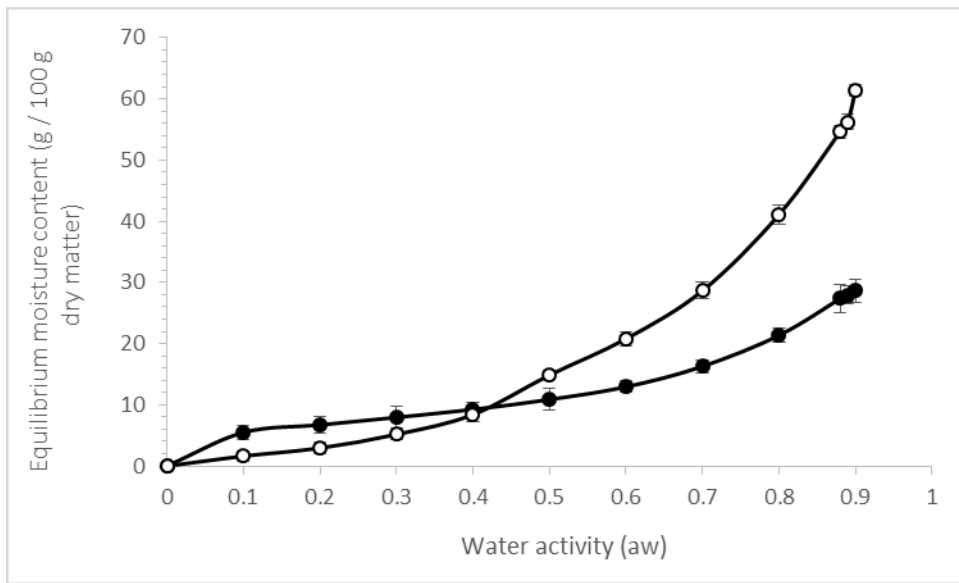


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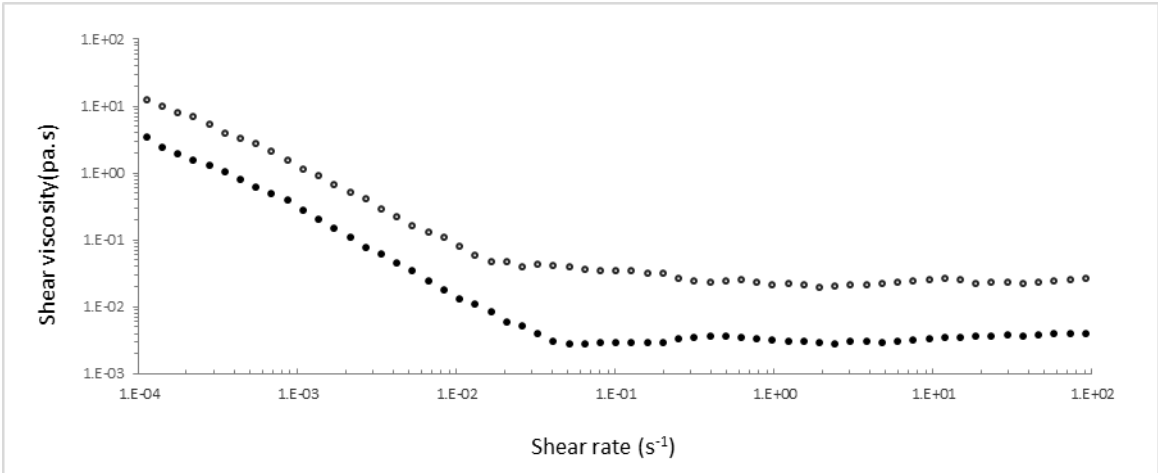


Figure 4:

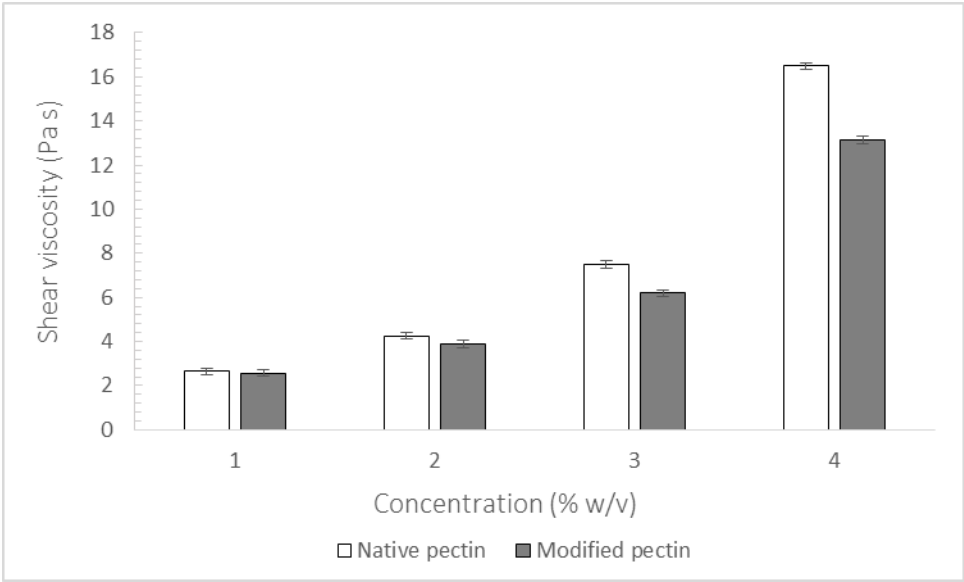


Figure 5:

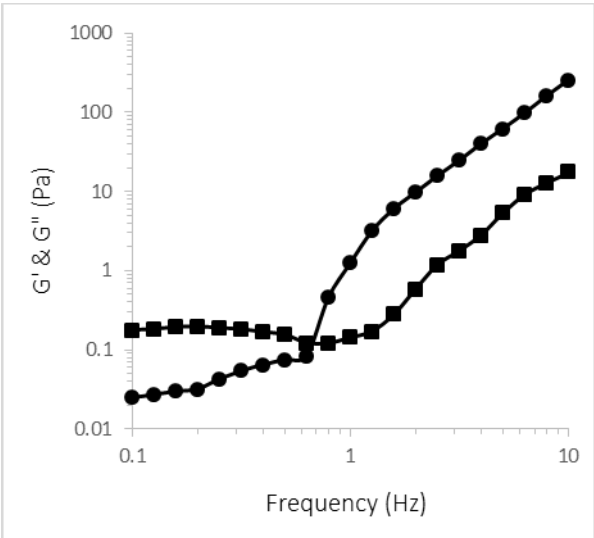
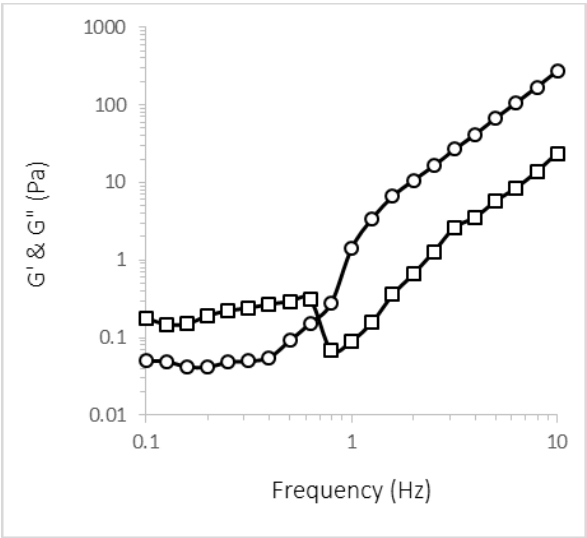


Figure 6:

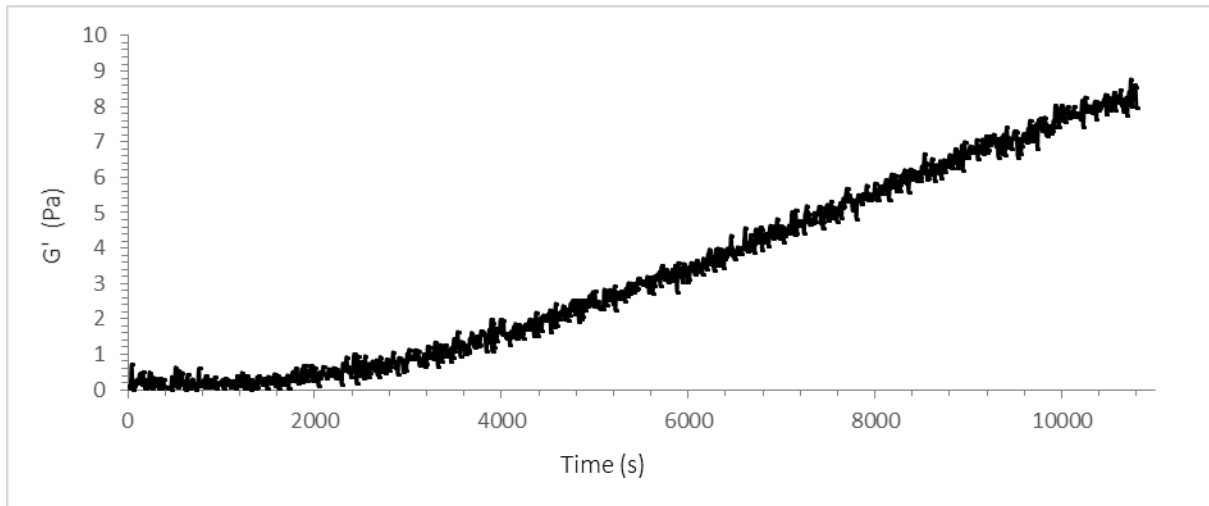


Table 1:

DSC analysis	T _g (°C)	T _c (°C)	T _m (°C)
Native pectin	60 ^a	-	180 ^a
Modified pectin	48 ^b	130	160 ^b

T_g: glass transition temperature. T_c: crystallization temperature. T_m: melting temperature. Each value is expressed as mean ± standard deviation (n = 3). Values not followed by the same letter in each column are significantly different at the 0.05% level (Duncan's test).

Table 2:

	EC ₅₀ DPPH test (mg/mL)	EC ₅₀ ABTS test (mg/mL)
Native pectin	29.5 ± 0.3 ^a	116.2 ± 3.9 ^a
Modified Pectin	1.4 ± 0.2 ^b	11.2 ± 0.8 ^b

Each value is expressed as mean ± standard deviation (n = 3). Values not followed by the same letter in each column are significantly different at the 0.05% level (Duncan's test)