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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  $\alpha$ -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<sup>®</sup> (Novo Nordisk A/S, Bagsvaerdt, Denmark) was  
73 bought from the Society Novozymes under liquid form. The Suberase<sup>®</sup> 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  $1.15 \text{ g.mL}^{-1}$ . 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<sup>®</sup> (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 P<sub>2</sub>O<sub>5</sub> 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  $\pm 0.2$   
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<sup>-1</sup>. 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  $\text{CaCl}_2$  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<sup>•</sup> 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<sup>•</sup> 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<sup>•</sup> Scavenging ability (%) =  $(1 - \text{Abs sample} / \text{Abs control}) \times 100$

162 The EC<sub>50</sub> 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<sup>•+</sup> radical cation decolorization assay

166 The ABTS<sup>•+</sup> radical scavenging activity method was based on the ability of one  
167 compound to quench the ABTS<sup>•+</sup> radical cation. ABTS<sup>•+</sup> 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  $0.7 \pm 0.25$  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<sup>•+</sup> 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<sup>•+</sup> radical scavenging activity (%) =  $(1 - \text{Abs sample} / \text{Abs control}) \times 100$

175 ABTS<sup>•+</sup> radical scavenging activity was expressed as EC<sub>50</sub> value, which corresponded to the  
176 antioxidant concentration capable of reducing 50% of the ABTS<sup>•+</sup> 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  $0.0325 \text{ s}^{-1}$ , 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 ( $0.0511 \text{ s}^{-1}$ ). 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<sup>•</sup> and the ABTS<sup>•+</sup> 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 EC<sub>50</sub> 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.



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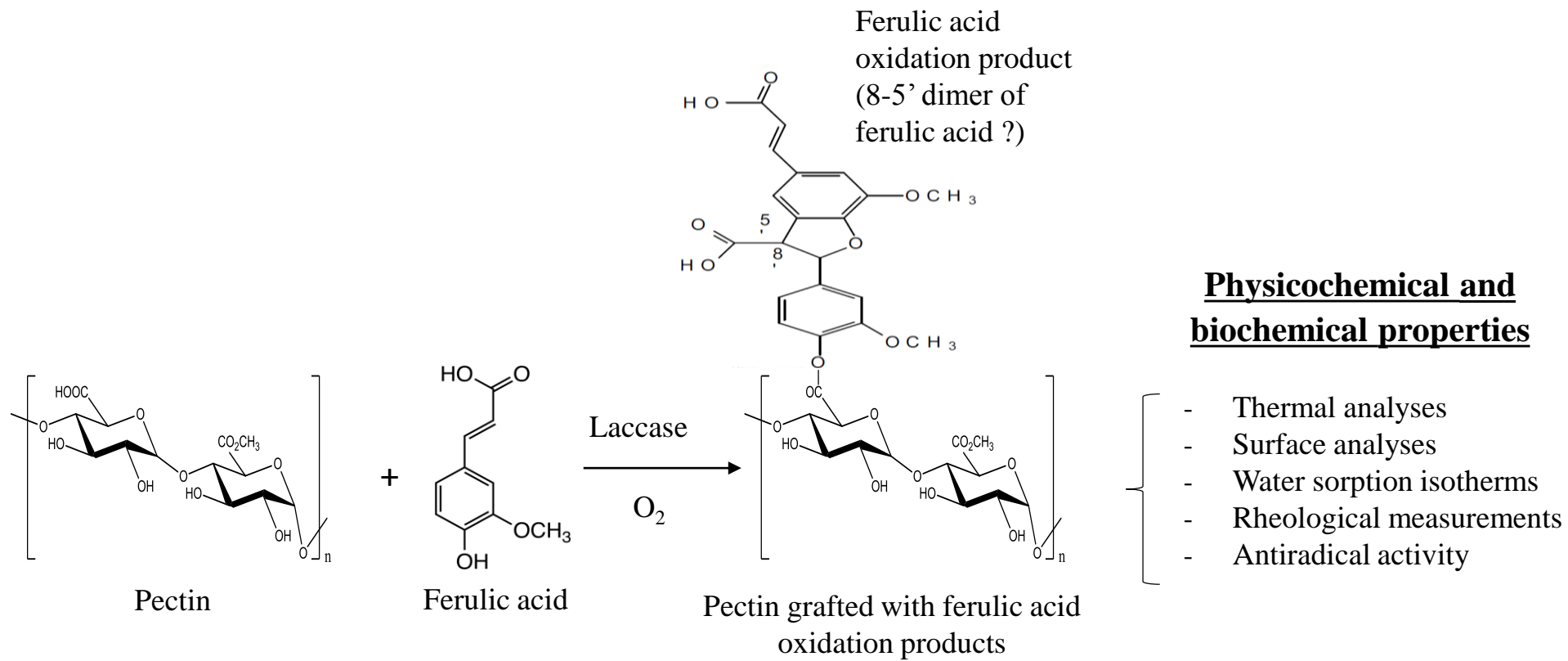
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527



**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  $25 \pm 1$  °C with cone-plate geometry (50 mm, 2°), sd. was  $\leq 4$  %

Figure 4: Shear viscosity as a function of the concentration in native and modified pectin. Viscosities were measured at  $25 \pm 1$  °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<sup>•</sup> and ABTS<sup>•+</sup> species, expressed as EC<sub>50</sub> values

Figure 1:

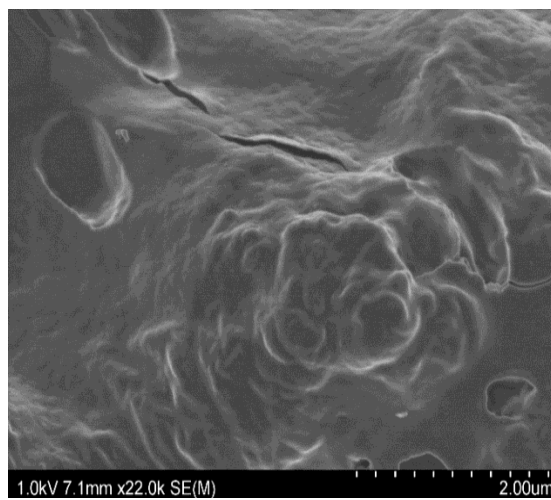
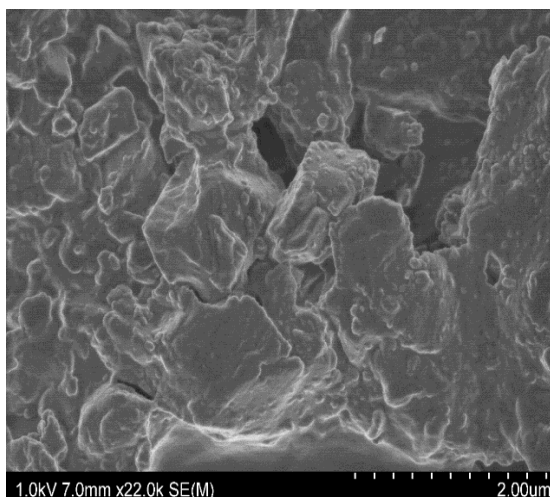




Figure 2:

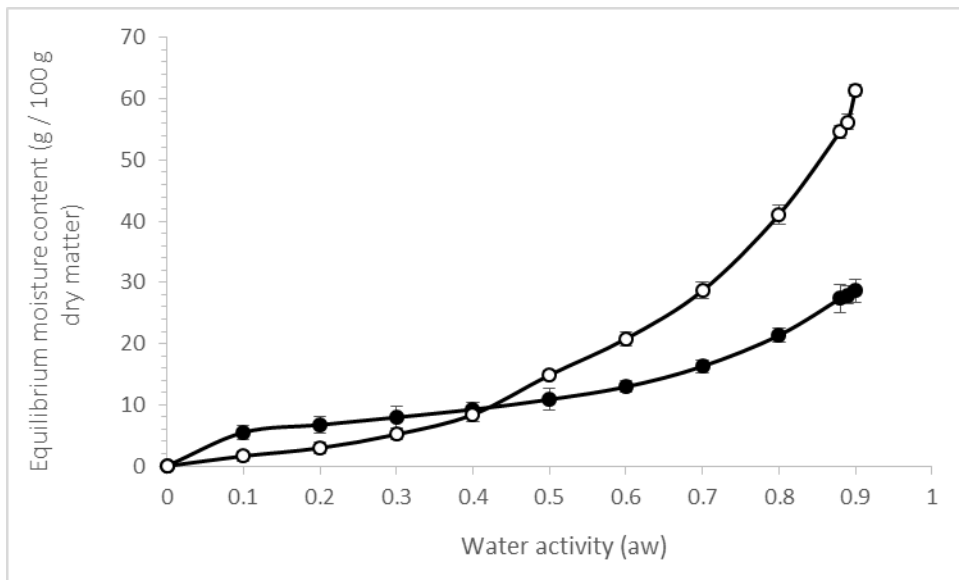


Figure 3:

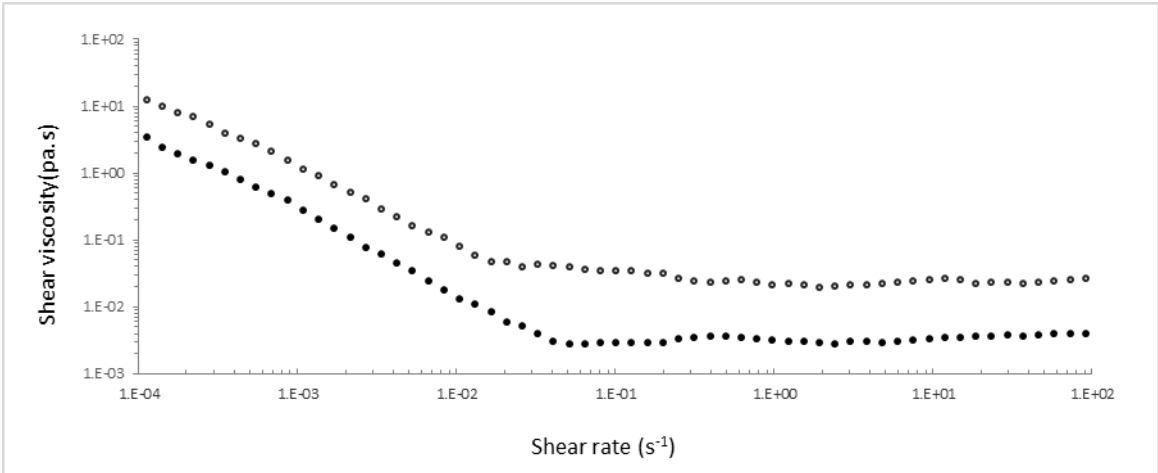


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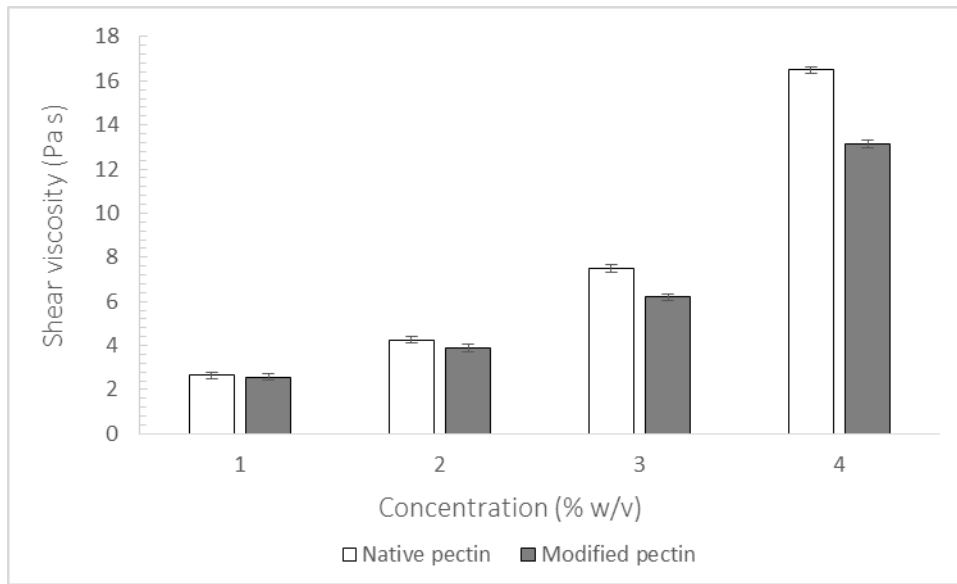


Figure 5:

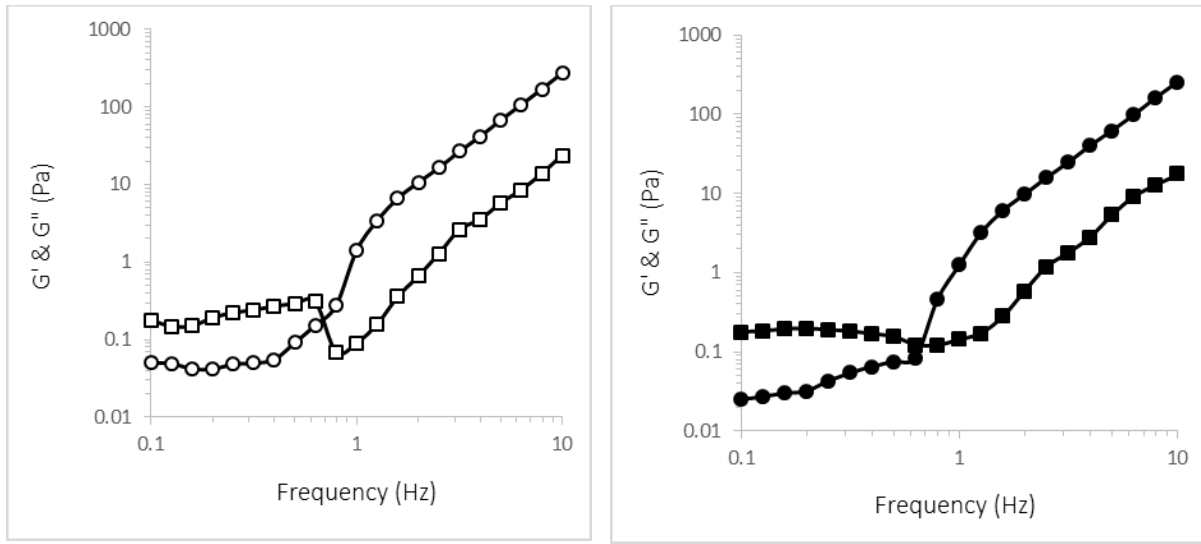


Figure 6:

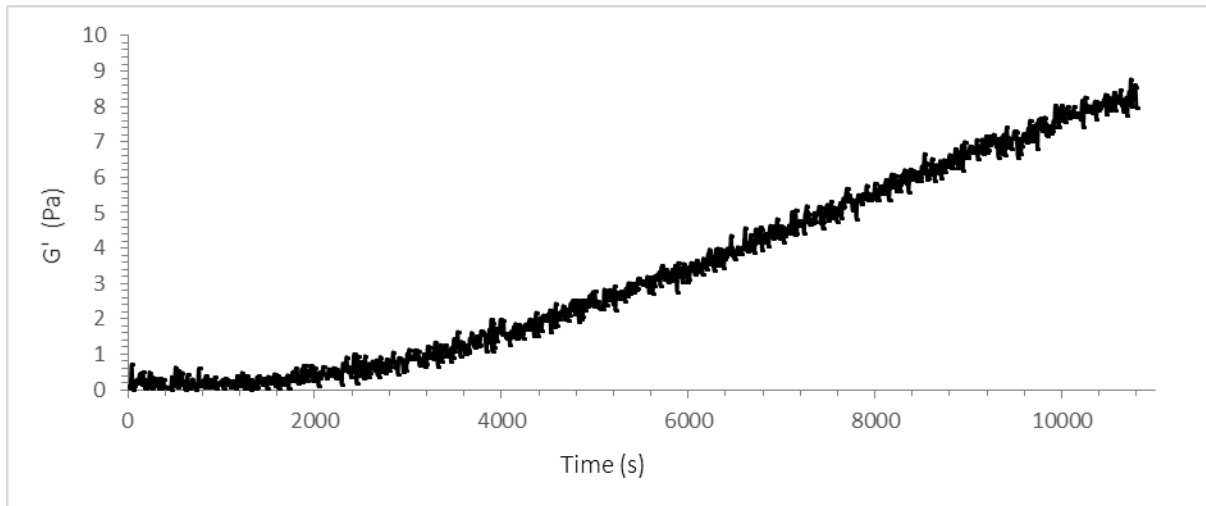


Table 1:

DSC analysis	T <sub>g</sub> (°C)	T <sub>c</sub> (°C)	T <sub>m</sub> (°C)
Native pectin	60 <sup>a</sup>	-	180 <sup>a</sup>
Modified pectin	48 <sup>b</sup>	130	160 <sup>b</sup>

T<sub>g</sub>: glass transition temperature. T<sub>c</sub>: crystallization temperature. T<sub>m</sub>: 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 <sub>50</sub> DPPH test (mg/mL)	EC <sub>50</sub> ABTS test (mg/mL)
Native pectin	29.5 ± 0.3 <sup>a</sup>	116.2 ± 3.9 <sup>a</sup>
Modified Pectin	1.4 ± 0.2 <sup>b</sup>	11.2 ± 0.8 <sup>b</sup>

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)