



23 **Abstract**

24 **BACKGROUND:** The greatest hurdle to the commercial marketing of fresh-cut fruits is  
25 related to their higher susceptibility to enzymatic browning, tissue softening, and  
26 microbial growth. The aim of this study was to test the efficacy of a pectin-based edible  
27 coating and low oxygen modified atmosphere packaging (MAP) to control enzymatic  
28 browning and reduce microbial growth of fresh-cut 'Rojo Brillante' persimmon. The  
29 survival of *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes*  
30 artificially inoculated on fresh-cut fruit was also assessed. The pectin coating was  
31 amended with 500 IU mL<sup>-1</sup> nisin (NI) as antimicrobial agent and 10 g kg<sup>-1</sup> citric acid and  
32 10 g kg<sup>-1</sup> calcium chloride as antibrowning and firming agents, respectively. Persimmon  
33 slices were dipped in the coating or in water (control) and packed under 5 kPa O<sub>2</sub> (MAP)  
34 or in ambient atmosphere for up to 9 days at 5 °C. Microbial growth, package gas  
35 composition, colour, firmness, polyphenol oxidase (PPO) activity, visual quality and  
36 overall sensory flavour of persimmon slices were measured during storage.

37 **RESULTS:** Coating application combined with active MAP significantly reduced the  
38 CO<sub>2</sub> emission and O<sub>2</sub> consumption in the package. The coating was effective to reduce  
39 browning and also inhibited the growth of mesophilic aerobic bacteria. Coating also  
40 reduced the populations of *E. coli*, *S. enteritidis* and *L. monocytogenes*.

41 **CONCLUSION:** The combination of the pectin-based edible coating and active MAP  
42 proved to be the most effective treatment to maintain the sensory and microbiological  
43 quality of persimmon slices for more than 9 days of storage.

44

45 **Keywords:** Minimally processed persimmon, food-borne human pathogens, antibrowning  
46 agents, antimicrobial activity, shelf-life

47

48 **INTRODUCTION**

49 The demand for fresh-cut fruits and vegetables is continuously increasing, being the  
50 convenience factor and health promoting benefits associated with their consumption the  
51 main reasons for such an increment. ‘Rojo Brillante’ is the most important persimmon  
52 cultivar in Spain. This cultivar, mainly grown in the Ribera del Xúquer area (Valencia,  
53 Spain) has experienced in the last decade an important increase in planted surface and  
54 production due to the fruit good sensory characteristics and nutritional properties. When  
55 harvested, the fruit is astringent, but the exogenous application of high levels of CO<sub>2</sub>  
56 allows the removal of astringency without affecting fruit firmness, which enables this  
57 cultivar to be commercialized as a fresh-cut commodity. However, physical damage  
58 during peeling, cutting or slicing processes increases respiration rate, metabolic changes  
59 and susceptibility to microbial spoilage, which often result in degradation of the colour,  
60 flavour and firmness of the product.<sup>1</sup> Furthermore, cut surfaces can provide both  
61 attachment opportunities and entry points for microorganisms.<sup>2</sup> Recent studies have  
62 documented the exponential growth of *Escherichia coli* O157:H7, *Salmonella* spp. and  
63 *Listeria monocytogenes* in non acidic horticultural products as well as on a wide variety  
64 of acidic fresh produce, although the growth in the latest is thought to be limited because  
65 of the acidity.<sup>3</sup>

66 Main approaches to extend the shelf-life of fresh-cut products include chlorine  
67 sanitation, the use of low temperatures, modified atmosphere packaging (MAP) with low  
68 O<sub>2</sub> concentration, and the use of antioxidants and calcium salts. The effect of MAP to  
69 maintain the quality of fresh-cut products is related to a reduction in the product  
70 respiration rate, ethylene biosynthesis and action, water loss, phenolic oxidation, and  
71 aerobic microbial count.<sup>4</sup> However, the beneficial effects depend upon a number of

72 uncontrollable factors, such as the species, cultivar, cultural practices, stage of maturity,  
73 as well as controllable factors, including packaging material gas permeability, respiration  
74 rate, and storage conditions.<sup>5</sup> Thus, previous work by our group showed that controlled  
75 atmosphere conditions with high CO<sub>2</sub> concentrations (10 or 20 kPa) induced in fresh-cut  
76 ‘Rojo Brillante’ persimmons the darkening of some tissue areas associated with a flesh  
77 disorder known as ‘internal flesh browning’. The maximum fruit shelf life was achieved  
78 in samples stored in low O<sub>2</sub> atmospheres (5 kPa O<sub>2</sub>, balance N<sub>2</sub>), as this concentration  
79 effectively controlled enzymatic browning and prevented ‘flesh browning’. Subsequent  
80 studies confirmed the beneficial effect of active MAP (5 kPa O<sub>2</sub>) compared to passive  
81 MAP to improve the visual quality of fresh-cut ‘Rojo Brillante’ persimmon, showing a  
82 synergic effect with an antibrowning dip in citric acid and CaCl<sub>2</sub> (unpublished data).

83         Nowadays, edible coatings are gaining importance as an alternative treatment to  
84 reduce the deterioration caused by minimal processing fruits, as they provide a  
85 semipermeable barrier to gases and water vapour and, therefore, help to control  
86 respiration rate, enzymatic browning, and water loss. Furthermore, their protective  
87 function may be also enhanced by the addition of other ingredients such as antimicrobials,  
88 antioxidants, flavours, nutrients, etc.<sup>6</sup> It can be found in the literature numerous works  
89 remarking the effect of antioxidant edible coatings to control browning in fresh-cut fruits  
90 such as apple, pear, papaya, etc.<sup>7-9</sup> However, the incorporation of antimicrobial food  
91 additives into edible coatings to prevent microbial spoilage has been considerably less  
92 studied. In previous works by our group, the addition to a pectin-based edible coating of  
93 citric acid and CaCl<sub>2</sub>, as antibrowning and firming agents, and nisin as antimicrobial  
94 agent effectively prevented enzymatic browning of fresh-cut ‘Rojo Brillante’ persimmon  
95 and extended the commercial visual shelf-life up to 8 days of storage at 5 °C. In addition,

96 the coating also inhibited the growth of mesophilic aerobics and reduced the population of  
97 inoculated *E. coli*, *S. enteritidis* and *L. monocytogenes* on cut persimmons.<sup>10</sup>

98 Since it is known that the application of hurdle technologies can considerably  
99 improve the overall quality of fresh-cut fruits and vegetables, some attempts have also  
100 been focused on extending the shelf life of fresh-cut commodities by combining both  
101 edible coatings and MAP technologies. For this instance, the use of MAP as a second  
102 technology resulted in a significant benefit on the visual quality of fresh-cut kiwifruit  
103 coated with a sodium alginate coating amended with grape seed extract,<sup>11</sup> and improved  
104 the microbiological quality of fresh-cut strawberries coated with chitosan.<sup>12</sup> In fresh-cut  
105 ‘Rojo Brillante’ persimmon, the combination of a soy protein isolate-based coating  
106 containing antioxidants with active MAP packaging (5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>) showed a  
107 synergistic effect in controlling tissue browning and maintained the visual quality above  
108 the limit of marketability up to 8 days of storage at 5 °C.<sup>13</sup> However, no studies are  
109 available on the effect of antimicrobial and antioxidant edible coatings combined with  
110 MAP on enzymatic browning and microbial quality of fresh-cut persimmons. Therefore,  
111 the aim of this work was to study the combined effect of a pectin-based edible coating  
112 amended with antibrowning and antimicrobial food additives and low O<sub>2</sub> MAP on fruit  
113 quality and microbial growth of fresh-cut ‘Rojo Brillante’ persimmon. The survival of  
114 important food-borne human pathogens artificially inoculated on fresh-cut fruit was also  
115 assessed.

116

## 117 **MATERIALS AND METHODS**

### 118 **Plant material**

119 Persimmons (*Diospyros kaki* Thunb cv Rojo Brillante) harvested at commercial maturity  
120 were provided by a local packinghouse assigned to the persimmon geographical  
121 indication ‘Denominación de Origen Kaki Ribera del Xuquer’ (Valencia, Spain).  
122 Persimmons were harvested with an external colour index (CI=1000 a /L b) of  $15.1 \pm 4.0$ ,  
123 firmness of  $21.4 \pm 5.0$  N, total acidity of  $1.14 \pm 0.02$  g malic acid/100 g and a soluble  
124 solid content of  $18.20 \pm 0.09$  °Brix.

125

### 126 **Edible coating formulation**

127 The edible coating was elaborated from a base solution of apple pectin (Sigma-Aldrich,  
128 St. Louis, MO, USA) at  $10 \text{ g kg}^{-1}$ . The aqueous solution of apple pectin was prepared at  
129 mild heating. The pectin was emulsified with  $2.5 \text{ g kg}^{-1}$  oleic acid (Panreac Química,  
130 S.A., S.A., Barcelona, Spain) and  $2.5 \text{ g kg}^{-1}$  Tween 80 (Sigma-Aldrich) and glycerol  
131 (Panreac Química) was added as plasticizer at  $10 \text{ g kg}^{-1}$ . As antibrowning agents,  $10 \text{ g kg}^{-1}$   
132 citric acid (Quimivita, Barcelona, Spain) and  $10 \text{ g kg}^{-1}$  calcium chloride ( $\text{CaCl}_2$ ) (Sigma-  
133 Aldrich) were incorporated into the coating formulation. Nisin (NI) was added as  
134 antimicrobial agent at 500 international units (IU)  $\text{mL}^{-1}$  (Coralim Aditivos S.L., Valencia,  
135 Spain). The coating emulsion was kept at  $5 \text{ }^\circ\text{C}$  until application.

136

### 137 **Pathogenic strains and inoculum preparation**

138 Stock cultures for the food-borne contamination-specific human pathogenic strains of *E.*  
139 *coli* serotype O157:H7 (CECT 4972; ATCC 700728), *S. enterica* subsp. *enterica* (CECT  
140 4300; ATCC 13076) and *L. monocytogenes* serovar 1 (CECT 7467; ATCC 19111) were  
141 obtained from the Microbiology Reference Laboratory (University of Valencia, Spain) in  
142 the form of agar slants. Strains were activated by streaking on MacConkey’s agar (AES

143 Laboratoire, Combourg, France) (*E. coli* and *S. enteritidis*) and tryptic soya agar + 50 g  
144 kg<sup>-1</sup> sheep's blood agar (BD, New Jersey, USA) (*L. monocytogenes*) plates, followed by  
145 incubation for 48 h at 37 °C. Single colonies were grown individually in Luria-Bertani  
146 broth (Luria-Bertani<sup>®</sup>, Barcelona, Spain) (*E. coli* and *S. enteritidis*) or tryptone soya yeast  
147 extract broth (Sigma-Aldrich) (*L. monocytogenes*) for 24 h at 37 °C. Bacterial cells were  
148 harvested by centrifugation at 3,000 rpm for 10 min at 10 °C and then resuspended in  
149 saline peptone to obtain a concentrated suspension. The process was repeated 3 times.  
150 Finally, cell pellets were resuspended in maximum recovery diluent to obtain a culture  
151 optical density of 0.2 at 600 nm. This corresponded to a final inoculum concentration of  
152 6.0 log cfu mL<sup>-1</sup>.

153

#### 154 **Persimmon processing and packaging**

155 Natural astringency of 'Rojo Brillante' persimmons was eliminated by placing them for  
156 24 h in closed chambers at 20 °C with an atmosphere containing 95±2 kPa CO<sub>2</sub>.  
157 Chambers used for deastringency consisted of hermetically sealed, transparent  
158 polymethyl methacrylate cabinets (82 x 62 x 87 cm) fitted with outlet and inlet ports  
159 through which CO<sub>2</sub> (Alphagaz, Air Liquide España S.A., Madrid, Spain) were injected  
160 until the desired concentration was achieved. The cabinets were also fitted with internal  
161 basal water trays that allowed achieving a high relative humidity (RH of 95 ± 5%). CO<sub>2</sub>  
162 level, temperature, and RH were continuously monitored by means of the computer-  
163 controlled system (Control-Tec<sup>®</sup>, Tecnidex S.A., Paterna, Valencia, Spain). After  
164 removing them from the chambers, fruit were stored in air at 5 °C for 1 day until  
165 processing. Persimmons were sanitized in a 150 mg L<sup>-1</sup> NaClO solution for 2 min, rinsed  
166 with tap water, and dried prior to cutting operations. For the physico-chemical, sensory

167 and microbiological analyses, persimmons were peeled, cut into eight wedges with a  
168 sharp stainless-steel knife to reduce mechanical bruising and dipped into the pectin-based  
169 coating or in water as control for 3 min. After dipping, persimmon pieces were removed  
170 and left to dry at 5 °C. Then, four persimmon pieces ( $115 \pm 10$  g) were placed on  
171 polypropylene trays (17.4 x 12.9 x 3.6 cm, Ilpra Systems, Barcelona, Spain) and sealed  
172 with 64- $\mu$ m thickness, microperforated polypropylene-polyethylene terephthalate film  
173 (P12-2050PXNP, ILPRA Systems España S.L. Mataró, Spain). Oxygen and carbon  
174 dioxide permeance of the film were 110 and 500 mL m<sup>-2</sup> d<sup>-1</sup> bar<sup>-1</sup> respectively, at 23 °C.  
175 Coated and uncoated samples were divided into two groups. Half of the fruit was packed  
176 in air and the other half under active MAP of 5 kPa O<sub>2</sub> balanced with N<sub>2</sub>. To ensure that  
177 the atmosphere on the trays that were packed in air was not modified, and to study the  
178 effect of only the edible coating, the film was perforated with a needle (4 perforations, 1  
179 mm in diameter). A total of 9 trays per treatment and sampling time were prepared that  
180 corresponded to 3 trays for physico-chemical analysis, 3 trays for sensory and 3 trays for  
181 microbiological analysis. Samples were stored up to 9 days at 5 °C.

182

### 183 **Headspace gas composition**

184 Gas composition (O<sub>2</sub> and CO<sub>2</sub>) in the package headspace of fresh-cut persimmon were  
185 analyzed with a gas chromatograph (Trace GC, Thermo Fisher Scientific, Inc. Waltham,  
186 MA, USA) equipped with a thermal conductivity detector (TCD) and fitted with a  
187 Poropack QS 80/100 column (1.2 m x 0.32 cm i.d.). Temperatures for the oven, injector,  
188 and thermal conductivity detector were 35, 115, and 150 °C, respectively. Helium was  
189 used as a carrier gas at flow rate of 22 mL min<sup>-1</sup>. The gas sample was taken with a needle  
190 through an adhesive septum that had been stuck on the film. One milliliter of the gas



191 headspace was injected into the system. O<sub>2</sub> and CO<sub>2</sub> concentrations were calculated using  
192 peak areas from standard gas mixtures of 15.0:2.5% O<sub>2</sub>:CO<sub>2</sub>. Results were expressed as  
193 kPa. Five trays per treatment were analyzed.

194

#### 195 **Microbial growth in fresh-cut persimmon**

196 On days 0, 4 and 8, the total number of mesophilic and psychrophilic aerobic bacteria,  
197 yeasts and moulds was determined in triplicate. A representative sample of persimmon  
198 wedges (10 g) were removed aseptically from the package, transferred to a sterile plastic  
199 bag and blended for 2 min with 90 mL of phosphate buffer (pH=7) in a homogenizer  
200 (Stomacher<sup>®</sup> 400, Seward Ltd., Worthing, UK). Serial dilutions were prepared using  
201 sterile phosphate buffer. Then, 0.1 mL were plated onto plate count agar (PCA) (Sigma-  
202 Aldrich). Duplicate plates were incubated for 2 days at 35 °C and 10 days at 7 °C to  
203 enumerate mesophilic and psychrophilic aerobic bacteria, respectively. For moulds and  
204 yeasts, 0.1 mL of the dilutions were poured onto potato dextrose agar (PDA) (Sigma-  
205 Aldrich) and incubated for 5 days at 25 °C. After incubation, colonies were counted and  
206 the results were expressed as log<sub>10</sub> cfu per g of persimmon.

207

#### 208 **Populations of inoculated food-borne human pathogens on fresh-cut persimmon**

209 For pathogenic analysis, persimmons were cut into slices and plugs of 1.2 cm of diameter,  
210 1 cm long (weighting approx. 1 g) were prepared using a cork borer to achieve a uniform  
211 inoculation of the samples.<sup>3</sup> Persimmon plugs were inoculated by immersion in the  
212 bacterial inoculum (6 log<sub>10</sub> cfu g<sup>-1</sup>) for 2 min. Once dried, plugs were immersed for 3 min  
213 in the pectin-based edible coating or in water as control, dried in a flow cabinet to avoid

214 contamination of the samples, and packed as described above (active MAP or air  
215 conditions).

216 The concentration of *E. coli*, *S. enteritidis* and *L. monocytogenes* on persimmon  
217 plugs was determined just before (BT) and after (AT) the treatment (i.e. coating or water  
218 dips) and after 4 and 8 days at 5 °C. At each sample time, 10 g of inoculated and treated  
219 plugs were placed into sterile plastic bags and 90 mL of phosphate buffer (pH=7) were  
220 added. The mixture was homogenized in a stomacher blender (Stomacher®400) for 2 min.  
221 Serial dilutions were made using sterile phosphate buffer and 100 µL were then pour  
222 plated onto the corresponding plates. Counts of *E. coli* and *S. enteritidis* were made in  
223 MacConkey's agar after incubating at 37 °C for 24 h and 36 h, respectively. Counts of *L.*  
224 *monocytogenes* were made in tryptic soy agar plus 5 % sheep's blood agar after  
225 incubating for 2-3 days at 37 °C. There were three replicates per treatment for each  
226 pathogen and sampling time, and each assay was also repeated 3 times. The results were  
227 expressed as log<sub>10</sub> cfu per g of persimmon.

228

### 229 **Colour evaluation**

230 Colour (CIELAB parameters  $L^*$ ,  $a^*$ , and  $b^*$ ) was determined with a Minolta CR-400  
231 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan) on 12 pieces of fresh-cut fruit  
232 per treatment. Each measurement was performed randomly at 3 different locations per  
233 sample piece. A standard white calibration plate was employed to calibrate the apparatus.  
234 The results were expressed as the mean of 12 samples per treatment.

235

### 236 **Firmness measurements**

237 The firmness of fresh-cut persimmon was evaluated using an Instron Universal Machine  
238 (Model 3343, Instron Corp., Canton, MA, USA) by measuring the force required for an 8-  
239 mm diameter rod to penetrate the sample to a depth of 2 mm at a speed of 5 mm s<sup>-1</sup>.  
240 Twelve samples per treatment were measured and the results were expressed in newtons  
241 (N).

242

#### 243 **Polyphenol oxidase (PPO) activity**

244 For the enzyme extraction, 15 g of fresh persimmon was blended and mixed with a  
245 McIlvaine buffer solution (1:1) at pH 6.5, containing 1 M sodium chloride and 5%  
246 polyvinylpyrrolidone (Ultraturrax, IKA, Germany). Then, the homogenate was  
247 centrifuged at 12,000 rpm and 4 °C for 30 min. The supernatant was collected to its  
248 activity measurement. Two extractions were done per each replicate.

249 To determine enzyme activity, 3 mL of 0.05 M 4-methylcatechol was added to  
250 100 µL of enzyme extract. The changes in absorbance were determined every 5 s in a  
251 spectrophotometer (UV-1, Thermo Electron Corporation, UK) at 420 nm for up to 2 min  
252 from the time the enzyme extract was added. Three replicates per treatment were  
253 measured. Activity was expressed in absorbance per minute. All the reagents used were  
254 obtained from Sigma-Aldrich (St. Louis, MO, USA).

255

#### 256 **Sensory quality**

257 During storage, persimmon slices were evaluated visually by 15 trained judges. Fruit  
258 from each treatment was presented to the panelists in trays that contained 12 persimmon  
259 pieces to account for sample variability, and labelled with a 3-digit random code. Visual  
260 quality, based on general visual appearance, was determined by using the following visual

261 scale: 9=excellent, just sliced; 7=very good; 5 = good, limit of marketability; 3=fair, limit  
262 of usability; and 1=poor, inedible.<sup>14</sup> A colour photograph of samples rated with this scale  
263 was used by the judges to score the samples.

264 The panellists also evaluated off-flavours, firmness and overall flavour of fresh-  
265 cut ‘Rojo Brillante’ persimmon pieces. Off-flavour was rated on a 5-point scale, where  
266 1=absence and 5=marked presence. Firmness was rated in a 5-point scale, where 1=very  
267 soft and 5=very firm. Overall flavour was rated on a 9-point scale, where 1 to 3  
268 represented a poor quality range, 4 to 6 an acceptable quality range, and 7 to 9 an  
269 excellent quality range. These attributes were evaluated in 2 persimmon slices randomly  
270 selected from each treatment to compensate for the biological variation of the materials.  
271 The samples were presented to the panellists on trays labelled with the 3-digit codes and  
272 served at room temperature (25±1 °C). Spring water was used for palate cleansing  
273 between samples. To avoid discrimination due to colour, samples were illuminated with  
274 appropriate lighting to completely mask browning.

275

## 276 **Statistical analysis**

277 The statistical analysis was performed with the software Statgraphics 5.1 (Statpoint  
278 Technologies Inc., Warrenton, VA, USA). Specific differences among treatments were  
279 determined by the least significant difference (LSD) test when the analysis of variance  
280 (ANOVA) showed significant *P*-value. Significant differences were defined at  $P \leq 0.05$ .

281

## 282 **RESULTS AND DISCUSSION**

### 283 **Headspace gas composition**

284 Figure 1 shows the effect of the pectin-based edible coating on the content of O<sub>2</sub> and CO<sub>2</sub>  
285 inside packages of fresh-cut 'Rojo Brillante' persimmon under air and active MAP  
286 conditions. Samples packed in air maintained O<sub>2</sub> and CO<sub>2</sub> levels close to atmospheric  
287 values and no differences were observed between coated and uncoated samples. In  
288 contrast, in active MAP, the O<sub>2</sub> concentration decreased and reached the equilibrium by  
289 storage day 7, with values of 1 and 2 kPa, and the CO<sub>2</sub> concentration steadily increased  
290 during the 9 days of storage, with values of 6 and 7 kPa for coated and uncoated samples,  
291 respectively ( $P < 0.05$ ). Therefore, the effect of the pectin-based edible coating to reduce  
292 respiration rate of persimmon slices was only observed in samples packed under active  
293 MAP. Several studies have described the effect of polysaccharide-based edible coatings  
294 on reducing the respiration rate of fresh-cut products. For example, a depletion of  
295 respiration was reported in fresh-cut apple and melon dipped in an alginate-based edible  
296 coating when compared to uncoated samples<sup>15,16</sup> and in apple and mango slices coated  
297 with cassava starch.<sup>17</sup>

298

### 299 **Microbial growth in fresh-cut persimmon**

300 Growth of moulds, yeasts and aerobic psychrophilic bacteria was not observed during  
301 storage at 5 °C in all fresh-cut persimmons, including control samples dipped in water  
302 (data not shown). Figure 2 shows the development of mesophilic bacteria on fresh-cut  
303 persimmon slices during cold storage at 5 °C. The antimicrobial pectin-based edible  
304 coating effectively controlled the growth of mesophilic bacteria during storage  
305 independently of the packaging conditions; whereas bacterial growth increased in  
306 uncoated samples. In uncoated samples, no effect of the packaging condition was  
307 observed on storage day 4, with bacteria counts of 3.0 log<sub>10</sub> cfu g<sup>-1</sup>, but on day 8 these

308 values increased to  $4.0 \log_{10} \text{ cfu g}^{-1}$ , while they did not increased in persimmon slices  
309 packed in active MAP ( $P < 0.05$ ). The effect of low  $\text{O}_2$  and high  $\text{CO}_2$  concentrations on the  
310 growth of Gram negative bacteria, moulds, and aerobic microorganisms is well known.  
311 For example, packaging under active and passive MAP significantly inhibited the growth  
312 of spoilage microorganisms in fresh-cut pear, melon, honey pomelo, and mushroom  
313 slices, among others<sup>8,18,19</sup> and reduced the development of aerobic psychrotrophic  
314 bacteria and *Pseudomonas* in leaf spinach.<sup>20</sup> The effectiveness of MAP activity depends  
315 on the type and concentration of the microorganism, as well as on  $\text{O}_2$  and  $\text{CO}_2$   
316 concentrations and ripeness stage of the commodity at processing. Oms-Oliu et al.<sup>21</sup>  
317 observed that active MAP (2.5 kPa  $\text{O}_2$  + 7 kPa  $\text{CO}_2$ ) inhibited bacterial growth, and yeast  
318 and mould proliferation in mature-green pears, but did not control microbial growth in  
319 partially ripe and ripe pears. In our case, the effect of active MAP on mesophilic bacteria  
320 was only observed in uncoated samples after 8 days of storage, when  $\text{CO}_2$  concentration  
321 was close to 7 kPa and microbial population high.

322 In a previous work, a similar pectin-based edible coating amended with 500 IU  
323  $\text{mL}^{-1}$  NI totally inhibited aerobic mesophiles in fresh-cut 'Rojo Brillante' persimmon.<sup>10</sup>  
324 NI has a broad activity spectrum against gram-positive bacteria, but do not significantly  
325 inhibit gram-negative bacteria, yeasts or moulds.<sup>22</sup> Activity of NI, which is known to  
326 destabilize the cytoplasmic membrane of bacteria via an electrostatic interaction when  
327 contact is produced, has been shown to be enhanced at low pH.<sup>23</sup> In our coating  
328 formulation, CA and  $\text{CaCl}_2$  were added as antibrowning and firming agents and conferred  
329 a final pH to the formulation of 2.30. Furthermore, the use of the additives CA or  $\text{CaCl}_2$   
330 alone or incorporated to other edible coatings has also been reported to confer some

331 antimicrobial activity in fresh-cut commodities such as fresh-cut apple and melon, which  
332 was related to their chelating activity.<sup>24,25</sup>

333

#### 334 **Populations of inoculated food-borne human pathogens on fresh-cut persimmon**

335 The effect of the pectin-based edible coating and MAP on the growth of *E. coli*, *S.*  
336 *enteritidis* and *L. monocytogenes* in artificially inoculated persimmon plugs is shown in  
337 Figure 3. The application of the pectin antimicrobial coating significantly reduced the  
338 initial population of *E. coli* and *L. monocytogenes* by 1.5 and 1.0 log<sub>10</sub> units (AT  
339 application), respectively, whereas *S. enteritidis* was reduced by more than 2.0 log<sub>10</sub> units.  
340 The antimicrobial activity of the coating resulted in a further reduction of the population  
341 of the pathogens during storage at 5 °C to achieve a complete inhibition of *E. coli* and *S.*  
342 *enteritidis* in the samples under MAP (Coating-MAP) or air conditions (Coating),  
343 respectively, by the end of the 8-day storage period. The population of *L. monocytogenes*  
344 in coated samples stored in MAP also dropped significantly, being more than 5.0 log<sub>10</sub>  
345 units lower by the end of the storage period, whereas coated samples stored in air  
346 exhibited a slow decline in the population of *L. monocytogenes*, with only 2.0 log<sub>10</sub> units  
347 reduction.

348 These results revealed the potential growth-inhibition effect of NI added to the  
349 pectin-based edible coating in order to reduce populations of these pathogens in fresh-cut  
350 ‘Rojo Brillante’ persimmon. A previous study by our group showed that the application  
351 of a similar coating also reduced pathogen populations in artificially inoculated  
352 persimmon when compared to other antimicrobial food additives.<sup>10</sup> In that experiment,  
353 however, the impact upon the population of *L. monocytogenes* was greater, whereas in the  
354 present study, the coating was more effective on the reduction of *E. coli* and *S. enteritidis*

355 populations for both packaging conditions (active MAP and air). The effectiveness of NI  
356 in inhibiting the growth of Gram-positive bacteria is well-known. For example, NI  
357 inhibited the growth of *L. monocytogenes* in processed mangoes or melons.<sup>26,27</sup> However,  
358 some works have described a resistance of *L. monocytogenes* to NI, which was explained  
359 by a mutation of the bacteria that caused changes in the fatty acid composition of the cell  
360 membrane hindering NI insertion into the membrane.<sup>28-30</sup> Nevertheless, the effect  
361 observed in our study could be due to other factors, since the population of *L.*  
362 *monocytogenes* in coated samples packed in MAP conditions was significantly reduced  
363 after 8 days of storage at low temperature.

364 On the other hand, in the absence of other preservation methods, NI does not  
365 inhibit Gram negative bacteria or yeasts and moulds.<sup>22</sup> Therefore, NI is often used in  
366 combination with other preservation methods such as lowering the pH, addition of high  
367 salt concentrations, or the use of other chelating agents to achieve a bactericidal effect  
368 toward both Gram-positive and Gram-negative bacteria. In these cases, the effect of NI on  
369 Gram-negative bacteria is achieved so long as the outer bacteria cell membrane, which  
370 acts as a shield, is destroyed. For instance, treatments with NI and some chelators such as  
371 EDTA or certain acids, reduced the population of Gram-negative bacteria.<sup>31,32</sup> Therefore,  
372 the low pH of the pectin-based edible coating (pH 2.30), due to the addition of CA, might  
373 have enhanced the effect of NI against the different food-borne pathogens tested.

374

### 375 **Colour and polyphenol oxidase (PPO) activity of fresh-cut persimmon**

376 Figure 4 shows the effect of the pectin-based edible coating and packaging conditions on  
377 hue angle and a\* values of fresh-cut persimmon during storage at 5 °C. Coated  
378 persimmon slices maintained lower a\* and higher hue values than uncoated samples



379 during the 9 days of storage, which indicates the positive effect of the pectin coating to  
380 control enzymatic browning of the samples. In uncoated samples, the use of MAP helped  
381 maintaining lower  $a^*$  and higher hue values of persimmon slices than air conditions  
382 (Control). The application of MAP to the coated samples further reduced initial enzymatic  
383 browning compared to those samples stored in air conditions, as reflected by a decrease in  
384  $a^*$  and an increase in hue values after 2 days of storage at 5 °C. However, the differences  
385 were reduced as storage time at 5 °C increased. In a previous work, the use of active MAP  
386 (5 kPa O<sub>2</sub>) significantly reduced the enzymatic browning of untreated persimmon slices  
387 compared to those packed under passive MAP conditions, whereas packaging conditions  
388 did not affect the colour parameters of antioxidant-treated samples (unpublished data).

389 Several works have reported the effectiveness of polysaccharide coatings to  
390 control enzymatic browning of fresh-cut fruits and vegetables when antioxidants are  
391 incorporated into base formulations. The effect of coatings on browning control greatly  
392 depends on intrinsic factors such the antibrowning substance incorporated and the edible  
393 coating selected. In preliminary work conducted by our group, pectin and hydroxypropyl  
394 methylcellulose-based edible coatings containing CA and CaCl<sub>2</sub> proved more effective to  
395 extend the commercial shelf life of fresh-cut persimmon than soy protein isolate- or whey  
396 protein isolate-based coatings containing the same antibrowning agents (unpublished  
397 data). Other research works have also reported the positive effect of the incorporation of  
398 antioxidants to polysaccharide edible coatings to control enzymatic browning of fresh-cut  
399 fruits. Thus, pectin-, gellan-, and alginate coatings containing N-acetylcysteine and  
400 glutathione as antioxidants were effective in avoiding browning of fresh-cut pears<sup>8</sup> and  
401 the incorporation of ascorbic acid into an alginate-based coating contributed to colour  
402 retention of fresh-cut mango.<sup>33</sup>

403           On the other hand, some attempts have also been focused on extending the shelf  
404 life of fresh-cut commodities by combining both edible coatings and MAP. For example,  
405 the combination of soy protein coatings with antibrowning agents and MAP has been  
406 evaluated by our group on fresh-cut artichoke, eggplant, and persimmon.<sup>13,34,35</sup> MAP  
407 conditions included passive MAP, active conventional MAP (5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>),  
408 and high O<sub>2</sub> MAP (>50 kPa, balanced with N<sub>2</sub>) and they were compared to atmospheric  
409 conditions as control. Coating application in atmospheric packaging conditions provided  
410 the best and cheapest approach for extending the shelf life of fresh-cut eggplants and  
411 artichokes.<sup>34,35</sup> On the contrary, the combination of soy protein coating with active  
412 conventional MAP showed a synergic effect in controlling tissue browning of fresh-cut  
413 ‘Rojo Brillante’ persimmon.<sup>13</sup>

414           The effect of the pectin-based coating to control browning correlated with a lower  
415 PPO activity in coated persimmons compared to uncoated ones (Fig. 5), whereas the use  
416 of active MAP slightly affected the enzyme activity compared to atmospheric conditions.  
417 The effect of the coating on PPO activity can be attributed to the effect of the  
418 antibrowning ingredients (citric acid and CaCl<sub>2</sub>). Carboxylic acids such as citric acid have  
419 been reported to exhibit a double inhibitory effect by chelating copper, a key component  
420 of the PPO activity, and reducing the pH below that necessary for optimal PPO activity.<sup>36</sup>  
421 In persimmon, the optimum PPO activity has been reported to fall within the pH range of  
422 5.5-7.5, depending on the substrate.<sup>37,38</sup> Thus, the low pH of the pectin-based coating (pH  
423 2.3) might be the main factor that contributed to reduce the PPO activity. On the other  
424 hand, although low O<sub>2</sub> MAP has been reported to affect the PPO activity of fresh-cut  
425 commodities, its effect in this work was only observed for uncoated samples after 7 days  
426 of storage.

427

428 **Firmness of fresh-cut persimmon**

429 Firmness of fresh-cut persimmon decreased from an initial value of  $33.3 \pm 1.7$  N to values  
430 close to 15 N after 9 days of storage, except for uncoated samples stored in active MAP,  
431 which maintained firmness values above 20 N (Fig. 6). It is well known the effect of  
432 MAP with high CO<sub>2</sub> and low O<sub>2</sub> on reducing softening during postharvest storage of  
433 fruits and vegetables, which has been attributed to the reduction of either the activity of  
434 cell-wall-degrading enzymes or the metabolic activity of the product.<sup>39</sup> For example, the  
435 application of 4 kPa O<sub>2</sub> + 5 kPa CO<sub>2</sub> delayed firmness loss in fresh-cut pineapple<sup>40</sup>, and  
436 similar atmospheric conditions positively influenced firmness in fresh-cut apples  
437 compared to air conditions.<sup>41</sup> In this work, the application of active MAP only helped to  
438 retain firmness of uncoated samples, whereas coated samples were not benefited by MAP.  
439 The effectiveness of edible coatings on preventing firmness depends on many factors,  
440 such as coating composition, commodity or stage of maturity. Thus, for example, Shon  
441 and Haque<sup>42</sup> reported no effect of calcium caseinate or whey protein-based coatings on  
442 the firmness of fresh-cut apples and potatoes, whereas Lee et al.<sup>43</sup> observed that the  
443 application of whey protein concentrate-based coatings to minimally processed apples  
444 reduced losses of firmness compared to uncoated samples. On the contrary, a  
445 carrageenan-based coating did not improve apple firmness and the addition of citric acid  
446 induced tissue softening. Previous work with fresh-cut 'Rojo Brillante' persimmon  
447 showed that acidic antibrowning agents such as citric or ascorbic acid, although effective  
448 in preventing enzymatic browning, led to major tissue softening and their combination  
449 with CaCl<sub>2</sub> was required to prevent excessive softening and maintain firmness within the  
450 same range than the control samples.<sup>44</sup>

451

452 **Sensory quality of fresh-cut persimmon**

453 The application of the pectin-based edible coating and active MAP maintained the visual  
454 quality of fresh-cut 'Rojo Brillante' persimmon within the limit of marketability during  
455 the 9 days of storage at 5 °C, whereas uncoated samples packaged in air conditions were  
456 scored below this limit after 2 days of storage (Fig. 7). Overall, the judges scored the  
457 samples subjected to the combination of coated and active MAP with a value of 7 (very  
458 good) during the 9 days of storage, whereas samples subjected to each technology  
459 separately were scored as 5 (limit of marketability) by day 5, which indicates the synergic  
460 effect of both treatments to extend the shelf-life of 'Rojo Brillante' persimmon during  
461 storage at 5 °C.

462 The combination of coating and MAP induced a slight off-flavour (scored as 2) to  
463 persimmon slices after 7 days of storage (Table 1). Despite of this, at the end of the 9-day  
464 storage period all the persimmon slices were evaluated with an overall flavour within the  
465 limit of acceptability (5.6-7.0 range). Coated persimmon slices were evaluated as less  
466 firm than uncoated samples at the end of storage, which confirmed the results from the  
467 instrumental texture analysis (Table 1; Fig. 6). However, the judges were not able to  
468 differentiate sensory firmness in uncoated samples subjected to different packaging  
469 conditions.

470

471 **CONCLUSION**

472 The application of a pectin-based coating formulated with antibrowning agents and NI as  
473 antimicrobial significantly extended the shelf-life of 'Rojo Brillante' persimmon slices by  
474 controlling enzymatic browning and reducing the growth of total aerobic mesophilic

475 bacteria during storage at 5 °C. Overall, the combination of the edible coating and active  
476 MAP (5 kPa O<sub>2</sub>) proved to be the most effective treatment to maintain the visual quality  
477 of persimmon slices, being evaluated as very good at the end of the 9-day storage period,  
478 while the overall flavour fell within the limit of acceptability. The antimicrobial pectin  
479 coating also effectively stunted the growth of *E. coli*, *S. enteritidis* and *L. monocytogenes*  
480 in artificially inoculated fresh-cut persimmon.

481

## 482 **ACKNOWLEDGEMENTS**

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488

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625

626 **Table 1.** Sensory quality of uncoated or pectin-based coated fresh-cut ‘Rojo Brillante’  
 627 persimmon packed in air or active modified atmosphere packaging (MAP; 5 kPa O<sub>2</sub>, balance  
 628 N<sub>2</sub>) during 9 days at 5 °C.

Treatment		Days of storage			
		2	5	7	9
Off-flavour	Coating - MAP	1.6 ± 0.3a	1.6 ± 0.2a	2.1 ± 0.3a	2.1 ± 0.3a
	MAP	1.2 ± 0.1a	1.5 ± 0.3a	1.1 ± 0.1b	1.1 ± 0.1b
	Coating	1.6 ± 0.2a	1.5 ± 0.2a	1.3 ± 0.1b	1.3 ± 0.1b
	Control	1.5 ± 0.3a	1.2 ± 0.1a	1.3 ± 0.2b	1.3 ± 0.2b
Flavour	Coating - MAP	6.5 ± 0.4a	6.3 ± 0.4a	5.0 ± 0.3b	5.6 ± 0.5b
	MAP	6.6 ± 0.4a	7.1 ± 0.3a	6.9 ± 0.2a	6.9 ± 0.3a
	Coating	6.4 ± 0.4a	6.6 ± 0.3a	6.5 ± 0.2a	6.0 ± 0.4ab
	Control	6.9 ± 0.4a	6.7 ± 0.4a	6.7 ± 0.4a	7.0 ± 0.3a
Firmness	Coating - MAP	2.9 ± 0.3b	2.8 ± 0.2b	2.6 ± 0.2b	2.7 ± 0.2b
	MAP	3.8 ± 0.2a	3.8 ± 0.2a	3.4 ± 0.2a	3.3 ± 0.3a
	Coating	3.2 ± 0.2ab	3.5 ± 0.2ab	3.2 ± 0.2ab	2.5 ± 0.2b
	Control	3.1 ± 0.3ab	4.0 ± 0.2a	3.6 ± 0.2a	3.3 ± 0.2a

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Sensory quality of persimmon fruit at processing: Off-flavour = 1.0 ± 0.0; Flavour = 7.8 ± 0.2; Firmness = 4.1 ± 0.2.  
 For each parameter and storage time, different letters indicate significant differences among treatments by the least significant difference (LSD) test ( $P \leq 0.05$ ).  
 Data are mean ± standard error.

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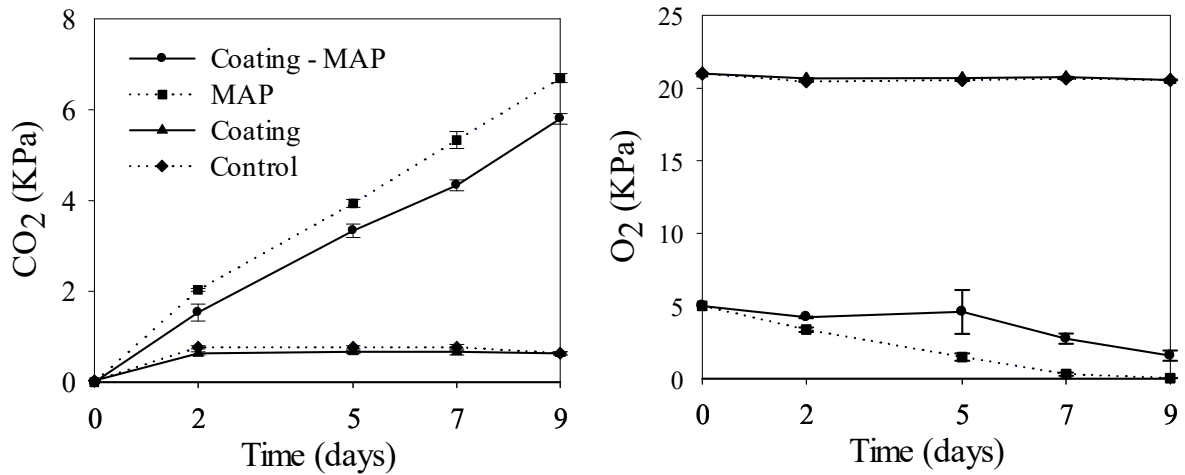
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648 **Figure 1.** Concentration of CO<sub>2</sub> and O<sub>2</sub> in the headspace gas composition of uncoated and  
649 pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or active modified  
650 atmosphere packaging (MAP; 5 kPa O<sub>2</sub>, balance N<sub>2</sub>) and stored for 9 days at 5 °C.  
651 Vertical bars represent the standard error.

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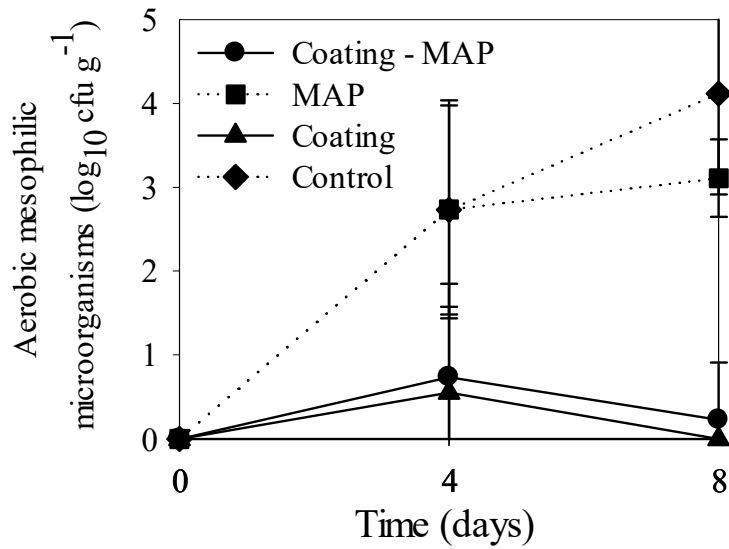
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**Figure 2.** Growth of aerobic mesophilic bacteria in uncoated and pectin-based coated fresh-cut ‘Rojo Brillante’ persimmon packed in air or active modified atmosphere packaging (MAP; 5 kPa O<sub>2</sub>, balance N<sub>2</sub>) and stored for 8 days at 5°C. Vertical bars show the standard error.

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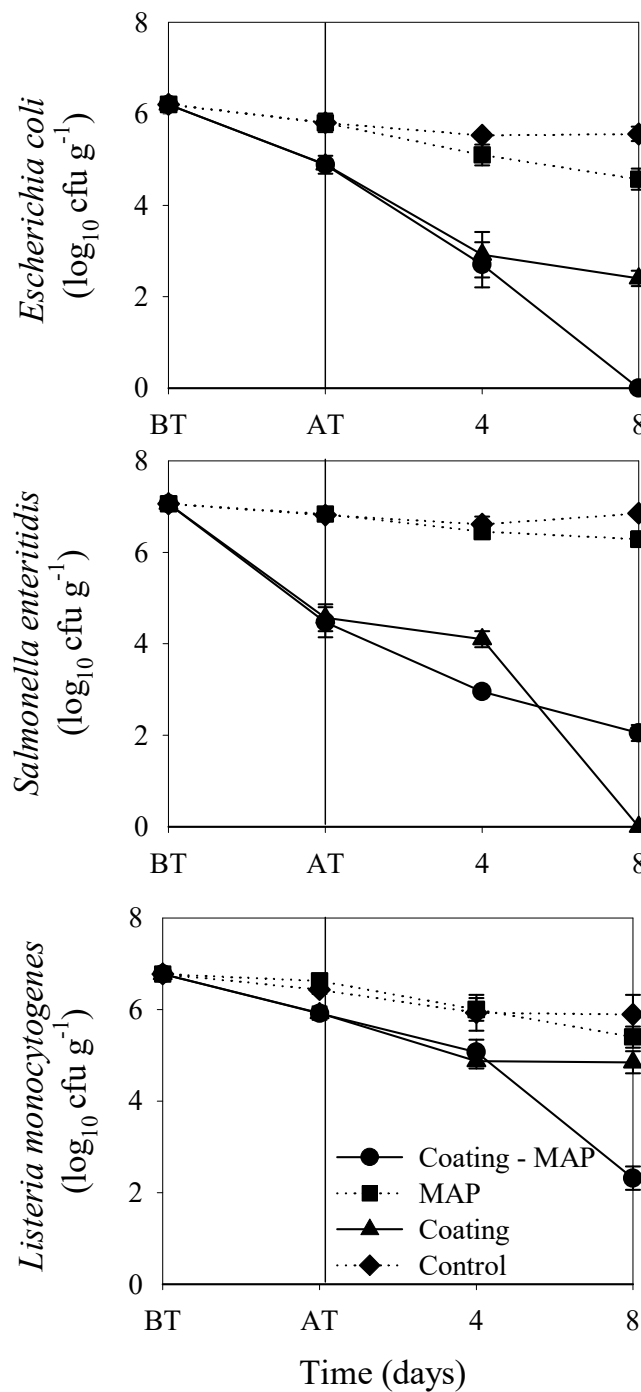
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691 **Figure 3.** Populations of *Escherichia coli*, *Salmonella enteritidis* and *Listeria*

692 *monocytogenes* in uncoated and pectin-based coated minimally processed ‘Rojo

693 Brillante’ persimmon plugs before (BT) and after treatment (AT) (coating or water dips),

694 and after 4 and 8 days of storage at 5 °C in air or active modified atmosphere packaging

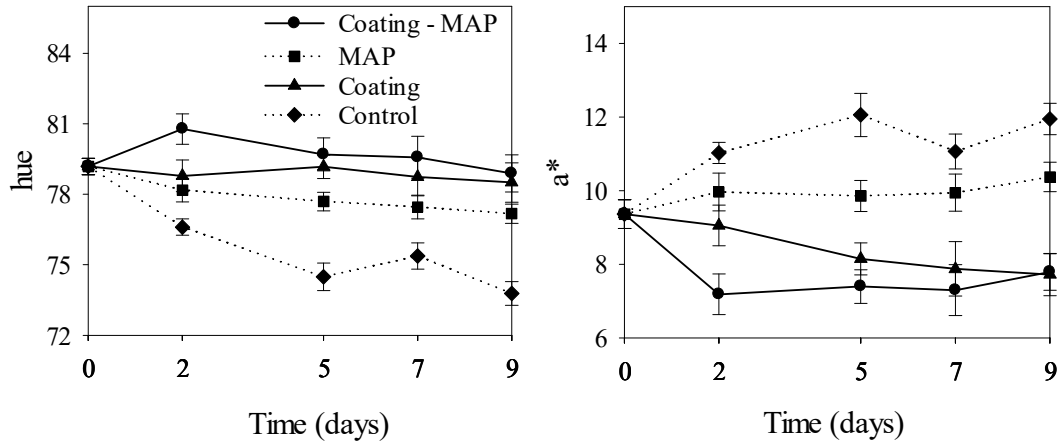
695 (MAP; 5 kPa O<sub>2</sub>, balance N<sub>2</sub>). Vertical bars show the standard error.

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701 **Figure 4.** Flesh color hue and  $a^*$  values of uncoated and pectin-based coated fresh-cut

702 'Rojo Brillante' persimmon packed in air or modified atmosphere packaging (MAP; 5

703 kPa  $O_2$ , balance  $N_2$ ) and stored for 9 days at 5 °C. Vertical bars show the standard error.

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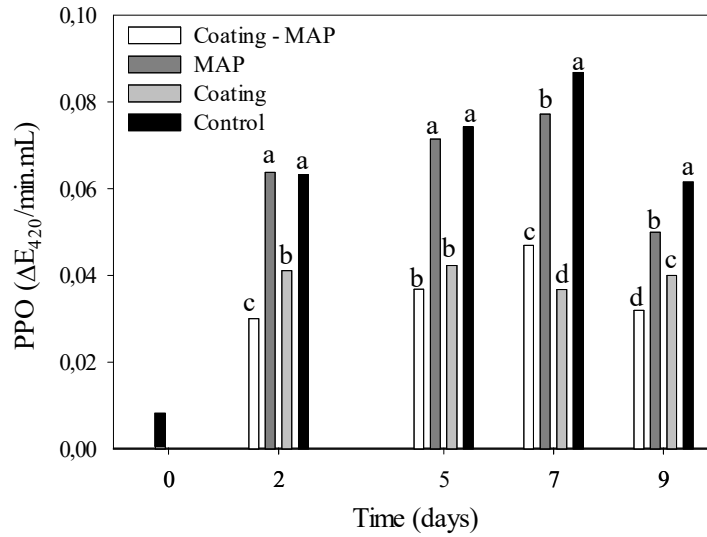
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719 **Figure 5.** Polyphenol oxidase (PPO) of uncoated and pectin-based coated fresh-cut ‘Rojo

720 Brillante’ persimmon packed in air or modified atmosphere packaging (MAP; 5 kPa O<sub>2</sub>,

721 balance N<sub>2</sub>) and stored for 9 days at 5 °C. For each storage time, bars with a different

722 letter are significantly different at the 95% level.

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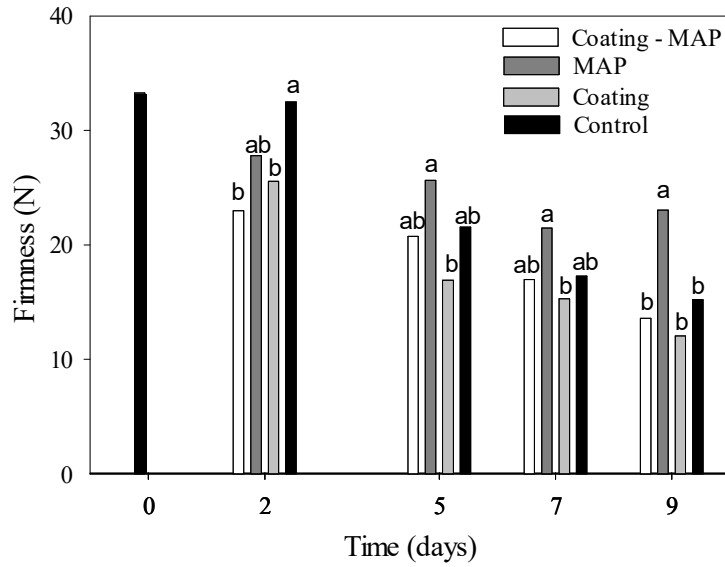
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**Figure 6.** Firmness of uncoated and pectin-based coated fresh-cut ‘Rojo Brillante’ persimmon packed in air or modified atmosphere packaging (MAP; 5 kPa O<sub>2</sub>, balance N<sub>2</sub>) and stored for 9 days at 5 °C. For each storage time, bars with a different letter are significantly different at the 95% level.

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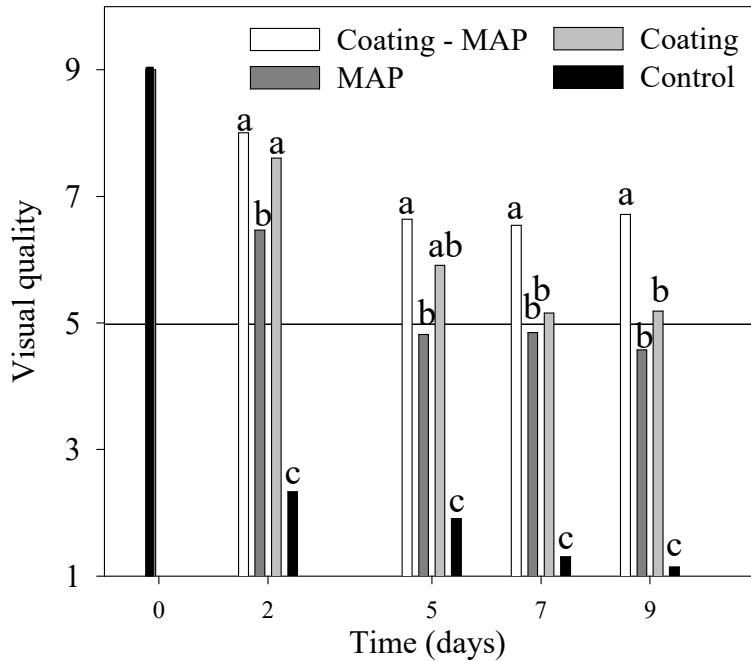
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**Figure 7.** Visual quality of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmons packed in air or modified atmosphere packaging (MAP; 5 kPa O<sub>2</sub>, balance N<sub>2</sub>) and stored for 9 days at 5 °C. Visual scale: 9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible. For each storage time, bars with a different letter are significantly different at the 95% level.