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Additional Information

Impact of high hydrostatic pressure and pasteurization on the structure and the extractability of bioactive compounds of persimmon 'Rojo Brillante'

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Keywords:	microstructure, Persimmon, bioactive compounds, high pressure processing, pasteurization

For Review

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Journal of Food Science Editor-in-chief

Dear Sir/Madam,

I am sending to you the revised manuscript **JFDS 2013-1245-R1** 'Impact of high hydrostatic pressure and pasteurization on the structure and the extractability of bioactive compounds of persimmon 'Rojo Brillante' (M. Hernández-Carrión, J.L. Vázquez-Gutiérrez, I. Hernando, A. Quiles), together with the changes suggested by the reviewer, as well as the responses to the reviewers.

For Peer Review

Yours sincerely,

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Reviewers' Comments to Author:
Reviewer: 1
Comments to the Author
This is a well-written manuscript that will be of interest to scientists and technologists working in high-pressure processing of foods. The excellent microscopy is relevant to fruits and vegetables in general, not just to persimmons. I would like to see the figures giving compositional, antioxidant, color and texture measurements that is presented as bar charts replaced with tables. Scientists may want to compare the carotenoid or tannin data, for example, in this paper with other worker's results. It is impossible to extrapolate three significant figures from these bar charts. The figures do not clarify or make the data more understandable. A table (or tables) can present the information much more efficiently.

The figures 4, 5, 6, and 7 have been replaced by tables 1, 2, 3, and 4, respectively in order to make the data more understable.

Suggestions on some minor issues:

Pg 2, l 38– Replace "Nowadays, the consumer…" with "Todays' consumer demands…"

This change has been made in the revised text. (Line 38)

Pg 4, l 77. Give citations of at least 2 review articles after "…have been reviewed extensively."

Several review articles have been included in the revised text and the corresponding references have been included in the reference section. (Lines 78-79; 455-457; 471-476)

Pg 4, l 95 "Fruit was not treated for astringency". Suggest you give a brief explanation or description of what treatment for astringency encompasses.

This type of persimmon is mainly consumed in fresh. As it has astringency characteristics, it has to be treated for its consumption with carbon dioxide. Carbon dioxide precipitates the tannins and so eliminates astringency. The authors also want to check if HHP favors the precipitation of tannins. This is why fruit was no treated for astringency. If the reviewer considers it necessary, this explanation can be included in the manuscript.

Pg 5, l 101. Replace "Other third of the bags" with "Another third of the bags."…

This change has been made in the revised text. (Line 103)

Pg 5, l 03, Replace "The last third…" with "The final third of the bags…"

This change has been made in the revised text. (Line105)

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1 Food Chemistry

2 **Impact of high hydrostatic pressure and pasteurization on the structure and the**
3 **extractability of bioactive compounds of persimmon 'Rojo Brillante'**

4

5 **M. Hernández-Carrión*, J. L. Vázquez-Gutiérrez, I. Hernando, A. Quiles**

6

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14

15 Word count of the body text: 5052

16 Short version of title: Persimmon bioactive and structural study…

17

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18 **Abstract**

19 Rojo Brillante is an astringent oriental persimmon variety with high levels of bioactive
20 compounds such as soluble tannins, carotenoids, phenolic acids and dietary fiber. The
21 purpose of this study was to investigate the effects of high hydrostatic pressure (HHP)
22 and pasteurization on the structure of the fruit and on the extractability of certain
23 bioactive compounds. The microstructure was studied using Light Microscopy,
24 Transmission Electron Microscopy and Low Temperature Scanning Electron
25 Microscopy and certain physicochemical properties (carotenoid and total soluble tannin
26 content, antioxidant activity, fiber content, color and texture properties) were measured.
27 The structural changes induced by HHP caused a rise in solute circulation in the
28 tissues that could be responsible for the increased carotenoid level and the unchanged
29 antioxidant activity in comparison with the untreated persimmon. In contrast, the
30 changes that took place during pasteurization lowered the tannin content and
31 antioxidant activity. Consequently, HHP treatment could improve the extraction of
32 potentially bioactive compounds from persimmons. A high nutritional value ingredient to
33 be used when formulating new functional foods could be obtained using HHP.
34 **Keywords:** microstructure, persimmon, bioactive compounds, high hydrostatic
35 pressure, pasteurization.
36

37 **Practical application**

38 ~~Nowadays, the~~Todays' consumer demands foods rich in bioactive compounds which
39 have beneficial health effects. In this sense, persimmons are among the fruits with the
40 highest levels of bioactive antioxidant compounds such as carotenoids and
41 polyphenols such as tannins. High hydrostatic pressure (HHP) processing is
42 considered one of the most economically viable of the non-thermal technologies and
43 could help to obtain persimmons with high nutritional and quality parameters. HHP
44 causes structural changes in the persimmon tissue and increases the extractability of

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45 some bioactive compounds. Consequently, food industry could use HHP treatment to
46 obtain persimmon extracts for formulating new functional foods.
47

48 **Introduction**

49 Oriental persimmons or kakis (*Diospyros kaki* L. f.) are among the fruits with the
50 highest levels of bioactive compounds (Jung and others 2005). They contain vitamins
51 and minerals, particularly provitamin A (β-carotene), vitamin C and potassium (Wright
52 and Kader 1997; De Ancos and others 2000). As well as β carotene, they contain other
53 carotenoid compounds with considerable antioxidant activity. They also have high
54 phenolic acid and dietary fiber contents (Gorinstein and others 2001) and large
55 quantities of tannin, an antioxidant that is responsible for the fruit's astringency. The
56 Rojo Brillante variety, specifically, is an astringent type persimmon (Tárrega and others
57 2012). This variety has a high soluble tannin content that gradually falls as the fruit
58 ripens.
59 The greater or lesser extent to which the bioactive compounds in fruit and vegetables
60 are accessible in the digestive tract depends on many factors, including the variety,
61 stage of ripeness, structure of the plant matrix, interaction with other components of the
62 plant matrix and how the food has been processed (Parada and Aguilera 2007).
63 Previous studies have shown a significant increase in carotenoid extraction from
64 persimmons and persimmon puree subjected to high pressure treatment (De Ancos
65 and others 2000; Plaza and others 2012), which could indicate that this non thermal
66 treatment could favor the extractability of bioactive compounds when the food is
67 ingested. High hydrostatic pressure (HHP) treatments also seem to increase the
68 bioavailability of vitamins and other low molecular weight compounds in orange juice
69 and *gazpacho* [a cold tomato soup] (Oey and others 2008b).
70 The main aim of HHP processing is to obtain healthy and suitable foods of high
71 sensory quality. HHP facilitate the production of food products that have the quality of

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fresh foods but the convenience and profitability associated with shelf life extension (McClements and others 2001). HPP can be applied to a range of different foods, including juices and beverages, fruits and vegetables, meat-based products, fish and pre-cooked dishes, with meat and vegetables being the most popular applications (Norton and Sun 2008). The potential and limitations of processing foods with HHP have been reviewed extensively (Hendrickx and others 1998; Oey and others 2008a; 2008b). Most of the studies of this method have focused on its microbe and enzyme inactivating effects. The effects of this technology on nutritional and bioactive compounds and on the microstructure of the food have received less attention. To understand the bioavailability of certain nutritional components of foods such as carotenoids, it is essential to characterize the microstructure of plant tissues and the changes that take place during their industrial processing.

The aim of the present study was to compare the effects of an emerging non thermal treatment such as HHP and of a conventional thermal treatment on the structure of persimmons and the extractability of certain bioactive compounds. In this way, improving their nutritional properties, it would be possible to make use of astringent persimmon varieties in functional food formulations.

Materials and methods

Sample preparation

Persimmon fruits cv. Rojo Brillante“ were harvested in Carlet (Valencia, Spain) at the beginning of November of 2011. The maturity index was selected following the method of Salvador et al. (2007) where six maturity stages are accordingly defined, ranging from I (yellow green) to VI (orange red). Stage IV of this scale was studied in this work. Fruit was not treated for astringency. Cubes (15 mm) were taken from the equatorial area and heat-sealed in 110 x 220 mm plastic bags (Doypack type®, Amcor, Spain). Each bag contained approximately 80 g of sample. One third of the bags was placed

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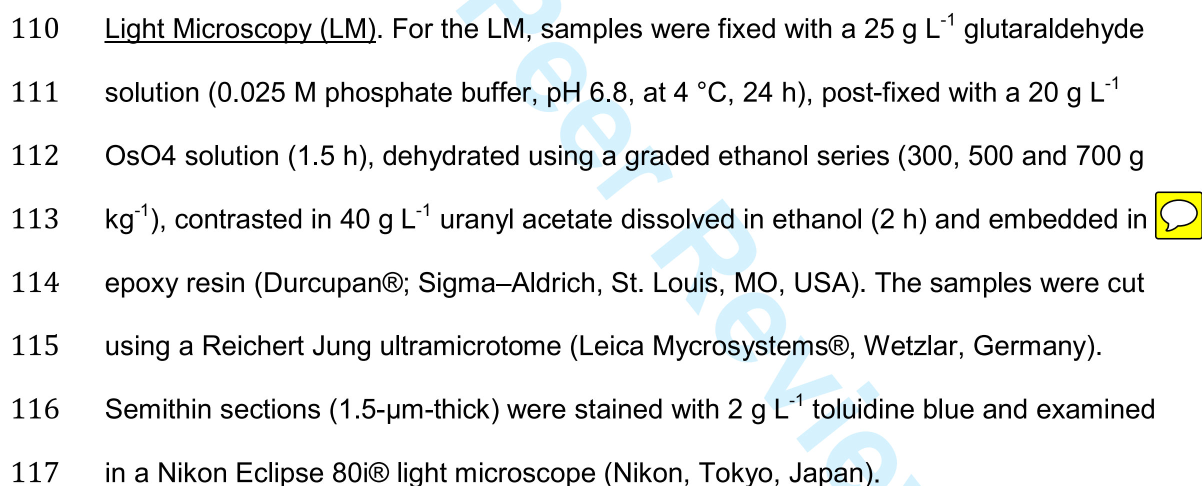
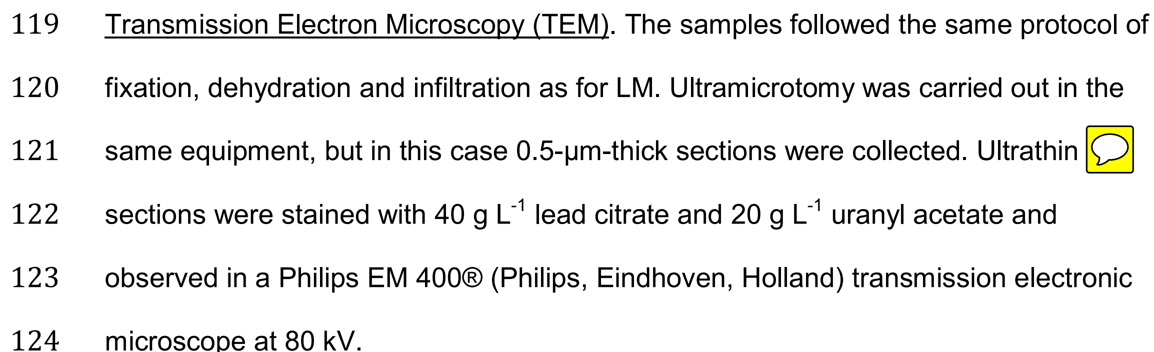
inside a hydrostatic pressure unit (HHP-treated samples) with a 2350 mL capacity and water was used as the pressure medium (GEC Alsthom ACB 900 HP®, type ACIP 665, Nantes, France). The pressure employed in the treatment was 200 MPa during 6 minutes at 25 ºC, based on previous studies (Plaza and others 2012). ~~Other~~ Another third of the bags was submitted to a pasteurization process (pasteurized samples) in a water bath at 70 ºC during 15 min. The ~~last~~ final third of the bags was not submitted to treatment (untreated samples). Then, the bags were stored at 4 ºC until their analysis. Microstructure, color, and texture properties were analyzed within 24 h after the treatment.

Microstructural analysis

Light Microscopy (LM). For the LM, samples were fixed with a 25 g L^{-1} glutaraldehyde solution (0.025 M phosphate buffer, pH 6.8, at 4 °C, 24 h), post-fixed with a 20 g L^{-1} OsO4 solution (1.5 h), dehydrated using a graded ethanol series (300, 500 and 700 g kg^{-1}), contrasted in 40 g L^{-1} uranyl acetate dissolved in ethanol (2 h) and embedded in epoxy resin (Durcupan®; Sigma–Aldrich, St. Louis, MO, USA). The samples were cut using a Reichert Jung ultramicrotome (Leica Mycrosystems®, Wetzlar, Germany). Semithin sections (1.5-μm-thick) were stained with 2 g L^{-1} toluidine blue and examined in a Nikon Eclipse 80i® light microscope (Nikon, Tokyo, Japan).



Transmission Electron Microscopy (TEM). The samples followed the same protocol of fixation, dehydration and infiltration as for LM. Ultramicrotomy was carried out in the same equipment, but in this case 0.5-μm-thick sections were collected. Ultrathin sections were stained with 40 g L^{-1} lead citrate and 20 g L^{-1} uranyl acetate and observed in a Philips EM 400® (Philips, Eindhoven, Holland) transmission electronic microscope at 80 kV.



Low Temperature Scanning Electron Microscopy (CryoSEM). A JSM5410® SEM

127 microscope (JEOL, Tokyo, Japan) was used with a Cryo CT500 C® unit (Oxford
128 Instruments,Witney,UK) for the CryoSEM observation. Samples (1-mm-thick) were
129 placed in the holder, fixed with nitrogen slush (T≤−210 °C), transferred frozen to the
130 Cryo unit, fractured, etched (−90 °C), and gold-coated (10^{-2} bar and 40 mA). Samples
131 were then transferred to the microscope and examined at 15 kV, −130 °C, and at a
132 working distance of 15 mm.
133
134 **Image analysis**
135 The image analysis was carried out using ImageJ software (Rasband, W.S., ImageJ v.
136 1.43s, National Institute of Health, Bethesda, Maryland, USA). The area of the cells
137 was determined using LM images, while the thickness of the cell walls was determined
138 using TEM images. Both, area and thickness were assessed from at least 6 randomly
139 acquired LM and TEM images, respectively. The cells and cells walls were manually
140 labeled and their area (μm^2) and thickness (μm) measured from each image.
141
142 **Physicochemical analysis**
143 Persimmon purée preparation. 120 grams of cubes of persimmon were homogenized
144 during 90 s. The persimmon purée was then stored in hermetically sealed glass jars at
145 -40 °C in a deep freezer until further analysis, and it was thawed at room temperature
146 to determine the bioactive compounds content.
147
148 Extraction and quantification of carotenoids. Total carotenoids were extracted
149 according to Hornero-Méndez and Mínguez-Mosquera (2001) with modifications.
150 Persimmon purée (5 g) was extracted five times with 25 mL cool acetone using an
151 Ultraturrax® (IKA Ultraturrax T25 Basic) and vacuum filtered, until no more color was
152 extracted. The extract was added gradually over 50 mL ethyl ether contained in a
153 decanting funnel. With each addition of extract, enough NaCl solution (100 g L^{-1}) was
154 added to separate the phases and to transfer the pigments to the ether, and the

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155 aqueous phase was removed. The process was carried out in several steps to ensure
156 the highest elimination of aqueous phase. The organic phase was treated several times
157 with anhydrous Na_2SO_4 (20 g L^{-1}) to remove residual water and evaporated to dryness
158 in a rotary evaporator (model RII; Büchi Labortechnik, Flawil, Switzerland) at a
159 temperature lower than 35 ºC. Finally, the pigments were collected with acetone to a
160 volume of 100 mL and the absorbance was measured at 450 nm using a
161 spectrophotometer (model Helios Zeta UV Visible; Thermo Fisher Scientific Inc.,
162 Cambridge, UK). The calibration curve was performed with different concentrations of
163 β–carotene in acetone. Results were expressed as mg β carotene/100 g of fresh
164 weight. Carotenoid extractions were made three separate times and measurements
165 were performed in triplicate.
166
167 Total soluble tannin content. Total soluble tannin content of the samples was
168 determined with a spectrophotometer (Helios Zeta UV Visible) using the Folin Denis
169 colorimetric method as described by Arnal and Del Río (2004). Persimmon purée (5 g)
170 was homogenized in an Ultraturrax with 25 mL of 800 g kg^{-1} methanol. Homogenates
171 were centrifuged (14500 rpm, 20 min, 4 ºC) and filtered. The supernatant was kept.
172 More supernatant was extracted from the pellet with 25 mL of 800 g kg^{-1} methanol and
173 added to the first supernatant. The total supernatant was brought to 100 mL with 800 g
174 kg^{-1} methanol. In a test tube, 1 mL of the extract and 6 mL distilled water were mixed
175 and vortexed. Thereafter, 0.5 mL of Folin Ciocalteu reagent was added. After 3 min, 1
176 mL saturated Na_2CO_3 was added, vortexed, and 1.5 mL distilled water was added.
177 Absorbance was measured after 90 min at 725 nm. The calibration curve was
178 performed with different concentrations of gallic acid in 800 g kg^{-1} methanol. Results
179 were expressed as g gallic acid/100 g of fresh weight. Total soluble tannin extractions
180 were made three separate times and measurements were performed in duplicate.
181

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182 Antioxidant activity. Antioxidant activity was measured by ferric reducing antioxidant
183 power assay (FRAP). Extracts were obtained in the same way as for total soluble
184 tannin content determination but using 960 g kg^{-1} ethanol. Distilled water (30 μL),
185 sample (30 μL) and FRAP reagent (900 μL) were placed in each cuvette. Cuvettes
186 were incubated during 30 min in a water bath at 37 ºC and the absorbance was
187 measured at 595 nm. The calibrated curve was performed using different
188 concentrations of Trolox in 960 g kg^{-1} ethanol. Results were expressed as μmol
189 Trolox/g of sample. Extracts were made three separate times and measurements were
190 performed in triplicate.
191
192 Total and insoluble dietary fiber. Total dietary fiber (TDF) and insoluble dietary fiber
193 (IDF) were determined according to AOAC official method 991.43 (AOAC 1992) using
194 Fibertec E system® (model TM1023, Foss Analytical AB, Höganäs, Sweden). For this
195 purpose, 1 g lyophilized sample was used. Duplicate samples underwent sequential
196 enzymatic digestion by heat stable α amylase, protease, and amyloglycosidase to
197 remove starch and protein. For TDF, enzyme digestate was treated with ethanol to
198 precipitate soluble dietary fiber before filtering, and TDF residue was washed with
199 ethanol, dried and weighed. For IDF, enzyme digestate was filtered, and residue (IDF)
200 was washed with warm water, dried and weighed. TDF and IDF residue values were
201 corrected for protein, ash, and blank. Results were expressed as g/100 g of dry weight.
202
203 Color. The measurements were carried out with a Chroma meter CR400® (Konica
204 Minolta Sensing Americas, Inc., Ramsey, NJ). The results were expressed in
205 accordance with the CIELAB system with reference to illuminant C and a visual angle
206 of 2º. The colorimeter was calibrated with a white standard pattern (Y = 92.9; x =
207 0.3137; y = 0.3198). The parameters determined were: lightness (L*), a* (green red
208 hue) and b* (blue yellow hue). Hue (h_{ab}) and chroma (C_{ab}^*) were determined using

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209 equations 1 and 2, respectively.

210 $$h_{ab} = \arctan (b^*/a^*) \quad (1)$$

211

212 $$C_{ab}^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

213

214 Texture properties. Flesh firmness, cohesiveness and shear force were determined at
215 room temperature with a TA.XTplus Texture Analyzer® (Stable Micro Systems). Flesh
216 firmness was expressed as the load in newtons (N) required breaking the flesh of the
217 persimmon cubes with a 4 mm diameter flat tipped cylindrical probe at 1 mm s^{-1} test
218 speed. A texture profile analysis was performed to determine cohesiveness.
219 Cohesiveness was calculated as the ratio of the area under the second curve to the
220 area under the first curve. The samples were axially compressed in two consecutive
221 cycles at 1 mm s^{-1} test speed and 75% compression, three seconds apart, with a 50
222 mm diameter flat plunger. Shear force was determined as the load in newtons (N)
223 needed to cut the persimmon cubes with a knife blade at 1 mm s^{-1} test speed.
224 Firmness, cohesiveness and shear force values were an average of the measurements
225 from ten cubes.

226

227 **Statistical analysis**

228 Data was subjected to variance analysis (ANOVA), using the least significant difference
229 (LSD) test with a 95% confidence interval for the comparison of the test averages
230 (Statgraphics Plus 5.1, Manugistics, Inc., Rockville, MA, USA).

231

232 **Results and discussion**

233 **Microstructural study**

234 The parenchymal tissue of untreated Rojo Brillante persimmons is made up of turgid
235 cells with a rounded appearance measuring 21792.9 ± 6270.2 μm^2 in close contact

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236 with each other. The tissue contains intercellular spaces, mostly triangular (Figure 1A).
237 The cell walls, approximately 0.700 ± 0.026 μm thick, stained uniformly (Figure 1D) and
238 well bundled cellulose fibrils (Figure 1E) and an unbroken continuous middle lamella
239 (Figure1B) can be seen. The cell membranes (plasmalemma and tonoplast) remain
240 close to the cell wall in most of the cells (Figure 1B, 1E). A dense eutectic artefact can
241 be seen in the parenchymal cell interiors, indicating high soluble matter content (Figure
242 1C, 1F). Precipitated solutes can be observed in some cells (Figure 1A). These are
243 probably tannins which were beginning to turn insoluble, a natural effect of ripening in
244 this fruit. The presence of tannin cells can also be seen with CryoSEM (Figure 1C).
245 Most of the intercellular spaces appear to be empty although solutes can be seen in
246 some, generally in larger spaces than the triangular ones (Figure 1C, 1D, 1F). These
247 persimmons appear to possess an active apoplastic pathway.
248 Treating persimmons with high hydrostatic pressures (HHP) causes structural
249 modifications. In general, the parenchymal tissues of the persimmons subjected to
250 HHP treatment display a more compact structure containing little air (Figure 2C). The
251 cells have a mean surface area of 22110.977 ± 5723.972 μm^2, their perimeters are
252 deformed and they are spaced further apart from each other than in the untreated
253 persimmon, so large intercellular spaces can be seen (Figure 2A). The cell walls are
254 approximately 0.604 ± 0.026 μm thick, their cellulose fibrils present less bundling
255 (Figure 2B) than in the untreated persimmon and their middle lamella is thicker and
256 has broken down in some areas (Figure 2E). Breakdown of the cellulose 'cements'
257 encourages the walls of adjoining cells to separate (Figure 2A, 2B). Despite the HHP
258 treatment, the cell membranes have remained intact and are still close to the cell wall
259 in many areas (Figure 2B, 2E). Eutectic artefacts indicating the presence of solutes can
260 be observed both in the interior of the cells and in practically all the intercellular spaces
261 (Figure 2C, 2D). The tannin cells appear to be filled with a compact mass of insoluble
262 matter (Figure 2C) indicating that HHP could encourage tannin precipitation and,

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therefore, tannin cell formation. The small triangular air filled spaces that predominated in the untreated persimmon have disappeared with the HHP treatment, giving rise to large solute filled intercellular spaces (Figure 2D, 2F). The structural changes brought about by HHP treatment favor solute movement at cell level, probably using the apoplastic, symplastic and transmembrane transport routes, which could influence the extractability of some bioactive compounds by encouraging their diffusion from the interior to the exterior of the cell.

Pasteurizing the persimmons also gave rise to changes in the parenchymal tissue microstructure in comparison with untreated persimmons and ones subjected to HHP. The cells are smaller, with surface areas of 12545.163 ± 2863.148 μm^2, and the cell walls are more deformed (Figure 3A) than those of the untreated persimmons and those subjected to HHP. Adjoining cells have drawn apart from each other and the parenchyma presents large intercellular spaces (Figure 3D). The cell walls are approximately 0.511 ± 0.021 μm thick (Figure 3B, 3E), thinner than those of the untreated and HHP-treated fruit. The cell walls are generally more faintly stained (Figure 3A) and show a certain loss of fibril bundling, and the middle lamella has broken down in some areas (Figure 3E). Although intact, the cell membranes have drawn away from the cell wall and towards the middle of the cell (Figure 3B, 3E). In the pasteurized persimmon parenchyma, the eutectic artefact is mainly located in the cell interior (Figure 3C), most of the large intercellular space appear empty, as in the untreated persimmon (Figure 3F), and groups of tannin cells can be seen. As HHP treatment, pasteurization also would seem to favor tannin precipitation and tannin cell formation (Figure 3A, 3C).

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Carotenoid content measurement

~~Figure 4A~~Table 1 shows the mean carotenoid contents of the three types of persimmon studied (untreated, HHP-treated and pasteurized). It can be seen that the treated persimmons (HHP and pasteurization) had a significantly higher carotenoid content

291 than the untreated fruit (P < 0.05). Of the two treatments studied, the rise in the
292 carotenoid content was more significant with HHP (P < 0.05). Plaza et al. (2012)
293 obtained similar results on studying the influence of HHP treatment on the carotenoid
294 content of persimmons. They showed that applying a HHP treatment at 200 MPa for 1,
295 3 or 6 minutes induced a significant increase in the total carotenoid content (P < 0.05).
296 Of the three treatments they tested, 200 MPa for 6 minutes gave the highest level of
297 carotenoid compound extraction.
298

299 **Total soluble tannin content measurement**

300 No statistically significant differences (P > 0.05) were observed between the mean total
301 soluble tannin content (~~Figure 4B~~Table 1) of the HHP-treated persimmon and
302 pasteurized fruit, whereas the tannin content of the untreated sample was significantly
303 higher (P < 0.05). The lower soluble tannin content of the HHP-treated and pasteurized
304 persimmon could be due to the tannin insolubilization (tannin precipitation and tannin
305 cell formation) already observed in the microstructural study (Figures 2, 3), which could
306 be related to the loss of astringency. These results are in agreement with previous
307 studies (Vázquez-Gutiérrez and others 2011) that established that the application of
308 HHP provoked the precipitation of soluble tannins in 'Rojo Brillante' persimmons which
309 could be related with the lower soluble tannin content detected in those samples.
310

311 **Antioxidant activity measurement**

312 The mean antioxidant activity values of the three types of persimmon analyzed are
313 shown in ~~Figure 4C~~Table 1. No statistically significant differences in antioxidant activity
314 (P > 0.05) were found between the untreated persimmon and the fruit subjected to
315 HHP. However, the thermal treatment led to a significant fall (P < 0.05) in the
316 antioxidant activity of the pasteurized persimmons. Several researchers (Butz and
317 others 2002; 2003) have studied the influence of HHP on the antioxidant activity of very
318 different foods without finding any statistically significant differences (P > 0.05)

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319 between the controls and the samples treated with HHP. Other authors (Fernández-
320 Garcia and others 2001; Sanchez-Moreno and others 2005) established that for short
321 treatment times (500 and 800 MPa/20 ºC/5 min or 400 MPa/40 ºC/1 min), no changes
322 in antioxidant activity of orange juice and tomato puree were found after HHP
323 treatments. The reduction in the antioxidant activity of the pasteurized persimmons
324 could be related to the lower soluble tannin content of these samples and the
325 degradation of other antioxidant compounds caused by thermal processing (Oey and
326 others 2008b). The HHP samples maintain a similar antioxidant activity to the untreated
327 ones due to their high carotenoid content.

328

329 **Total and insoluble dietary fiber content measurement**

330 The results for total dietary fiber (TDF) and insoluble dietary fiber (IDF) are shown in
331 ~~Figure 5A, 5B respectively~~Table 2. No statistically significant differences in TDF and
332 IDF values (P > 0.05) were found between the different types of persimmon under
333 study. Consequently, it would appear that neither the HHP treatment nor pasteurization
334 affected the dietary fiber content of the persimmons. So, persimmon seems to be a rich
335 source of dietary fiber.

336

337 **Color**

338 Color is an indicator of prime importance in relation to the different attributes that define
339 the quality of plant products and is considered the major quality attribute that influences
340 the consumer's choice (Quitão-Teixeira and others 2008). ~~Figure 6~~Table 3 shows the
341 color parameters luminosity (L*), hue (h_{ab}) and chroma (C_{ab}*). With regard to L* ~~(Figure~~
342 ~~6A)~~, it may be seen that both HHP and pasteurization induced a significant reduction in
343 luminosity (P < 0.05) and the non thermal HHP treatment generated the significantly
344 lowest values (P < 0.05). The lower L* values observed in HHP-treated and

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345 pasteurized persimmons could be associated with a higher browning reactions that
346 could take place in these samples.

347 Concerning hue (Table 3~~Figure 6B~~), statistically significant differences (P < 0.05) were
348 found between the three types of persimmon. In this case it was the thermal treatment,
349 pasteurization that led to the significantly lowest hue values (P < 0.05). Generally, hue
350 values of the three types of persimmon were between 80 and 90 º, corresponding to
351 the yellow coloring of the samples due to carotenoid pigments of persimmon. The lower
352 hue values of HHP-treated and pasteurized samples could be related to browning
353 reactions, because the lower hue values, the higher redness the samples are. The
354 decrease in the hue values was higher for pasteurization than for HHP-treated
355 samples.

356 In the case of chroma (~~Figure 6C~~Table 3), no statistically significant differences (P >
357 0.05) between the untreated and pasteurized persimmons were observed but the
358 persimmons treated with HHP registered significantly lower chroma values (P < 0.05).

359 So, both preservation treatments caused changes in the color parameter values.
360 Pasteurized samples showed higher L* and chroma values than HHP persimmons.
361 These variations in the color parameters of the treated (HHP and pasteurization)
362 persimmons could be indicative of greater activity by the enzymes responsible for
363 enzymatic browning, such as polyphenol oxidase (PPO) and peroxidase (Quitão-
364 Teixeira and others 2008). The microstructural changes in the cell walls and
365 membranes caused by the HHP and pasteurization treatments could favor contact
366 between the enzyme and its substrates, which had previously remained separate in
367 different compartments of the untreated persimmon cells (Rastogi and others 2007).
368 This contact could encourage browning reactions.

369

370 **Texture properties**

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~~Figure 7~~Table 4 shows the texture properties of firmness ~~(Figure 7A)~~, cohesiveness ~~(Figure 7B)~~ and shear force ~~(Figure 7C)~~ of the three types of persimmon under study. No statistically significant differences in these properties ($P > 0.05$) were observed between the untreated and pasteurized persimmons. However, the persimmons treated with HHP presented significantly lower firmness, cohesiveness and shear force ($P < 0.05$). The structural modifications together with a greater movement of solutes at cell level could explain the lower texture parameter values of the HHP-treated persimmons. These results are in agreement with previous studies that observed lower firmness and cohesiveness in persimmons treated with HHP (Vázquez-Gutiérrez and others 2012). Texture changes could be related to transformations in cell wall polymers due to enzymatic and non enzymatic reactions (Sila and others 2008). Due to cell structure changes, HHP processing facilitates the occurrence of enzymatic and non enzymatic reactions. Substrates, ions and enzymes which are located in different compartments in the cells can be liberated and interact with each other during and after HHP treatment. At the same time, pressure can enhance the action of pectinmethylesterase (PME) and polygalacturonase (PG), causing the softening of persimmon and decrease of texture properties (Oey and others 2008b).

Conclusions

Both HHP treatment and pasteurization cause structural changes in the parenchymal tissues of persimmons. The fruit subjected to HHP presents a more compact structure containing little air and with intercellular spaces filled with cell material, indicating increased solute movement through the tissue. These microstructural changes could be responsible for the modifications in the bioactive compounds content of persimmon. Both preservation treatments lead to a fall in the total soluble tannin content and maintain the dietary fiber content of untreated persimmon. The decrease in the total soluble tannin content could be related to the loss of astringency and could make the

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398 persimmon more suitable for consumption. However, HHP processing improves the
399 extraction of carotenoids and keeps the antioxidant properties of the fruit. Treating
400 persimmon with HHP allows obtaining a high nutritional value ingredient to be used
401 when formulating new functional foods.
402

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410

411 Author contributions

412 M. Hernández-Carrión collected test data, interpreted the results and drafted the
413 manuscript.
414 I. Hernando interpreted the results and revised the manuscript.
415 A. Quiles designed the study and interpreted the results.
416

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Table 1. Carotenoid content, total soluble tannin content, and total antioxidant activity of untreated, HHP, and pasteurized persimmon.

	Carotenoid content (mg β-carotene/100 g f.w.)	Total soluble tannin content (g gallic acid/100 g f.w.)	Antioxidant activity [Trolox] (μmol/g)
Untreated	0.581[a] (0.130)	0.468[a] (0.059)	31.143[a] (0.165)
HHP	1.695[b] (0.046)	0.260[b] (0.031)	31.154[a] (0.135)
Pasteurized	1.237[c] (0.134)	0.251[b] (0.038)	25.445[b] (2.253)

f.w.: fresh weight

Values within a column without the same letter reveal significant difference ($P < 0.05$) according to the LSD multiple range test.

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Table 2. Total dietary fiber (TDF) and insoluble fiber of untreated, HHP, and pasteurized persimmon.

	TDF (g/100 g d.w.)	IDF (g/100 g d.w.)
Untreated	14.877[a] (2.751)	9.387[a] (1.735)
HHP	14.961[a] (2.845)	8.411[a] (1.600)
Pasteurized	15.308[a] (3.069)	8.744[a] (1.753)

d.w.: dry weight

Values within a column without the same letter reveal significant difference ($P < 0.05$) according to the LSD multiple range test.

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Table 3. Lightness, hue, and chroma of untreated, HHP, and pasteurized persimmon.

	Lightness	Hue	Chroma
Untreated	67.584[a] (2.102)	84.446[a] (1.787)	46.474[a] (4.385)
HHP	48.839[b] (3.031)	82.721[b] (1.352)	31.856[b] (3.257)
Pasteurized	62.791[c] (3.486)	80.947[c] (1.488)	43.944[a] (3.446)

Values within a column without the same letter reveal significant difference (P < 0.05) according to the LSD multiple range test.

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Table 4. Firmness, cohesiveness, and shear force of untreated, HHP, and pasteurized persimmon.

	Firmness (N)	Cohesiveness	Shear force (N)
Untreated	5.915[a] (1.256)	0.084[a] (0.016)	11.959[a] (2.131)
HHP	3.526[b] (1.029)	0.059[b] (0.004)	10.118[b] (1.894)
Pasteurized	5.234[a] (1.329)	0.074[a] (0.014)	12.919[a] (1.884)

Values within a column without the same letter reveal significant difference ($P < 0.05$) according to the LSD multiple range test.

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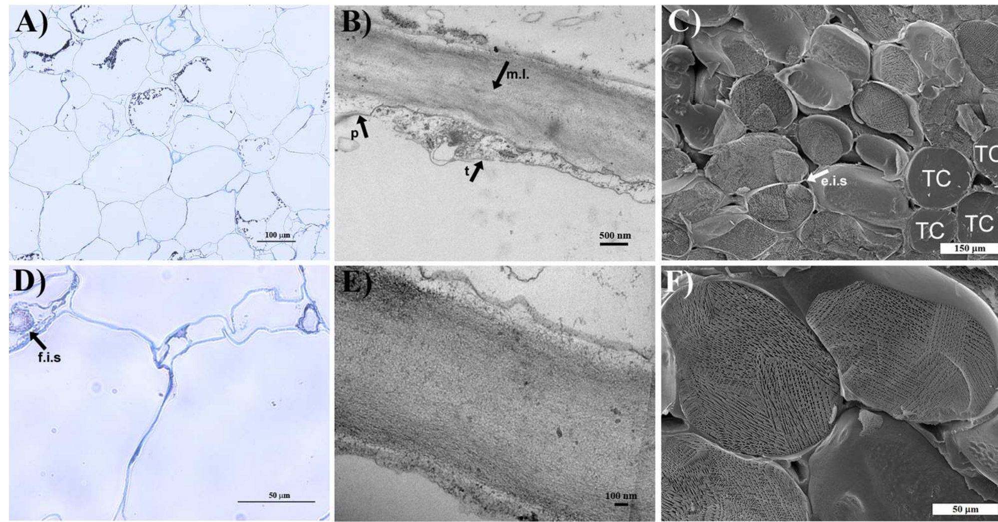

Figure 1. Light Microscopy (A, D), Transmission Electron Microscopy (B, E), and Cryo-SEM (C, F) micrographs of untreated persimmon. m.l.:middle lamella; p: plasmalemma; t: tonoplast; TC: tannin cell; e.i.s.: empty intercellular space; f.i.s.: full intercellular space.
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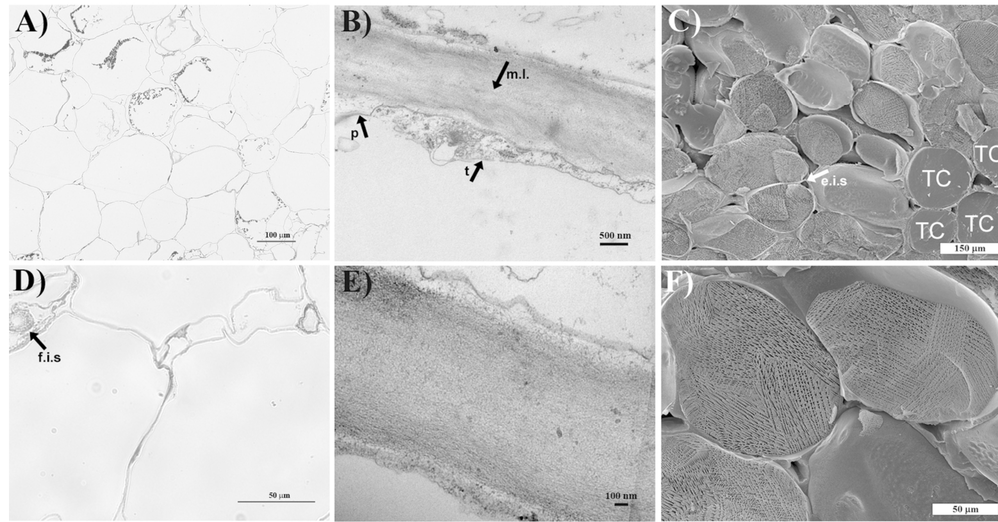

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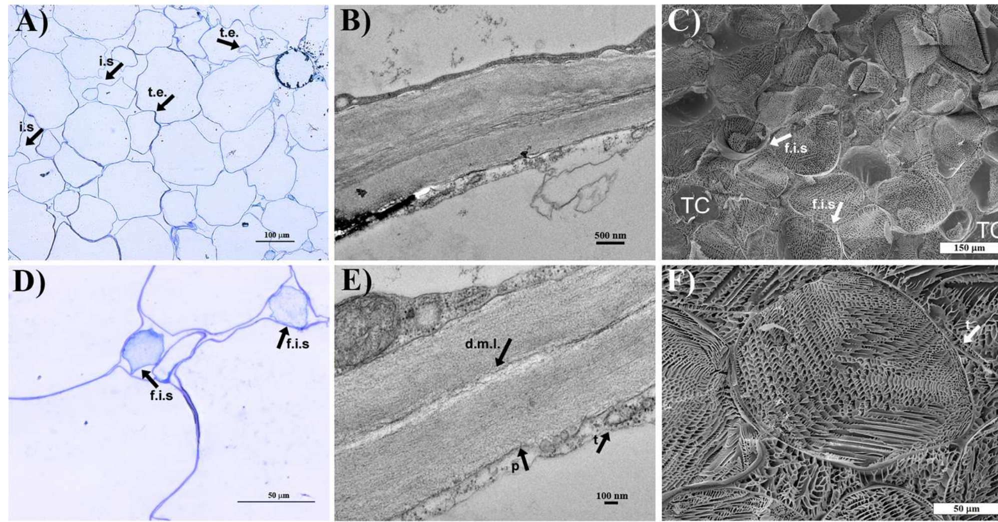

Figure 2. Light Microscopy (A, D), Transmission Electron Microscopy (B, E), and Cryo-SEM (C, F) micrographs of HHP-treated persimmon. t.e.: twisted edges; i.s.: intercellular space; d.m.l.: dissolved middle lamella; p: plasmalemma; t: tonoplast; TC: tannin cell; f.i.s.: full intercellular space.
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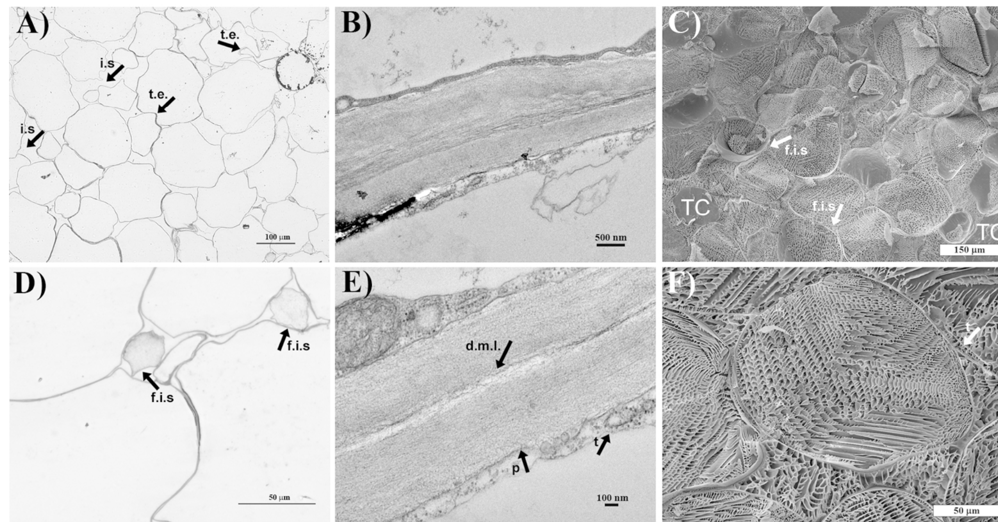

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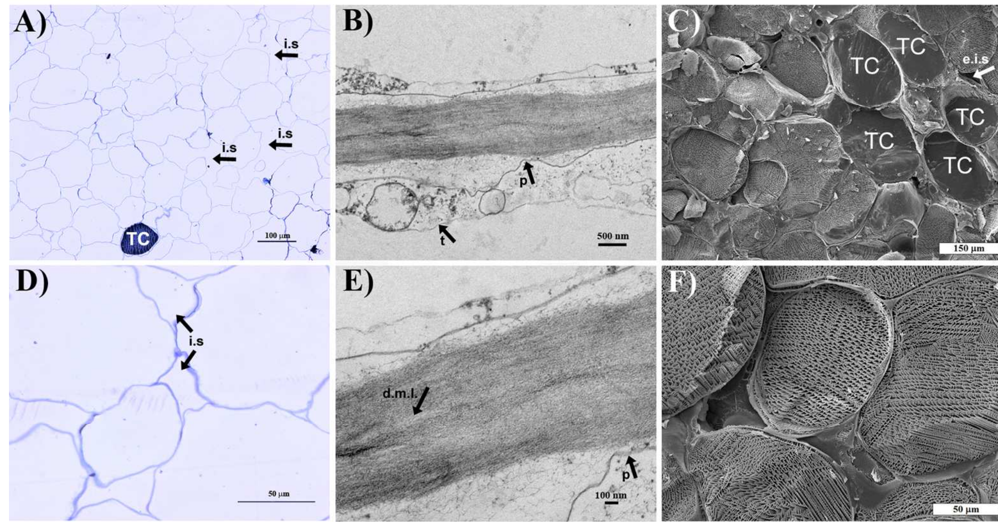

Figure 3. Light Microscopy (A, D), Transmission Electron Microscopy (B, E), and Cryo-SEM (C, F) micrographs of pasteurized persimmon. i.s.: intercellular space; d.m.l.: dissolved middle lamella; p: plasmalemma; t: tonoplast; e.i.s.: empty intercellular space; TC: tannin cell.
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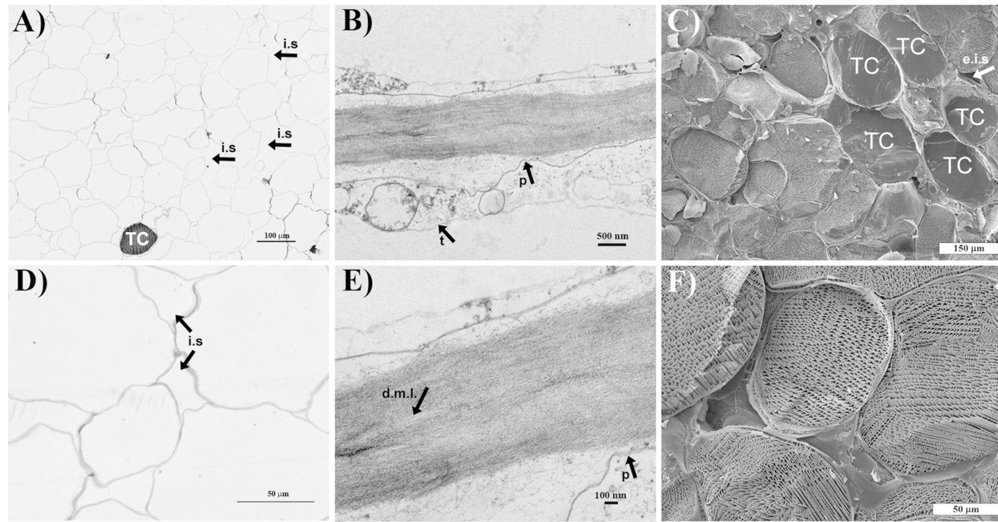

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