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Additional Information

1 **INFLUENCE OF CALCIUM LACTATE AND MODIFIED ATMOSPHERE ON**
2 **RESPIRATION RATE, OPTICAL AND MECHANICAL PROPERTIES OF SLICED**
3 **PERSIMMON**

4
5 **ABSTRACT**

6
7 The aim of this study was to evaluate the effect of a modified atmosphere (5% and 10%
8 of CO₂) and calcium lactate treatment on the respiratory metabolism of minimally
9 processed persimmon. A static system to measure changes in the composition of the
10 headspace was used. Composition, texture and colour were also analysed. Persimmon
11 slices were evaluated immediately after the washing treatment and after the O₂
12 composition had decreased to 17% to avoid changes in the metabolic pathway. All
13 samples were stored at 4°C. The results showed that modified atmosphere did not affect
14 compositional properties, although there was a slight increase in pH values at the end of
15 each treatment. Calcium lactate treatment reduced the respiration rate, in terms of O₂, in
16 samples kept in air. Additionally, a calcium lactate effect was immediately observed on
17 mechanical properties after the washing stage. On the other hand, luminosity and b*
18 coordinate decreased in unwashed and calcium lactate samples kept in 5% CO₂.

19
20 Keywords: fresh-cut persimmon, modified atmosphere, calcium lactate, respiration rate,
21 physicochemical properties

22
23 **INTRODUCTION**

24
25 The demand for minimally processed products has increased due to many factors such
26 as the reduction in time required to prepare meals, the smaller size of families and the
27 growing concern about fresh and healthy foods. Therefore the sector of minimally
28 processed fruits and vegetables is at its peak.

29 Minimally processed products, which are also called ready-to-used, fresh-cut or IV
30 gamme products, are fruits and vegetables that have been exposed to certain
31 mechanical processes such as washing, peeling, coring, slicing and cutting before
32 packaging (Izumi & Watada, 1994; Barry-Ryan & O'Beirne, 1998; Degl'Innocenti et al.,
33 2007). However, these products are more perishable than intact fruits or vegetables
34 because of physical stress (Watada et al., 1996). Texture and appearance are two
35 important factors which have a great influence on consumers' acceptability (Toivonen &
36 Brummel, 2008). It is also essential to prevent the development of strange flavours and
37 to guarantee microbiological stability.

38 There are numerous researchers that have studied different methods to extend and
39 improve the shelf life of fresh-cut products (Montero-Calderón et al., 2008; Aguayo et al.,
40 2008; Rojas–Graü et al., 2007; Marrero & Kader, 2006; Soliva-Fortuny et al., 2004;
41 Luna–Guzmán & Barrett, 2000). In fact, modified atmosphere packaging (MAP) is one
42 of the most widely used conservation techniques. MAP involves the modification of the
43 composition of the atmosphere inside the package, which is achieved as a result of the
44 interplay between the respiration of the product and the transfer of gases through the
45 packaging material (Fonseca et al., 2002; Sandhya, 2010). This interaction leads to a
46 decrease in the O₂ concentration and an increase in CO₂ inside the container (Fonseca
47 et al., 2002). If the permeability of the packaging material is suitable for the respiration
48 of the product, the product will have a longer shelf life (Sandhya, 2010). Nevertheless,
49 the CO₂ concentration inside the package might not achieve the required level, meaning
50 that it cannot be used as either a fungicide or bactericide (Serrano et al., 2008). In fact,
51 Soliva-Fortuny et al. (2004) studied the microbial shelf life and certain biochemical
52 changes in apple cubes under different MAP conditions, observing that microbial growth
53 was partially inhibited, which led them to conclude that poor O₂ and/or high CO₂
54 atmospheres had neither bactericidal nor fungicidal effects. Therefore the combination
55 of MAP and the application of some substances such as antioxidants, calcium salts,

56 antimicrobial agents etc. could also be useful for extending the shelf life of fresh-cut fruits
57 or vegetables.

58 In this regard, calcium salts have been used to maintain the mechanical properties of
59 minimally processed products (Silveira et al., 2011; Alandes et al., 2009; Martín-Diana
60 et al., 2006). Furthermore, the antimicrobial effect of calcium lactate has been evidenced
61 by many researchers (Aguayo et al., 2008; Torres et al., 2008; Moraga et al., 2009).
62 Thus, the use of this salt together with modified atmosphere packaging could maintain
63 the shelf life of minimally processed products from a sensorial, physicochemical and
64 microbial point of view.

65 Additionally, persimmon production has greatly increased in recent decades due to the
66 use of techniques to remove astringency, which keeps the fruit's texture firm. In fact, the
67 production of persimmon in the Ribera del Xúquer area (Valencia, Spain) was multiplied
68 by 140 from 1992 to 2002 (Llácer & Badenes, 2002). Moreover, according to GVA, 2013
69 the average of production between 2002 and 2011 was 49.312 tonnes, while 134.600
70 tonnes were produced in 2012, showing a continuous growing (GVA, 2013).
71 Consequently, there is an important surplus of this product and different types of
72 marketing of minimally processed product have been relied on to improve its distribution
73 and consumption.

74 Therefore, the aim of this study was to evaluate the effect of both a CO₂-rich atmosphere
75 and the application of lactate calcium on the composition, respiration rate, colour and
76 texture properties of fresh-cut persimmon.

77

78 **MATERIALS AND METHODS**

79

80 ***Raw materials***

81

82 For the purpose of carrying out the required experiments, fruits of persimmon (*Diospyros*
83 *kaki*) of the variety "Rojo Brillante" were used. They were stored for 24 h at 4 °C before

84 being processed. Fruits were selected according to their ripening stage, colour and
85 general appearance in order to optimize homogeneity of the samples.

86

87 ***Treatment of persimmon samples***

88

89 After selecting the persimmon they were washed in tap water with commercial sodium
90 hypochlorite (Amukina, Laboratories Angelini, Farma-Lepori, Barcelona, Spain) using
91 the recommended dose: 0.02 (v/v) for 1 min. The samples were then dried with
92 absorbent paper and the fruits were cut in slices which were approximately 1.5 cm thick.

93 All the samples were mixed to randomize the variability provided by the raw material.

94 The persimmon slices were dipped into a solution of calcium lactate pentahydrate (2%)
95 (Panreac, Barcelona, Spain) in a 3:1 (L/kg) water:fruit ratio for 5 min. The samples were
96 then drained for 5 min. Cut fruit that was not dipped and cut fruit dipped only in tap water
97 were used as controls.

98

99 ***Application of modified atmosphere***

100

101 300 g of persimmon slices were packaged in glass jars (1.937 L). Prior to storage at 4°
102 C, the headspace composition of the glass jars was modified by a gas mixer (WITT-KM-
103 ME 100-3 GB. WITT-GASETECHNICK, Witten Germany). The gas mixer was connected
104 to a valve at the top of the glass jars. The mixture of gas was circulated for 3 min, and
105 the valves were then closed, following which the composition of the headspace was
106 immediately measured. The atmosphere composition studied was 5% CO₂+20% O₂ and
107 10% CO₂+20% O₂ (balanced with N₂), and both of these were compared to the
108 composition of the air.

109

110 ***Analytical determinations***

111

112 The persimmon slices were evaluated immediately after the washing treatment, and also
113 when the O₂ composition decreased to 17% (48-72 h), in order to prevent changes in
114 metabolic behaviour from a lower availability of oxygen.

115

116 *Moisture content, soluble solids content pH, water activity and titratable acidity*

117 Moisture content was determined by drying the fruit to constant weight at 60 °C in a
118 vacuum oven at 10 kPa (adaptation of method 934.06 AOAC, 2000). Soluble solids were
119 measured in previously homogenized samples using a refractometer (Zeiss, ATAGO
120 model NAR-3T, Japan). Water activity (a_w) was measured with a hygrometer (Fast-lab,
121 GBX, France). pH was obtained directly from the homogenized sample by means of a
122 pH-meter (“Seven Easy” METTLER TOLEDO - United States) with a contact electrode.
123 Titratable acidity was determined by potentiometric titration with 0.1 N NaOH (Panreac,
124 Barcelona, Spain) of up to pH 8.1-8.2. Distilled water (40 mL) was added to the
125 homogenized sample (9 g). Results were expressed as g of malic acid per 100 g of
126 sample.

127

128 *Determination of respiration rate*

129

130 A closed system was chosen to measure the respiration rate. Persimmon slices (about
131 300 g) were placed in 1.937 L hermetic glass jars with a septum in the lid for sampling
132 the gas in the headspace at different times. The jars were stored in a temperature
133 controlled chamber (P Selecta, Hot-Cold M 4000668, Barcelona, Spain). Gas sampling
134 was carried out every 30 or 60 min by means of a needle connected to a gas analyser
135 (PBI Dansensor-CheckMate 9900 O₂/ CO₂, Ringsted, Denmark). Experimental points
136 were considered in the time range where a linear relationship was observed between
137 gas concentration and time. This means that no changes in the respiration pathway of

138 the samples occurred in this period, meaning that changes in the composition of the
139 headspace did not lead to notable alterations in their metabolism.

140 The respiration rate (R_i , mL \cdot kg $^{-1}\cdot$ h $^{-1}$) of the samples in terms of CO $_2$ emission and O $_2$
141 consumption was determined from the slope of the fitted linear equation, according to
142 Eq. (1), where y_{it} is the gas concentration (%O $_2$, %CO $_2$) at time t , i being O $_2$ or CO $_2$, m is
143 the mass of the fresh samples and V , the volume (mL) of headspace.

144

$$145 \quad y_{it} = y_{it_0} \pm 100 \times R_i \times \frac{m}{V} \times t \quad (\text{Eq. 1})$$

146

147 *Analysis of optical parameters*

148

149 The colour of the persimmon samples was measured by means of a spectrophotometer
150 Minolta (CM-3600 d, Tokyo, Japan) with a window of 7 mm in diameter. The colour was
151 analysed immediately after the dipping stage and at the end of each experiment. CIE-
152 L*a*b* coordinates were obtained using D65 illuminant and 10° observer as reference
153 system. It was measured in triplicate and four areas of the persimmon sliced were taken
154 for the purpose of determining their colour. Furthermore, the difference in colour (ΔE) as
155 compared to initial colour values was estimated using the following equation (2):

156

$$157 \quad \Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (\text{Eq. 2})$$

158

159 *Measurement of mechanical properties*

160

161 Mechanical properties were analysed using a texture analyser (TA.XT2 Texture Analyzer
162 Aname, Stable Micro Systems, Haslemere, England) by means of a puncture test (5 mm
163 diameter punch) to achieve a relative deformation of 95% at a speed of 1.5 mm/s. The
164 parameters analysed were: maximum force (F, N) and the distance at which the

165 maximum force took place (d, mm). The analysis was performed immediately after the
166 washing stage and at the end of each experiment. The parameters were measured in
167 triplicate and four areas of the sliced persimmon sliced were taken to determine their
168 mechanical properties.

169

170 ***Statistical analysis***

171

172 An ANOVA analysis using Statgraphics Centurion Software was performed to evaluate
173 the effect of process variables (percentage of CO₂ in the composition of the headspace
174 gas and dipping treatment) on the results obtained with a significant level of 95%.

175

176 **RESULTS AND DISCUSSION**

177

178 ***Evolution of the compositional properties***

179

180 Table 1 shows water and soluble solid mass fraction, pH and titratable acidity for each
181 treatment and % of CO₂ applied, both after the dipping treatment (initial conditions) and
182 when the O₂ composition decreased to 17% (final conditions). A simple ANOVA was
183 performed to analyse the differences in compositional properties immediately after the
184 dipping treatment and at the end of the trial.

185 The soaking treatment led to an increase in water mass fraction as compared to
186 unwashed samples due to the entrance of water into the matrix of the fruit, and also to a
187 decrease in soluble solid mass fraction. In general, the initial values for these two
188 compositional properties were maintained at the end of each trial. The values relating to
189 water activity were also steady. The initial and final water activity averages were 0.984
190 (± 0.006) and 0.982 (± 0.005) respectively. These values were similar to others reported
191 in previous studies (Castelló et al.; 2006; Igual et al., 2008). On the other hand, there
192 was a slight increase in pH values in all samples that were exposed to CO₂-rich

193 atmospheres. This behaviour was also observed in samples treated with calcium lactate,
194 which were also preserved in an air composition atmosphere. Wright & Kader (1997a)
195 studied the effect of controlled atmosphere storage on fresh-cut persimmon and
196 strawberry. Generally, pH values of both persimmons and strawberries stored under
197 various atmospheres tended to increase. In addition, Holcroft & Kader (1999)
198 investigated the effects of low O₂ atmospheres alone, or in combination with 20 kPa CO₂
199 on 'Selva' strawberries. They observed that both external and internal tissue pH
200 increased over the 10-day storage period. However, Li & Kader (1989) did not observe
201 any significant effect on pH in 'Selva' strawberries stored in a controlled atmosphere. In
202 relation to titratable acidity, the control samples were slightly higher than control samples
203 for non astringent persimmon (0,117±0,008 g of malic acid per 100 g of sample) obtained
204 by Vázquez-Gutiérrez et al. (2012). Moreover, there was a decrease in titratable acidity
205 of water treated samples which were stored in air. This might be due to the advance in
206 the ripening process.

207

208 ***Respiration rate***

209

210 Figure 1 shows respiration rates in terms of O₂ consumption and CO₂ production for each
211 of the treatments performed and CO₂ concentrations used. In stored air, calcium lactate
212 treated samples had lower respiration rates than other samples. Albors et al. (2008) also
213 observed that persimmon slices, which were washed in ascorbic acid and calcium lactate
214 solution, had lower respiration rates than unwashed and water washed sliced persimmon
215 on the 4th day of storage. This decrease has also been noticed in other studies that have
216 used this calcium salt. Thus, Luna-Guzmán & Barret (2000) studied the effect of calcium
217 lactate (alone or in combination with heat) on the respiration (in terms of CO₂) of melon
218 cylinders. As a result, CO₂ production was not increased until day 6 for all samples.
219 Nevertheless, calcium lactate, water dipped and heat treated melon showed lower CO₂
220 levels than untreated samples. Silveira et al. (2011) also observed that calcium

221 propionate, tartrate, lactate, ascorbate and chloride treated melon had lower respiration
222 rates than the rest of the salts studied at the end of their shelf-life. On the other hand, O₂
223 consumption rate was reduced in the un-dipped samples in a CO₂-rich atmosphere. Li &
224 Kader (1989) measured respiration rate in terms of O₂ consumption in 'Selva'
225 strawberries held in CO₂-rich atmospheres (air +10%, 15% or 20% CO₂). The respiration
226 rate was lower in CO₂-rich atmospheres than when the fruit was kept in air.

227 In this research, the use of high CO₂ atmospheres led to an increase in oxygen
228 consumption in washed samples, especially with calcium lactate treatment. The
229 combination of calcium lactate and a high percentage of CO₂ could reverse the
230 retardation effect on the respiration rate in comparison to calcium lactate treatment in
231 air. The CO₂ production rate was significantly decreased in all treatments at high
232 concentrations of CO₂. This behaviour could be explained by the high susceptibility of
233 cut persimmon fruit to rich-CO₂ atmospheres which completely collapse the release of
234 this gas in its metabolic pathways.

235

236 ***Evolution of the optical properties***

237

238 Table 2 shows the initial colour analysis before storage. Neither a* nor b* coordinates
239 showed significant differences. However, there was a small difference in luminosity (L*)
240 depending on the treatment used before storing. Thus, these results showed that all
241 optical properties were uniform. Furthermore, dipping the fruit in water or calcium lactate
242 did not change its initial colour. The initial value for luminosity was similar to the results
243 obtained by Albors et al. (2008). However a* and b* coordinates were slightly higher in
244 this study than those reported by Albors et al. (2008).

245 Figure 2 shows graphic L*-a* and chromatic plane b*-a* as a function of the dipping
246 treatment and CO₂ concentration in the glass container at the end of the trial as a function
247 of the treatment and the concentration of CO₂ in the glass container both at the beginning
248 and at end of the experiment. In the graphic L*-a*, luminosity decreased in unwashed

249 and calcium lactate samples stored in a 5% CO₂ atmosphere. Samples washed with tap
250 water which were stored in 10% CO₂ also showed lower luminosity. Regarding the a*
251 coordinate, un-dipped and water dipped samples stored in air, turned a reddish colour.
252 This could be due to the fact these samples had ripened more. Furthermore, water
253 washed samples had a lower titratable acidity, which reinforces this theory. However,
254 calcium lactate samples did not show this behaviour under the same conditions. It is
255 possible that calcium had had a slowing effect on the synthesis of carotenoids. On the
256 other hand, CO₂-rich atmospheres kept a* coordinate values. High CO₂ atmospheres
257 could have also had slowing effect on the synthesis of carotenoids. However, Wright &
258 Kader (1997b) reported that atmospheres with 12% CO₂ and 2% O₂ kept better the
259 values of retinol equivalent in comparison with slices of persimmon stored under 2% O₂
260 or air. In addition, 5% CO₂ atmospheres reduced the b* coordinate in undipped and
261 calcium lactate samples. The decrease in b* coordinate values along with a lower
262 luminosity in these samples could be related to the browning phenomena. It is possible
263 that persimmon slices were subjected to some kind of stress under these conditions
264 which could cause browning reactions. In fact, Kader & Ben-Yehoshua (2000) reported
265 that the oxidation of phenolic compounds by polyphenol oxidase resulted from loss of
266 compartmentalization within the cells when exposed to physical and/or physiological
267 stresses. Nevertheless, a higher percentage of CO₂ could have counteracted the
268 polyphenoloxidase action.

269 Colour differences at the end of each test are shown in figure 3. Un-dipped and calcium
270 lactate persimmon slices stored under 5% CO₂ atmosphere showed the most significant
271 changes in colour. The effect of calcium was also observed by Martin-Diana et al., (2005)
272 who reported that high concentrations of calcium lactate in lettuce gave higher colour
273 variation than samples treated at low or intermediates concentrations. The possible
274 increase in the synthesis of carotenoids was not significantly reflected in samples
275 packaged in an air composition.

276

277 ***Evolution of the mechanical properties***

278

279 Figure 4 shows values of maximum force and distance of persimmon slices both at the
280 beginning, and also at the end of the experiment before the O₂ composition in the
281 headspace dropped to values lower than 17% O₂. Calcium lactate treated samples
282 showed the highest values of maximum strength immediately after the immersion stage.
283 This increase was maintained throughout the study. The maintenance of the cell wall
284 structure mainly depends on the calcium binding pectic components of the middle lamella
285 (Grant et al., 1973; Poovaiah, 1986; Quiles et al., 2004). Calcium salts make it possible
286 to maintain the mechanical properties of minimally processed products (Martín-Diana et
287 al., 2006; Rico et al., 2007). On the other hand, the firmness of un-dipped samples did
288 not significantly vary under any conditions studied. Samples stored in 10% CO₂
289 presented great differences most likely due to the variability of the samples. In fact, there
290 were no differences between the initial and final values for each treatment. In relation to
291 persimmon slices stored in 5% CO₂, washed samples showed a significant increase (α
292 <0.01) in maximum force at the end of storage. An improvement in the fruits' firmness
293 has been observed in many research studies analysing CO₂-rich atmospheres (Li &
294 Kader, 1989; Harker et al., 2000). In contrast, Wright & Kader (1997ab) observed that in
295 the case of sliced persimmon which was stored in a different controlled-atmosphere, their
296 firmness tended to decrease for all the treatments studied. In this study, higher firmness
297 was shown in calcium lactate samples stored in 10% CO₂. There were no differences in
298 the distance at which maximum force took place.

299

300 **CONCLUSIONS**

301

302 Calcium lactate reduced the respiration rate, in terms of O₂ in samples stored in air.
303 However, a high concentration of CO₂ in the headspace atmosphere could have
304 counteracted the calcium lactate slowing effect on respiratory metabolism. Additionally,

305 the calcium lactate treatment slowed down the reddish appearance of samples and
306 improved the firmness of persimmon slices.

307

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456

457 **Figure caption**

458 **Figure 1.** Respiration rate, in terms of O₂ consumption and CO₂ emission, for each
459 treatment applied and under different % CO₂ in the composition of the headspace.

460

461 **Figure 2.** Graphic of L*-a* and chromatic plane representation (b*-a*) of sliced
462 persimmon for each treatment applied and under different % CO₂ in the composition of
463 the headspace. Dark symbols (t=0) correspond to initial values.

464

465 **Figure 3.** Colour differences of sliced persimmon for each treatment applied and under
466 different % CO₂ in the composition of the headspace.

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468 **Figure 4.** Maximum force expressed in Newton (N) and the distance (d) at which it occurs
469 in millimetres in sliced persimmon for each treatment applied and under different % CO₂
470 in the composition of the headspace.

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Tables

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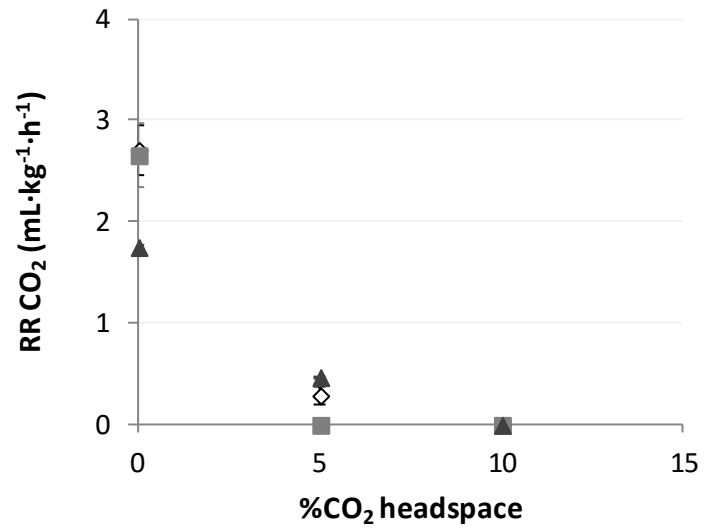
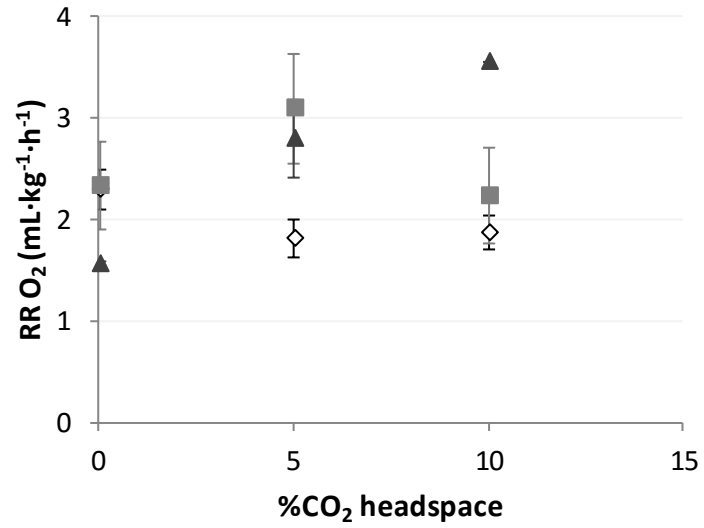
Table 1. Values for water and soluble solid mass fractions, pH and titratable acidity of sliced persimmon for each treatment at the beginning of the storage (t=0) and at the end storage (t=f).

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Table 2. Initial values for luminosity (L*) and a* and b* coordinates of sliced persimmon before the modification of the composition of the headspace.

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◇ Unwashed ■ Tap water ▲ Calcium lactate

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Figure 1

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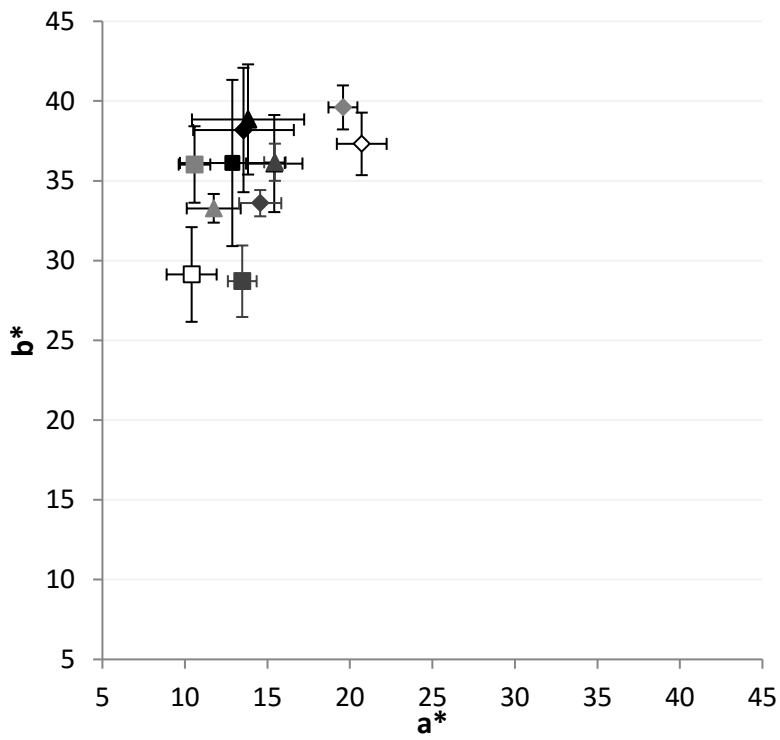
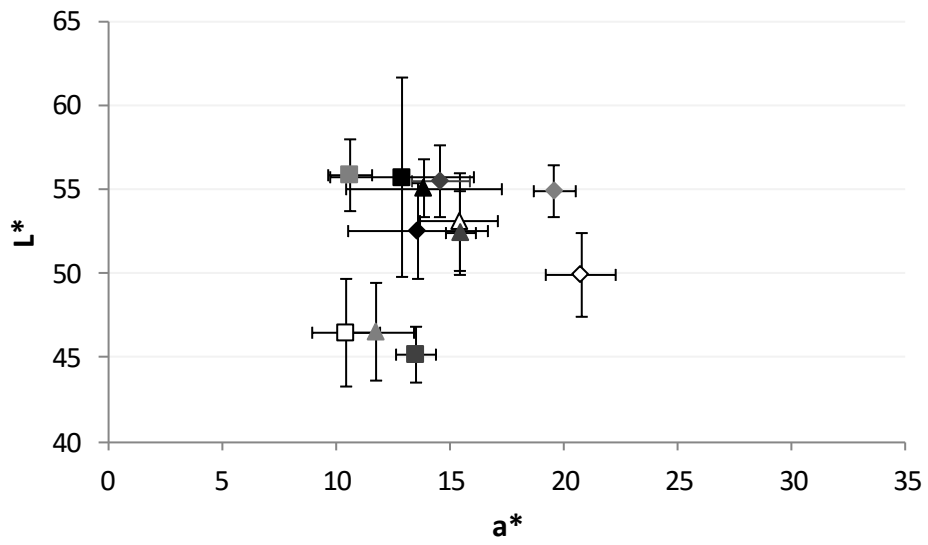
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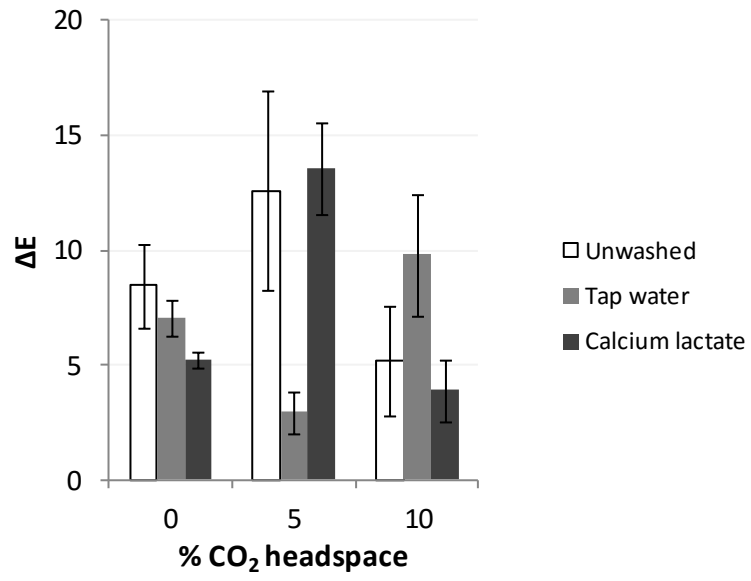
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- ◇ Unwashed-0% CO2 □ Unwashed-5% CO2 △ Unwashed-10% CO2
- ◆ Tap water-0% CO2 ■ Tap water-5% CO2 ▲ Tap water-10% CO2
- ◆ Calcium lactate-0% CO2 ■ Calcium lactate-5% CO2 ▲ Calcium lactate-10% CO2
- ◆ Unwashed (t=0) ■ Tap water (t=0) ▲ Calcium lactate (t=0)

Figure 2

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513 **Figure 3**

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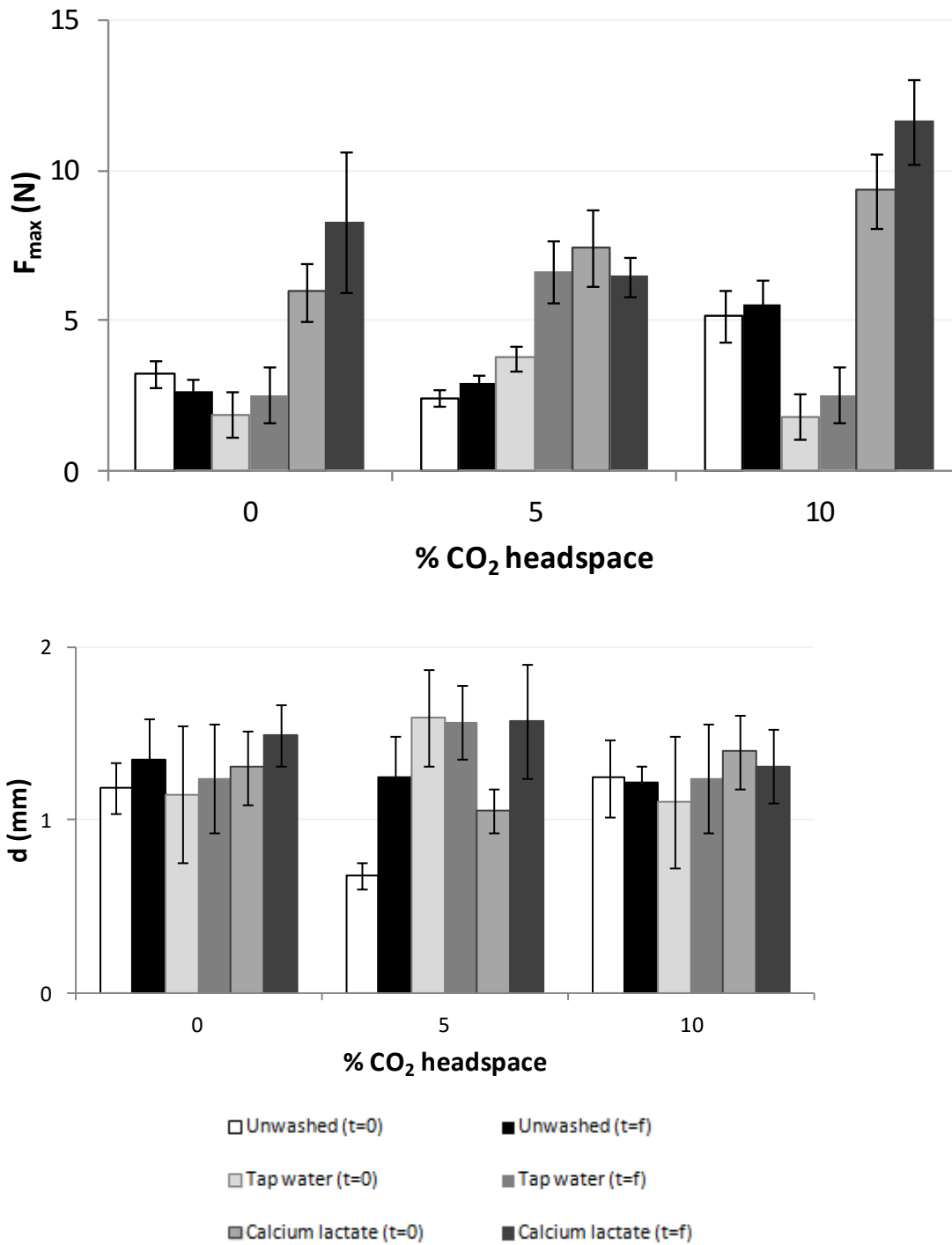
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Figure 4

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Table 1.

Treatment	% CO ₂	$x_{t=0}^w$	$x_{t=f}^w$	$x_{t=0}^{ss}$	$x_{t=f}^{ss}$	$pH_{t=0}$	$pH_{t=f}$	g malic acid/100 g sample (t=0)	g malic acid/100 g sample (t=f)
Control	0	0,807 (0,008)	0,805 (0,015)	0,168 (0,004)	0,163 (0,007)	5,947 (0,101)	5,99 (0,03)	0,19 (0,02)	0,176 (0,009)
	5	0,822 (0,007)	0,811 (0,012)	0,1679 (0,0012)	0,165 (0,002)	5,860 (0,014)	6,05 (0,02)*	0,203 (0,011)	0,205 (0,012)
	10	0,7962 (0,0008)	0,800 (0,008)	0,162 (0,002)	0,174 (0,013)	5,87 (0,03)	6,01 (0,04)*	0,171 (0,019)	0,195 (0,004)
Tap water	0	0,831 (0,004)	0,825 (0,008)	0,156 (0,003)	0,144 (0,003)*	6,03 (0,03)	6,10 (0,05)	0,187 (0,004)	0,160 (0,004)*
	5	0,845 (0,002)	0,843 (0,006)	0,13049 (0,00112)	0,132 (0,004)	5,94 (0,02)	6,06 (0,04)*	0,176 (0,004)	0,18 (0,02)
	10	0,836 (0,002)	0,8295 (0,0003)*	0,145 (0,002)	0,150 (0,005)	6,00 (0,03)	6,12 (0,02)*	0,167 (0,004)	0,163 (0,008)
Calcium lactate (2%)	0	0,833 (0,005)	0,829 (0,002)	0,150 (0,004)	0,145 (0,003)	5,880 (0,014)	6,06 (0,07)*	0,186 (0,013)	0,165 (0,004)
	5	0,846 (0,005)	0,8315 (0,0108)	0,141 (0,007)	0,147 (0,006)	5,80 (0,03)	6,01 (0,04)*	0,181 (0,018)	0,162 (0,007)
	10	0,820 (0,0012)	0,821 (0,005)	0,155 (0,008)	0,157 (0,006)	5,81 (0,06)	6,19 (0,06)*	0,171 (0,008)	0,174 (0,003)

Parenteses indicate standard deviation

*Indicates a significant difference (p-value<0.05)

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Table 2.

Treatment	Optical properties		
	L*	a*	b*
Control	53 (3) ^a	14 (3) ^a	38 (4) ^a
Tap water	56 (6) ^b	13 (3) ^a	36 (5) ^a
Calcium lactate (2%)	55 (2) ^b	14 (3) ^a	39 (3) ^a

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Parenteses indicate standard deviation
Same letters indicate homogeneous groups