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The final publication is available at:

<https://doi.org/10.1016/j.foodcont.2016.02.005>

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Accepted Manuscript

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PII: S0956-7135(16)30051-2

DOI: [10.1016/j.foodcont.2016.02.005](https://doi.org/10.1016/j.foodcont.2016.02.005)

Reference: JFCO 4861

To appear in: *Food Control*

Received Date: 11 December 2015

Revised Date: 4 February 2016

Accepted Date: 5 February 2016

Please cite this article as: Salinas-Roca B., Soliva-Fortuny R., Welte-Chanes J. & Martín-Belloso O., Combined effect of pulsed light, edible coating and malic acid dipping to improve fresh-cut mango safety and quality, *Food Control* (2016), doi: 10.1016/j.foodcont.2016.02.005.

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Combined effect of pulsed light, edible coating and malic acid dipping to improve fresh-cut mango safety and quality

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ABSTRACT

The impact of pulsed light (PL), alginate coating (ALC) and malic acid dipping (MA) treatments on quality and safety aspects of fresh-cut mango was studied. Fresh-cut mangoes were inoculated with *L.innocua* and then subjected to PL (20 pulses at fluence of $0.4 \text{ J}\cdot\text{cm}^{-2}/\text{pulse}$), ALC (2 %) or MA (2 %) treatments. Moreover, different combinations of PL, ALC and MA were assayed to evaluate possible synergisms among treatments. Microbial stability and quality parameters (colour, pH, soluble solids and firmness) of fresh-cut mango were examined throughout 14 days of storage at 4 °C.

Results show that MA-PL and PL-ALC-MA treatments additively reduced *L.innocua* counts by 4.5 and 3.9 logs, respectively. Microbial population in fresh-cut mango remained below 6 log CFU/g over 14 days. Differences between firmness values of untreated and treated fresh-cut mangoes were evident throughout storage. Namely, firmness of alginate-coated slices sharply increased and progressively decreased over storage. Colour parameters and total soluble solids content decreased in all treated mango slices throughout 14 days, while pH was kept similar to that of the fresh tissue. An optimal combination of different treatments enables to ensure safety of fresh-cut mango with minimal quality deterioration throughout storage.

Key words: Fresh-cut mango; combined treatments, *L.innocua*, pulsed light, edible coatings.

38 1. INTRODUCTION

39 The growing demand for ready-to-eat fruits and vegetables has led to an increasing interest in
40 the study of methods that enhance their safety while preserving freshness. Although fruits do
41 not generally pose a safety hazard, peeling and cutting operations make fresh-cut fruits more
42 susceptible to microbial attack. *Listeria sp* can be a hazardous contaminant of fresh-cut fruits
43 as it is able to survive in a wide range of pH and temperature conditions. In fruits of low
44 acidity, in which mango is included, *Listeria sp* may also find the conditions to survive and
45 multiply (Penteado, de Castro, & Rezende, 2014). Among fresh-cut melon, apple and
46 pineapple are the most commonly consumed and studied; however the demand for other fruits
47 such as mango is continuously growing (Siddiq, Sogi, & Dolan, 2013). Mango (*Mangifera*
48 *indica L.*) is one of the most harvested tropical fruits (FAO, 2012). It is widely demanded for
49 its yellow colour, fleshy texture and unique flavour. Freshness and appearance are the primary
50 criteria determining consumer satisfaction. Produce safety is also critical to maintain
51 consumer confidence. Therefore, developing adequate treatments to obtain fresh-cut mango
52 could help to promote its consumption and enable industry to satisfy the market trends.

53 Recent research in preservation methods for fresh-cut fruits has focused on assuring safety
54 and maintaining original characteristics of fruit, while avoiding the undesired effects caused
55 by handling and processing (Caminiti et al., 2011; Moody, Marx, Swanson, & Bermúdez-
56 Aguirre, 2014; Proctor, 2010). Pulsed light (PL) treatments are being studied as a feasible
57 alternative to conventional preservative processes (Oms-Oliu, Martín-Belloso, & Soliva-
58 Fortuny, 2008). This technology involves the application of very short high-intensity pulses of
59 broad spectrum light: (180 - 1100 nm). The composition of the spectrum and the energy
60 density has been shown to play an important role in microbial cell death by PL (Keklik,
61 Demirci, Puri, & Heinemann, 2012; Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny,

2011). Different studies have proposed the use of PL treatments for the decontamination of fresh-cut fruits; however, applications for fresh-cut tropical fruits are scarce. As far as we know, literature offers only a prospective study regarding the application of PL for the decontamination of fresh-cut mango (Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013). On the other hand, edible coatings based on polysaccharides such as sodium alginate have been proposed for extending the shelf-life of fresh-cut fruits (Rojas-Graü, Tapia, Rodríguez, Carmona, & Martín-Belloso, 2007). These coatings are commonly formed as a thin layer on the cut surface of fruits, acting as a barrier against gas exchange and transpiration. Edible coatings enable to retard the physiological response to mechanical stress and other physical disorders leading to moisture and solutes migration, gas exchange, respiration and increased oxidative phenomena that have a deleterious impact on the product quality (Oms-Oliu et al., 2010; Raybaudi-Massilia, Mosqueda-Melgar, & Tapia, 2010). However, their effects in preventing microbial inactivation are scarce (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008). The use of organic acids is another strategy which could be used to ensure safety of fresh-cut fruits. Malic acid dips have been shown to enable a decrease in microbial loads, thus ensuring safety and extending quality of fresh-cut produce over storage (Gómez et al., 2012; Raso & Barbosa-Cánovas, 2003; Rojas-Graü, Raybaudi-Massilia, et al., 2007; Tapia et al., 2007; Valencia-Chamorro, Palou, Del Río, & Pérez-Gago, 2011). Their antimicrobial activity could be attributed to the reduction of the medium pH, decrease of the intracellular pH by ionization of undissociated acid molecules.

As these strategies do not individually succeed in guaranteeing safety and quality maintenance of fresh-cut fruits, a combined methods approach stands as a good alternative to achieve this goal. Hence, the aim of the present work was to assess the effectiveness of combining PL, alginate coating and malic acid on the reduction of *Listeria innocua*

86 population as well as to evaluate microbial growth and physicochemical parameters (pH,
87 soluble solids, colour and firmness) of mango slices over refrigerated storage.

88

89 **2. MATERIALS AND METHODS**

90 **2.1. Mango slices preparation**

91 'Tommy Atkins' mangoes were purchased from a local market (Lleida, Spain) at commercial
92 maturity. Mango pH (3.46 ± 0.01) (Crison 2001 pH-meter; Crison Instruments S.A;
93 Barcelona, Spain), total soluble solids (13.9 ± 0.2 °Brix) (Atago RX-1000 refractometer,
94 Atago Company Ltd; Japan) and firmness (5.74 ± 0.7 N·s) (Texture Analyzer TA-XT2 Stable
95 Micro Systems Ltd., Surrey, England, UK) of the fruit flesh were determined before
96 processing. Whole mangoes were washed with an aqueous solution of sodium hypochlorite
97 ($300\mu\text{L/L}$) and then peeled and cut to obtain 5 mm-thick slices. Sliced mangoes were
98 inoculated and/or subjected to the different treatments, as described in the following sections.
99 Once treated, slices ($35\pm 1\text{g}$) were placed into transparent polypropylene trays and stored ($4 \pm$
100 1 °C) until analysis at days 0, 3, 7, 10 and 14.

101

102 **2.2. *Listeria innocua* culture and inoculation**

103 *L. innocua* IPL 1.17 (Institute Pasteur de Lille; Lille, France), as a surrogate of the pathogenic
104 *Listeria monocytogenes*, were provided from the culture collections of the Department of
105 Food Technology (University of Lleida, Spain). Stock culture of *L.innocua* was grown in
106 tryptone soy broth (TSB) with 0.6 % yeast extract (Bioakar Diagnostic; Beauvais, France) and
107 incubated at 35 °C with continuous agitation at 200 rpm for 15 h to obtain cells in stationary
108 growth phase (10^8 - 10^9 CFU/mL). Mango slices (35 g) were inoculated by spreading 100 μL

109 of *L.innocua* stock cultures over the entire upper surface with a sterile micropipette before
110 treatment and packaging (Ramos-Villarroel et al., 2011).

111

112 **2.3. Pulsed light treatment**

113 Pulsed light (PL) treatments were carried out with a XeMaticA-2L System (SteriBeam
114 Systems GmbH, Germany). The experiments were performed at a charging voltage of 2.5 kV.

115 The system is equipped with a lamp situated 8.5 cm above the sample holder. The lamp
116 delivered pulses of 0.3 ms with an overall radiant fluence of $0.4 \text{ J}\cdot\text{cm}^{-2}$ at the sample level.

117 The total light energy was measured according to the calibration of the equipment with a
118 standard light source estimated by photodiode readings and manufacturer's directions. The

119 emitted spectrum ranged from 180 - 1100 nm. To evaluate the effect of the wavelength of PL
120 on the inactivation of *L.innocua*, two types of UV filters were used: a 2 mm-thick Pyrex glass

121 filter that cuts off wavelengths below 305 nm hence allowing to pass some UVB, all UVA,
122 visible light (V) and infrared (IR) wavelengths (89 % of the emitted energy); and Makrolon

123 polycarbonate plastic filter that cuts all light below 400 nm, thus allowing only V and IR light
124 to pass through (83 % of the emitted energy). In addition, treatments with increasing number

125 of pulses (0, 10, 15, 20, 25, and 30) were assayed in order to evaluate the inactivation of
126 *L.innocua* as affected by PL.

127

128 **2.4. Alginate coating**

129 Film-forming solutions were prepared by dissolving 20 g of alginate coating (ALC) (FMC
130 Biopolymer Ladybum Works, USA) into 1000 mL of distilled water and homogenised with

131 an Ultra Turrax T25 (IKA WERKE, Germany). Calcium chloride (20 g) was dissolved into

132 1000 mL of distilled water to be used as a crosslinking agent (Sigma-Aldrich Chemic.

133 Steinhein, Germany). Mango slices were dipped into the sodium alginate solution (2 % w/v)
134 during 2 minutes and the excess was removed thereafter. A second dipping in calcium
135 chloride (2 % w/v) solution was performed for ALC-coated mango slices.

136

137 **2.5. Malic acid solution**

138 DL-Malic acid (20 g) (MA) (Fluka; Steinhein, Germany) was dissolved by stirring into 1000
139 mL of distilled water. Mango slices were dipped into MA solution during 2 minutes. It must
140 be noted that MA was incorporated to the calcium chloride solution when combined with the
141 edible coating.

142

143 **2.6. Combined treatments**

144 Different combinations of PL (20 pulses of broad-spectrum light), ALC (2 % w/v) and MA (2
145 %) were evaluated to elucidate possible synergistic, additive or antagonist effects. The
146 evaluated treatments were: ALC followed by PL (ALC-PL), MA followed by PL (MA-PL),
147 ALC followed by MA (ALC-MA), PL followed by ALC and MA (PL-ALC-MA) and ALC
148 followed by MA and PL (ALC-MA-PL). Untreated mango slices dipped in distilled water
149 were considered as a control reference treatment (C).

150

151 **2.7. Microbiological analyses**

152 Sliced mangoes (10 g) were placed into sterile plastic bags with 90 mL of saline peptone
153 water (Bioakar Diagnostic; Beauvais, France) and homogenized for 1 min in a stomacher
154 blender (IUL Instruments, Barcelona, Spain) for microbial analyses. Serial dilutions were
155 made and 100 µL were placed in Palcam agar plates (Bioakar Diagnostic; Beauvais, France)
156 and spread with a Drigalsky handle. The evaluation was made by duplicate for each dilution

157 and the plates were incubated for 48 h at 37 °C. Microbial population was evaluated and the
158 results expressed as \log_{10} CFU/g.

159 Enumeration of psychrophilic microorganisms on sliced mango was carried out by agar plate
160 counting (PCA) (Biokar Diagnostic; Beauvais, France), after incubation at 4 ± 1 °C for 10
161 days, following the ISO 17410 (2001) method. Mould and yeast counts were determined by
162 the ISO 7954 (1987) method using chloramphenicol glucose agar (CGA) (Biokar Diagnostic;
163 Beauvais, France) and incubating for 4 days at 25 ± 1 °C. Counts were expressed as \log_{10}
164 CFU/ g.

165

166 **2.8. Physicochemical determinations**

167 **2.8.1. pH and total soluble solids**

168 The pH (pH-meter Crison Instruments S.A. Barcelona, Spain) and total soluble solids (TSS)
169 (refractometer, Haake RS 80) were determined in a homogenate obtained from crushed
170 mango slices (20 g). Triplicate analyses were carried out and results were expressed as mean
171 and standard deviation.

172

173 **2.8.2. Colour parameters**

174 Colour was expressed as L^* , a^* and b^* , which indicate luminosity, chromaticity on a green (-)
175 to red (+) axis, and chromaticity on a blue (-) to yellow (+) axis, respectively. Lightness (L^*)
176 was determined with a tri-stimulus Minolta CR-400 colorimeter (Konica Minolta Sensing,
177 INC, Osaka, Japan) using a D65 illuminant and an observation angle of 10°. For reference, a
178 standard white tile ($Y=94.00$, $x=0.3158$, $y=0.3322$) was used. Based on the CIE L^* , a^* and b^*
179 values Hue angle (h°) was calculated (eq. 1). Colour parameters were obtained as the mean of
180 three determinations.

181 $h^{\circ} = \tan^{-1} (b^*/a^*)$ eq.1

182

183 **2.8.3. Firmness**

184 Firmness of sliced mangoes was analysed with a TA-XT2 Texture Analyser (Stable Micro
185 Systems Ltd., England, UK) by considering the impulse required to penetrate the fruit flesh
186 with a 4 mm diameter steel rod. To this purpose the area beneath the force-time curve was
187 recorded. The test speed was 4 mm/s and the distance of penetration was 4 mm. Results were
188 the mean of six measurements per sample and given as the delta firmness in N·s.

189

190 **2.9. Data analysis**

191 The treatments were conducted in duplicate; hence data were representative of two
192 independent experimental runs. Statistical analyses were performed using the Statgraphics
193 v.5.1 software (Manugistics, Inc. Rockville, MA, USA). The results were compared by
194 analysis of variance (ANOVA) followed by Tukey's multiple comparison test to determine
195 differences among means with a significance level of 5 %.

196 3. RESULTS AND DISCUSSION

197 3.1. Inactivation of *L. innocua* on mango slices

198 3.1.1. Effect of PL

199 The effect of PL spectral range and pulse number on the *L. innocua* population on mango
200 slices is shown in Fig. 1. Inactivation was higher as pulse number increased. However, no
201 additional inactivation was observed for treatments above 20 full spectrum pulses. Under
202 those conditions, corresponding to an energy of $8 \text{ J}\cdot\text{cm}^{-2}$, 3.15 log reductions of *L. innocua*
203 population were achieved. Similarly, Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny,
204 (2011) and Ramos-Villarroel, Aron-Maftei, Martín-Belloso, & Soliva-Fortuny (2014)
205 reported 2.61 and 2.97 log reductions of *L. innocua* population on fresh-cut avocado after
206 treatments of 15 and 30 pulses, respectively. In addition, microbial inactivation rates
207 decreased for intense treatments, probably due to the “shadow” effects caused by the
208 formation of biofilms, the product geometry and the internalization of microorganisms in the
209 fruit tissue. Furthermore, significant differences ($p < 0.05$) were observed between treatments
210 using full spectrum PL and spectral ranges in which the UV component was removed, either
211 partially ($\lambda = 305 - 1100 \text{ nm}$) or completely ($\lambda = 400 - 1100 \text{ nm}$). This is in line with other
212 studies describing a higher bactericidal effect for light wavelengths in the range of 250 - 270
213 nm than for those above 305 nm. Higher inactivation levels achieved when UV light was used
214 are related to the induction of DNA strand breaks and formation of pyrimidine dimers
215 (Guerrero-Beltrán, 2004; Keyser, Müller, Cilliers, Nel, & Gouws, 2008). However,
216 inactivation was yet significant for wavelengths above 400 nm, denoting a weak effect of light
217 on *L. innocua*. Although high-energy pulse light treatments present several drawbacks due to
218 the generation of heating on the product surface, no thermal effect was attributed to the

219 decrease of *L. innocua* population. Thus, inactivation was related with the amount of energy
220 received at the sample surface, wavelength and the microorganism type.

221

222 3.1.2. Effect of combined methods

223 The reduction of *L. innocua* counts on treated mango slices as affected by the combination of
224 PL, ALC and MA treatments is shown in Fig. 2. The assayed treatments led to a significant
225 ($p < 0.05$) reduction of the *L. innocua* survival fraction compared to those of untreated mango
226 slices. The three preservation factors applied individually led to a substantial reduction of the
227 initial counts. MA treatment was more effective than PL and ALC, in this order, leading to
228 2.9, 2.5 and 1.9 log reductions, respectively. Beyond the already discussed photochemical
229 effect of PL (Ross, Griffiths, Mittal, & Deeth, 2003), the antimicrobial effects of malic acid
230 have been reported to decrease the intracellular pH by ionization of un-dissociated molecules
231 (Ramos-Villarroel et al., 2014; Rathnayaka, 2013). Our results are in line with those reported
232 by Raybaudi-Massilia, Mosqueda-Melgar, Sobrino-López, Soliva-Fortuny, & Martín-Belloso,
233 (2009), who achieved 4 log reductions of *Listeria monocytogenes* counts in fresh-cut apples
234 dipped with MA. On the other hand, although sodium alginate has not been reported to
235 possess any antimicrobial effect, ALC treatments could remove part of microbial load or even
236 limit their ability to grow on the product surface.

237 Regarding the combined treatments, no synergistic effects were observed. However, the effect
238 of the combination of MA and PL was additive, leading to a maximum reduction of 4.49 log
239 cycles. It is noteworthy that the combination of the three preservation factors provided lower
240 inactivation levels, which at the same time depended on the order of the treatments
241 application. Hence, PL-ALC-MA and ALC-MA-PL treatments led to 3.92 and 3.03 log
242 reductions of the *L. innocua* populations, respectively. This fact was attributed to the

243 antagonistic action of the ALC factor, which may limit the effectiveness of the PL and MA
244 factors (Raybaudi-Massilia et al., 2010). Moreira, Tomadoni, Martín-Belloso, & Soliva-
245 Fortuny, (2015) observed that a gellan gum-based coating hindered the effectiveness of PL
246 applied to fresh-cut apples due to the blockage of a significant part of the UV-C radiation,
247 thus reducing the extent of photochemical inactivation. In this sense, our results are consistent
248 and suggest that PL treatments should be applied before alginate coating and malic acid
249 treatments. Although the feasibility of applying a combined methods strategy for reducing the
250 *L. innocua* populations growing on mango slices was demonstrated, a further experiment was
251 carried out to evaluate the impact of the assayed treatments on quality aspects throughout
252 refrigerated storage.

253

254 **3.2. Effects of combined treatments during storage on quality parameters of fresh-cut** 255 **mango**

256 **3.2.1. Microbial stability**

257 Among the naturally-occurring microbiota of untreated fresh-cut mangoes, moulds and yeasts
258 were initially predominant (Figure 3). However, after cutting, mould and yeast counts
259 remained almost stable, whereas psychrophilic bacteria abruptly increased. After the
260 application of the different treatments, both fungi and psychrophilic bacteria were reduced.
261 On the one hand, mould and yeast counts on the freshly cut untreated mango slices were
262 significantly ($p < 0.05$) reduced after the application of any of the individual or combined
263 treatments (fig. 3a). The lowest mould and yeast loads just after processing were observed for
264 mango slices subjected to PL or PL-ALC-MA, with reductions of 2.07 and 2.09 log cycles,
265 respectively. Mould and yeast inactivation achieved with the other treatments ranged from 1
266 to 1.5 log cycles. On the other hand, psychrophilic bacteria were less affected by the

267 treatments (fig. 3b). The highest inactivation of psychrophilic bacteria was obtained with the
268 PL treatment (1.37 log cycles), followed by the PL-ALC-MA treatment (1.09 logs cfu/g).
269 Mould and yeast counts observed after each treatment were maintained without substantial
270 change during the first week of storage. This lag period coincided with an increase in the
271 psychrophilic bacteria counts (Figure 3b) and might be directly attributed to the
272 environmental changes promoted by minimal processing. In general, psychrophilic bacteria
273 counts increased during the days thereafter processing but then they did not significantly
274 change during the storage. In contrast, an increase in the moulds and yeasts populations was
275 observed subsequently regardless the treatment applied. Microbial quality of mango slices
276 was best maintained after the application of the PL-ALC-MA treatment. Hence, those samples
277 exhibited the lowest counts for moulds and yeasts as well as for psychrophilic bacteria at the
278 end of the studied period.

279 In accordance with the results obtained for *L. innocua* inactivation, microbial growth was
280 influenced by the order of treatments combination. In this sense, PL application before
281 coating enabled light to more efficiently decontaminate mango surface from microorganisms.
282 Consistently, ALC, ALC-MA and ALC-PL treatments presented the highest microbial growth
283 over storage. However, when applied in the adequate order, alginate coatings could help to
284 maintain the integrity of damaged fruit tissues, thus limiting the presence of exudates and the
285 consequent proliferation of microorganisms, as in the case of mango slices subjected to the
286 PL-ALC-MA treatment.

287

288 **3.2.2. Physicochemical parameters**

289 **3.2.2.1. Total soluble solids (TSS) and pH**

290 Regarding TSS, significantly lower values, compared with the untreated, were observed just
291 after the processing with the exception of mango samples subjected to the PL treatment
292 (Table 1). These differences may be attributed to sugars lixiviation from the fruit slices when
293 immersed into the dipping solutions used in the ALC and MA treatments (Chiumarelli,
294 Ferrari, Sarantópoulos, & Hubinger, 2011; Hodges & Toivonen, 2008). Initial TSS values
295 were kept almost constant over storage although a slight decrease, probably caused by
296 microbial spoilage, was observed specially in untreated mango slices over the second week of
297 storage.

298 Concerning pH values, these were significantly affected by the type of treatment applied.
299 Malic acid dips resulted into a reduction of the natural pH of mango. However, this reduction
300 was not considered to play a significant role on quality stability, as the greatest pH change, as
301 much as 0.36 units, occurred after the PL-ALC-MA treatment. The decrease in pH may be
302 explained by the acidification of the cytoplasm which can be promoted by the production of
303 CO₂. As it is produced, this gas is partially dissolved in the water of the cellular tissues with
304 the consequent decrease of pH medium (A. Y. Ramos-Villarroel et al., 2011). PL applied
305 individually was not found to cause any pH modification. In contrast, alginate coated mango
306 slices exhibited increased pH values in comparison with those obtained for untreated sliced
307 mango. This may be attributed to the pH of the sodium alginate solution (pH = 4.3), which is
308 higher than that of untreated mango (pH = 3.5).

309 **3.2.2.2. Colour**

310 Lightness (L*) and hue angle (h°), of mango slices as affected by combined treatments are
311 displayed in Table 2. L* is an indicative parameter associated with the enzymatic browning of
312 fruit and vegetables. ALC treatments, either individually applied or in combination led to
313 decreased L* values compared to the colour of untreated mango slices (73.42 ± 2.53) or with

314 other combined treatments. L^* values of mango slices stored 14 days were similar to those of
315 the just processed although untreated mango slices increased L^* values up to 77.3 ± 3.5 . On
316 the other hand, mango slices had similar h° value between untreated (93.3 ± 1.1) and treated
317 mango slices at day 0. From then on, differences on h° values between treated and untreated
318 mango slices were not observed or were really scarce.

319 According to Chiumarelli et al., (2011) and Ramos-Villarroel et al. (2011) a decrease of L^*
320 and h° parameter in fresh-cut mango and avocado respectively was observed due to PL
321 treatment. Moreover, other authors suggested that mango cubes could develop undesirable
322 colour as a consequence of the exposure to visible light due to a decompartmentalization
323 process allowing colour substances such as phenolic compounds and carotenoids to come in
324 contact with oxidative enzymes (Charles et al., 2013; Gómez et al., 2012). Despite of this, the
325 present results indicated no signs of browning in treated mango slices. In this regard, the
326 chlorine wash may reduce the browning effect as reported by Chen, Zhu, Zhang, Niu, & Du
327 (2010) for fresh-cut lettuce. In addition, mango slices containing ALC preserved natural
328 pigments of mango, such as carotenoids, confined in the cells, thus, the oxidation was avoided
329 throughout the storage.

330 **3.2.2.3. Firmness**

331 Figure 4 shows the changes in firmness of mango slices as affected by combined treatments
332 and storage time. Treated mango slices, except those subjected to PL, ALC-MA and ALC-
333 MA-PL treatments, had higher firmness after processing than untreated mango slices. Fruits
334 are likely to soften mainly due to hydrolysis of the pectic acids found in cell walls.
335 Nevertheless, a protective effect of alginate and PL treatments against texture loss in mango
336 slices was observed during storage. This fact was attributed to the action of calcium ions,
337 which enable the crosslinking effect between the alginate polymer and calcium. Also, PL

338 could lead to the increase of polyamines, which could be related with a limitation of the
339 accessibility to the cell wall of the deleterious enzymes that promote softening (Charles et al.,
340 2013). Furthermore, an additive effect was observed when PL and alginate were combined. In
341 this sense, coating applied after PL treatment may have more influence on mango surface
342 texture than alone since pulsed light may have already increased the permeabilization of the
343 cell wall. Similarly, Gómez et al., (2012) observed, by light microscopy, that apple discs
344 treated by PL and dipping solution of ascorbic acid and calcium chloride increased the
345 resistance to rupture compared with untreated apple disc. This is in line with different studies
346 that described PL as a feasible treatment for firmness enhancement in fresh-cut fruits
347 (Gonzalez-Aguilar, Wang, Buta, & Krizek, 2001; Manzocco, Da Pieve, & Maifreni, 2011;
348 Ramos-Villarroel et al., 2011).

349

350

351 **CONCLUSIONS**

352 A PL treatment of 20 pulses of broad-spectrum light ($\lambda= 180 - 1100$ nm) with an overall
353 energy $8 \text{ J}\cdot\text{cm}^{-2}$ was most suitable for controlling the growth of *L. innocua* on fresh-cut
354 mango. The reduction could still be improved when MA-PL and PL-ALC-MA treatments
355 were used.

356 Moulds and yeasts and psychrophilic bacteria counts of mango slices were below 1×10^6
357 CFU/g after 14 days. In addition, the results suggested an additive effect on microbial load
358 reduction by treatments combination. In that sense, low microbial counts were obtained in
359 mango slices treated by those combined treatments where PL was applied first. PL, ALC, MA
360 and their combinations contributed to maintain the colour parameters of sliced mango for 14
361 days. Mango slices had high resistance to rupture when PL treatment was individually applied
362 or combined with both ALC and MA. Beyond confirming that PL plays an important role on
363 fresh-cut mango preservation, the present study indicated better quality parameters and
364 microbial stability in PL-ALC-MA treated mango slices.

365

366 **ACKNOWLEDGMENTS**

367 This work was supported by the University of Lleida (Spain) and financed by Tecnológico de
368 Monterrey (Research Chair Funds CAT-200 and CDB081)

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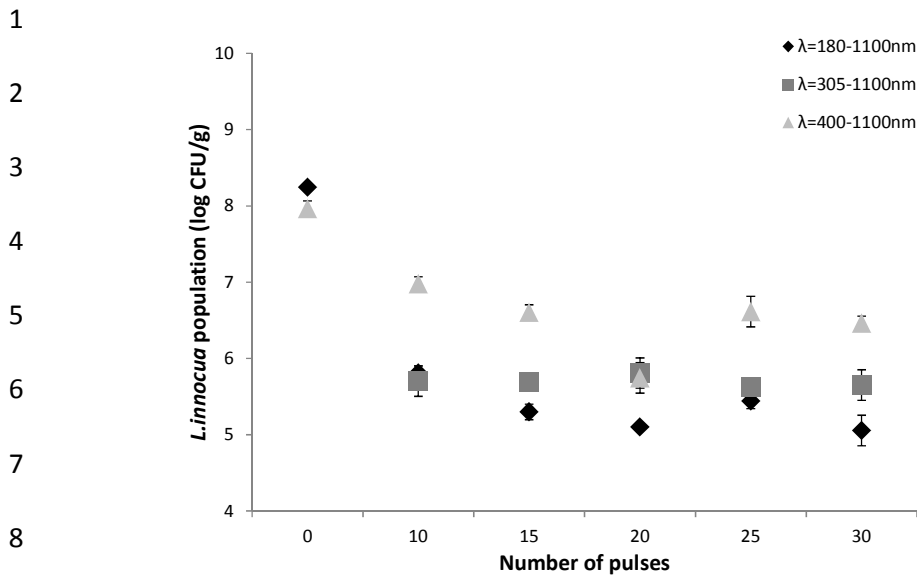
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1 **Table 1:** Changes in mango slices total soluble solids (TSS) and pH during 14 days of storage (4 °C) after treatments PL: pulsed light (20 pulses at $\lambda = 180$ -
 2 1100 nm and $0.4 \text{ J}\cdot\text{cm}^{-2}$ / pulse); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. Data shown are the mean of two replicate measurements
 3 \pm standard deviation. Values within a column followed by the same lowercase letter are not significantly different ($p < 0.05$). Values within the same line
 4 followed by the same uppercase letter are not significantly different ($p < 0.05$).

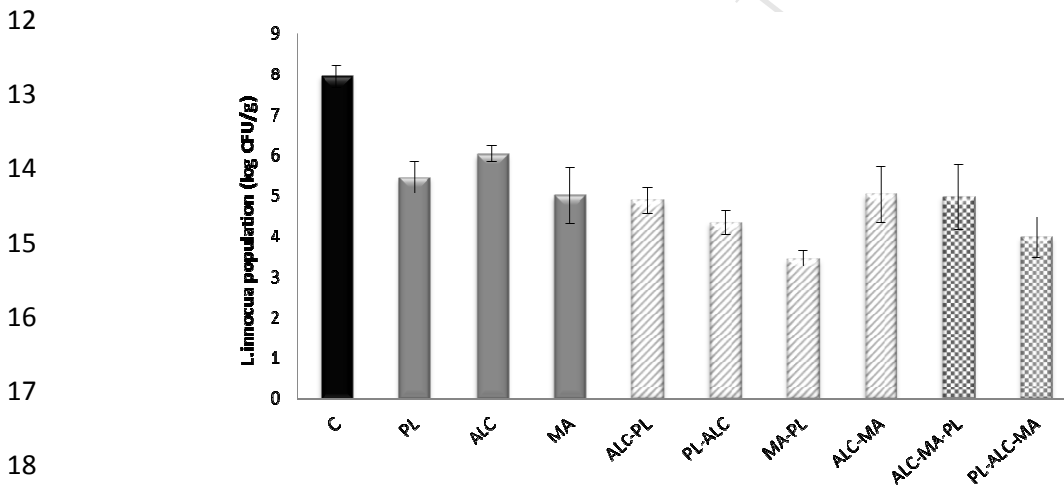
	TSS					pH				
	0	3	7	10	14	0	3	7	10	14
CONTROL	12.9 \pm 0.6 ^{Aa}	12.1 \pm 0.3 ^{Aa}	11.9 \pm 0.1 ^{ABa}	11.5 \pm 0.1 ^{Ba}	11.1 \pm 0.1 ^{Ba}	3.46 \pm 0.01 ^{Aa}	3.48 \pm 0.04 ^{Aa}	3.6 \pm 0.0 ^{Ba}	3.46 \pm 0.01 ^{Aa}	3.33 \pm 0.01 ^{Ca}
PL	12.6 \pm 0.9 ^{Aa}	12.6 \pm 0.8 ^{Aa}	12.4 \pm 0.1 ^{Ab}	13.1 \pm 0.5 ^{Bb}	13.6 \pm 0.1 ^{Ab}	3.37 \pm 0.05 ^{Aa}	3.45 \pm 0.09 ^{Ba}	3.5 \pm 0.0 ^{Ba}	3.33 \pm 0.01 ^{Ab}	3.56 \pm 0.01 ^{Bb}
ALC	11.3 \pm 0.9 ^{Abb}	11.3 \pm 0.7 ^{Aab}	10.6 \pm 0.2 ^{Ab}	10.9 ^A \pm 0.1 ^{Ac}	10.1 \pm 0.1 ^{Aa}	3.48 \pm 0.03 ^{Aa}	3.48 \pm 0.1 ^{Aa}	3.57 \pm 0.02 ^{Ba}	3.39 \pm 0.01 ^{Cb}	3.54 \pm 0.01 ^{Bb}
MA	11.3 \pm 0.2 ^{Abb}	10.9 \pm 0.1 ^{Bb}	11.0 \pm 0.1 ^{Ba}	10.4 \pm 0.1 ^{Bc}	10.5 \pm 0.1 ^{Ba}	3.28 \pm 0.03 ^{Ab}	3.13 \pm 0.1 ^{Bc}	3.41 \pm 0.05 ^{Cb}	3.36 \pm 0.01 ^{Ab}	3.57 \pm 0.01 ^{Cc}
PL-ALC	12.0 \pm 1.1 ^{Aa}	11.3 \pm 0.1 ^{Bab}	11.4 \pm 0.1 ^{Ba}	11.2 \pm 0.4 ^{Ba}	11.0 \pm 0.1 ^{Aa}	3.6 \pm 0.04 ^{Ac}	3.7 \pm 0.1 ^{Ab}	3.60 \pm 0.01 ^{Aa}	3.45 \pm 0.03 ^{Ba}	3.44 \pm 0.01 ^{Bc}
ALC-PL	10.9 \pm 0.2 ^{Ab}	11.5 \pm 0.8 ^{Aab}	11.1 \pm 0.1 ^{Aa}	10.9 \pm 0.5 ^{Ac}	10.4 \pm 0.1 ^{Bc}	3.69 \pm 0.01 ^{Ac}	3.63 \pm 0.06 ^{Ab}	3.61 \pm 0.01 ^{Aa}	3.51 \pm 0.02 ^{Bc}	3.64 \pm 0.01 ^{AcD}
MA-PL	12.0 \pm 0.9 ^{Aab}	12.0 \pm 0.6 ^{Aa}	11.3 \pm 0.1 ^{Ba}	11.6 \pm 0.1 ^{Ba}	11.5 \pm 0.1 ^{Aab}	3.22 \pm 0.03 ^{Ab}	3.22 \pm 0.02 ^{Ac}	3.3 \pm 0.0 ^{Ac}	3.51 \pm 0.01 ^{Bc}	3.53 \pm 0.02 ^{Cb}
ALC-MA	11.4 \pm 0.8 ^{Ab}	12.4 \pm 0.6 ^{Aa}	11.4 \pm 0.1 ^{Aa}	11.5 \pm 0.4 ^{Aa}	11.9 \pm 0.1 ^{Ac}	3.3 \pm 0.1 ^{Ab}	3.2 \pm 0.2 ^{Ac}	3.41 \pm 0.01 ^{Bb}	3.46 \pm 0.02 ^{Ba}	3.5 \pm 0.02 ^{Bb}
ALC-MA-PL	10.6 \pm 0.8 ^{Ab}	10.6 \pm 0.6 ^{Ab}	11.2 \pm 0.2 ^{Aa}	10.3 \pm 0.2 ^{Ac}	10.0 \pm 0.1 ^{Aa}	3.27 \pm 0.05 ^{Ab}	3.21 \pm 0.15 ^{Ac}	3.3 \pm 0.01 ^{Ac}	3.32 \pm 0.03 ^{Ab}	3.40 \pm 0.01 ^{Bc}
PL-ALC-MA	11.3 \pm 0.5 ^{Aab}	11.3 \pm 0.3 ^{Aab}	11.1 \pm 0.1 ^{Aa}	10.8 \pm 0.1 ^{Ac}	11.2 \pm 0.3 ^{Aa}	3.10 \pm 0.01 ^{Ab}	3.2 \pm 0.08 ^{Ac}	3.3 \pm 0.01 ^{Ac}	3.65 \pm 0.01 ^{Bcd}	3.45 \pm 0.01 ^{Bc}

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 6 **Table 2:** Changes in mango slices lightness (L^*) and hue angle (h°) during 14 days of storage (4 °C) after treatments PL: pulsed light (20 pulses at $\lambda = 180$ -
 7 1100 nm and $0.4 \text{ J}\cdot\text{cm}^{-2}$ / pulse); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. Data shown are the mean of two replicate measurements
 8 \pm standard deviation. Values within a column followed by the same lowercase letter are not significantly different ($p < 0.05$). Values within the same line
 9 followed by the same uppercase letter are not significantly different ($p < 0.05$).

	L^*					h°				
	0	3	7	10	14	0	3	7	10	14
CONTROL	73.42 \pm 2.53 ^{Aabc}	71.99 \pm 2.58 ^{Aa}	74.86 \pm 3.97 ^{Aa}	77.2 \pm 2.4 ^{Ba}	77.3 \pm 3.5 ^{Ba}	93.3 \pm 1.1 ^{Ac}	97.15 \pm 2.47 ^{Ba}	93.7 \pm 1.1 ^{Aa}	95.24 \pm 1.08 ^{Ba}	95.8 \pm 1.6 ^{Ba}
PL	76.41 \pm 1.96 ^{Ac}	73.57 \pm 0.19 ^{Ba}	72.04 \pm 2.31 ^{Ba}	78.4 \pm 0.3 ^{Ca}	76.57 \pm 2.37 ^{Aa}	94.2 \pm 0.2 ^{Aab}	94.83 \pm 1.20 ^{Ab}	94.14 \pm 2.36 ^{Aa}	93.86 \pm 0.55 ^{Bb}	92.6 \pm 0.8 ^{Bb}
ALC	73.85 \pm 1.05 ^{Aabc}	67.2 \pm 2.9 ^{Bab}	75.10 \pm 0.54 ^{Cb}	71.7 \pm 1.1 ^{Db}	72.2 \pm 2.1 ^{ADb}	94.81 \pm 0.44 ^{Aab}	97.01 \pm 0.23 ^{Ba}	94.74 \pm 0.17 ^{Aa}	92.94 \pm 0.04 ^{Cb}	95.51 \pm 1.67 ^{Aa}
MA	77.6 \pm 2.7 ^{Aabc}	72.7 \pm 1.4 ^{ABa}	77.20 \pm 0.04 ^{Ac}	75.6 \pm 2.0 ^{Aa}	76.8 \pm 4.1 ^{ABa}	95.3 \pm 0.5 ^{Aa}	97.33 \pm 0.55 ^{Aa}	96.5 \pm 0.4 ^{Bb}	92.84 \pm 0.26 ^{Cb}	95.23 \pm 2.16 ^{Aa}
PL-ALC	72.98 \pm 0.08 ^{Aab}	64.57 \pm 1.93 ^{Bab}	72.40 \pm 0.13 ^{Aa}	70.0 \pm 2.5 ^{Ab}	71.1 \pm 1.0 ^{Ab}	94.03 \pm 0.88 ^{Abc}	96.55 \pm 0.04 ^{Bc}	94.09 \pm 0.06 ^{Aa}	93.85 \pm 0.88 ^{Abc}	96.5 \pm 0.1 ^{Ba}
ALC-PL	69.61 \pm 1.68 ^{Aabc}	66.4 \pm 1.2 ^{Bab}	66.80 \pm 1.54 ^{Ac}	68.2 \pm 0.7 ^{Bc}	67.1 \pm 1.8 ^A	95.9 \pm 2.3 ^{Aa}	99.72 \pm 1.97 ^{BaD}	94.95 \pm 0.35 ^{Ca}	95.9 \pm 0.5 ^{Aa}	95.01 \pm 0.06 ^{Aa}
MA-PL	78.01 \pm 3.15 ^{Aabc}	71.26 \pm 2.66 ^{Bab}	71.7 \pm 3.9 ^{Aa}	72.47 \pm 0.78 ^A	72.90 \pm 2.46 ^{Ab}	95.20 \pm 3.22 ^{Aa}	97.37 \pm 2.78 ^{Aa}	92.97 \pm 0.45 ^{Bab}	93.03 \pm 0.59 ^{Bcb}	94.95 \pm 1.10 ^{Ca}
ALC-MA	72.8 \pm 1.2 ^{Aabc}	65.9 \pm 1.9 ^{Bab}	68.5 \pm 1.9 ^{Bc}	66.89 \pm 1.70 ^{Bc}	72.73 \pm 2.32 ^{Ab}	92.35 \pm 1.45 ^{Ac}	97.01 \pm 3.28 ^{Ba}	92.77 \pm 3.10 ^{Ab}	92.6 \pm 1.8 ^{Ab}	93.39 \pm 2.05 ^{Ab}
ALC-MA-PL	71.33 \pm 4.87 ^{Aa}	65.22 \pm 1.29 ^{Bab}	70.70 \pm 3.37 ^{Aa}	71.8 \pm 6.4 ^{Ab}	69.85 \pm 1.31 ^{Ac}	94.7 \pm 1.9 ^{Abc}	98.25 \pm 0.93 ^{BaD}	95.2 \pm 1.5 ^{Aab}	95.8 \pm 3.2 ^{Ba}	95.65 \pm 2.85 ^{Ba}
PL-ALC-MA	71.6 \pm 3.5 ^{Abc}	67.13 \pm 2.64 ^{Bab}	71.73 \pm 4.28 ^{Ba}	68.6 \pm 0.2 ^{Bc}	70.16 \pm 0.06 ^{Ac}	93.9 \pm 1.3 ^{Aa}	97.26 \pm 1.68 ^{BaD}	94.2 \pm 0.8 ^{Aab}	94.0 \pm 1.3 ^{Aab}	94.5 \pm 0.5 ^{Aa}



9 **Figure 1:** Influence of the spectral range on survival of *L. innocua* inoculated in mango
10 slices treated by pulsed light at different number of pulses. The values are the mean of
11 four determinations \pm SD.



19 **Figure 2:** Influence of different individual or combined treatments on survival of *L.*
20 *innocua* inoculated in mango slices. PL: pulsed light (20 pulses at $\lambda = 180- 1100$ and 0.4
21 $\text{J}\cdot\text{cm}^{-2}/\text{pulse}$); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. The
22 values are the mean of four determinations \pm SD.

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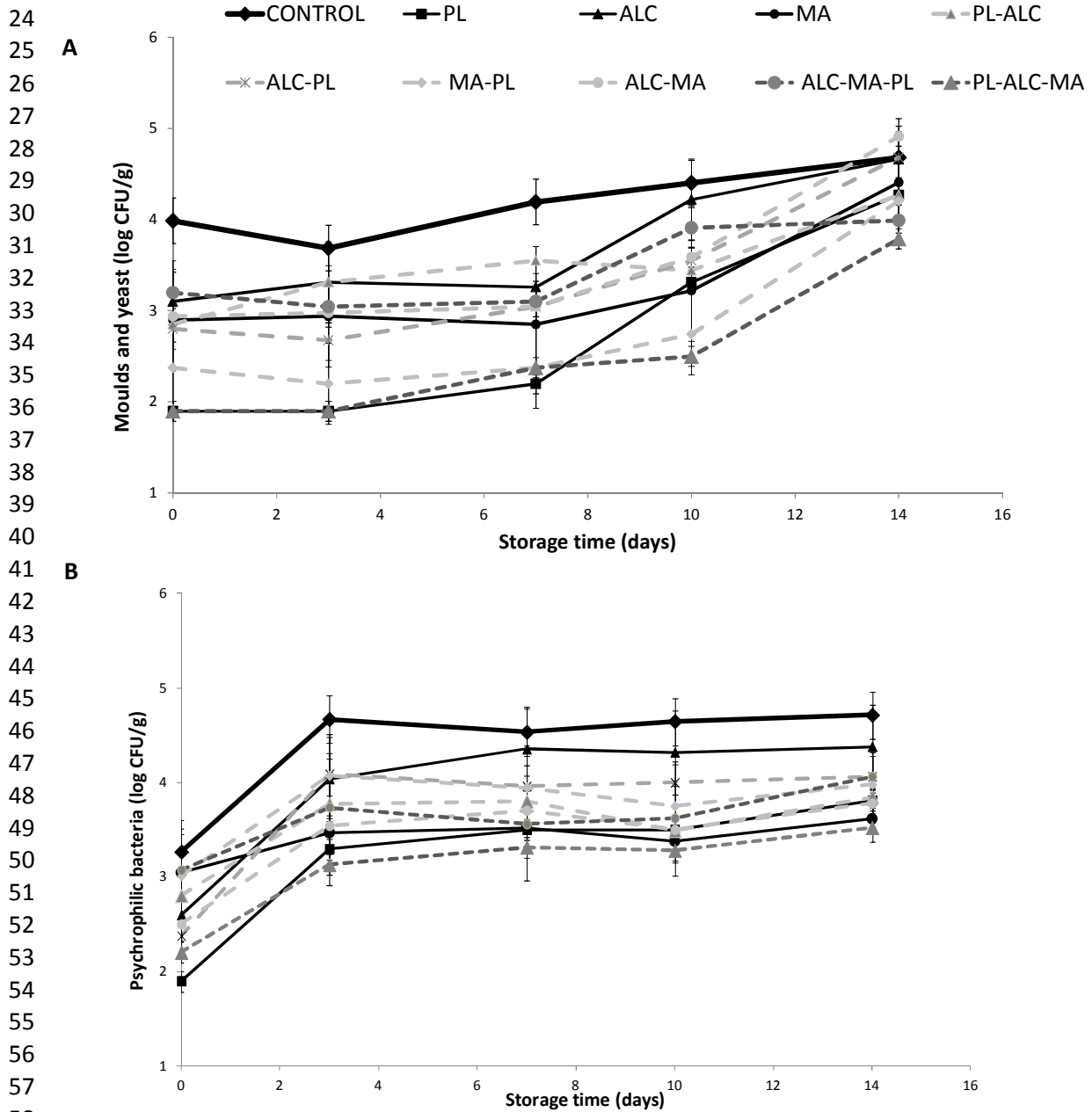
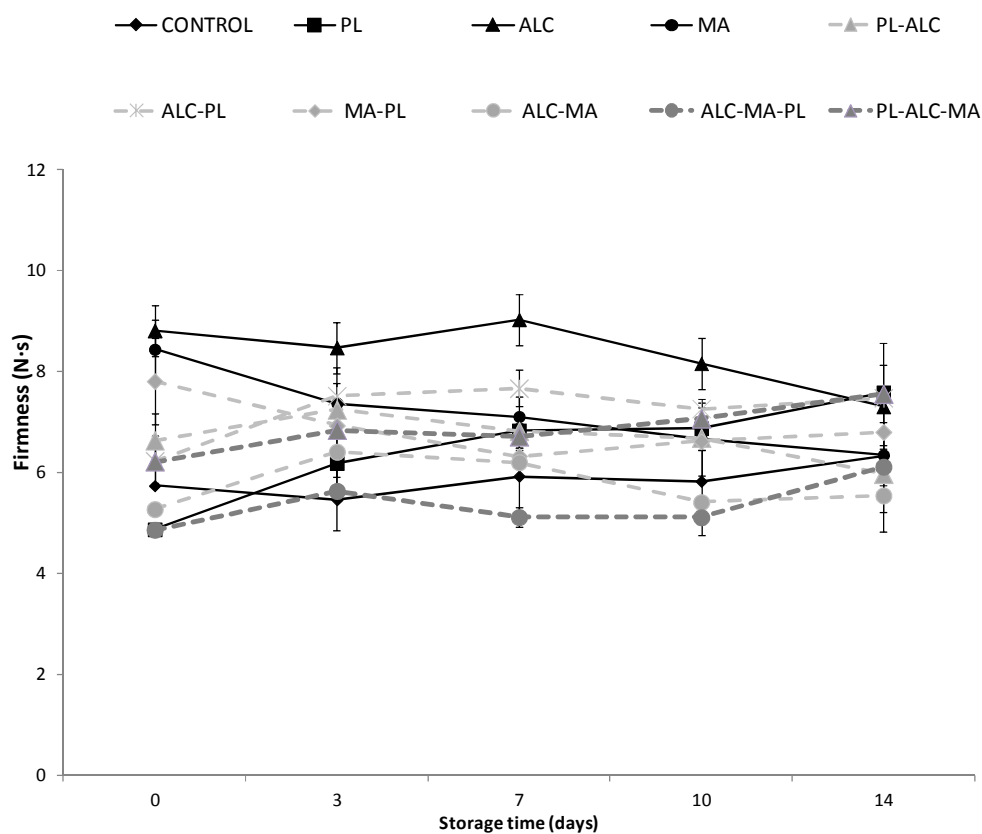


Figure 3: Growth of moulds and yeasts (A) and psychrophilic bacteria (B) on sliced mango submitted to different treatments throughout 14 days of storage at 4 °C. PL: pulsed light (20 pulses at $\lambda = 180- 1100$ and $0.4 \text{ J}\cdot\text{cm}^{-2}/\text{pulse}$); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. Data shown are the mean of two replicate measurements obtained from two replicate packages \pm standard deviation.



68
 69 **Figure 4:** Changes of firmness in stored mango slices after expose them to different
 70 treatments. PL: pulsed light (20 pulses at $\lambda = 180- 1100$ nm and $0.4 \text{ J}\cdot\text{cm}^{-2}/$ pulse);
 71 ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. Data shown are
 72 the mean of six measurements \pm SD.

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Highlights

- Broad spectrum pulsed light treatment reduced *Listeria innocua* population in fresh-cut mango.
- Pulsed light combined with edible coating and malic acid had an additive effect on microbial reduction in fresh-cut mango.
- Combined treatments maintained physical attributes of fresh-cut mango throughout storage.
- Pulsed light as a first hurdle in combination with the other treatments enhanced quality of fresh-cut mango for 14 days.