Preservation of Fresh-cut Apple Quality Attributes by Pulsed Light in Combination with Gellan Gum-Based Prebiotic Edible Coatings

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Abstract

Pulsed light (PL) has received considerable attention during the last years as a non-thermal method for the superficial decontamination of fresh foods. The aim of the present study was to evaluate the quality attributes of fresh-cut 'Golden Delicious' apples as affected by the combined application of a pulsed light treatment (12 J/ cm^2) and a gellan-gum based (0.5% w/v) edible coating enriched with apple fiber. Changes in color, firmness, antioxidant capacity, microbial growth and sensory attributes were determined during 14 days of storage at 4 °C. The combined application of coating and PL treatment retarded the microbiological deterioration of fresh-cut apples and maintained the sensory attribute scores above the rejection limits after prolonged storage. Incorporation of fiber in the coating formulation did not curb the sensory acceptability of apple cubes. Results show that the use of a gellan-gum based coating incorporating apple fiber followed by the application of a PL treatment significantly reduced softening and browning of apple pieces through storage.

Our results reveal that PL treatments applied to gellan-coated fresh-cut apples can be used to decontaminate the cut fruit surface without dramatically affecting its fresh-like quality attributes, thus conferring prebiotic potential and contributing to their shelf-life extension.

Keywords: edible coatings; fresh-cut apples; apple fiber; pulsed light; quality.

1. Introduction

Minimal processing is emerging as an alternative for the provision fresh-like, highly nutritious, convenient and healthful commodities. However, mechanical bruises caused during processing and handling may compromise the safety and appearance of fresh-cut produce, leading to an increase in the respiratory rates and triggering multiple biochemical reactions that underlie microbiological spoilage and quality deterioration (Moreira, Roura, & Ponce, 2011; Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008; Ramos, Miller, Brandao, Teixeira, & Silva, 2013; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007).

Different technologies are currently investigated with the aim of decontaminating fresh-cut produce avoiding physical and chemical changes associated to processing. Pulsed light (PL) is a non-thermal technology based on the application of intense pulses of short duration to effectively inactivate microorganisms contained either in light-transmitting media or on opaque surfaces (Gómez-López, Ragaert, Debevere, & Devlieghere, 2007; Marquenie, Michiels, Van Impe, & Nicolai, 2003). The treatment has been demonstrated to be cost effective and feasible for the microbial inactivation of both solid and liquid food products (Ramos-Villarroel, Aron-Maftei, Martín-Belloso, & Soliva-Fortuny, 2014). On the other hand, the use of edible coatings is another alternative under investigation to extend the shelf-life of fresh-cut products (Alvarez, Ponce & Moreira, 2013; Tharanathan, 2003). Gellan gum, a microbial polysaccharide secreted by the bacterium *Pseudomonas elodea*, exhibits unique colloidal and gelling properties and, therefore, good ability to form coatings. These coatings may also serve

62 as carriers of food additives such as antibrowning and antimicrobial agents, colorants,
63 flavors, nutrients, spices and nutraceuticals (Oms-Oliu et al, 2008; Oms-Oliu, Martín-
64 Belloso, & Soliva-Fortuny, 2010a; Robles-Sanchez, Rojas-Graü, Odriozola-Serrano,
65 Gonzalez-Aguilar, & Martín-Belloso, 2013). Among these last compounds, fiber was
66 one of the first ingredients associated with health and has been used in food industry
67 since 1980s (MoraesCrizel, Jablonski, Oliveira, Rios, & Rech, 2013). However, the fiber
68 intake in most developed countries fells below the levels recommended by health
69 authorities, which usually suggest amounts of total dietary fiber above 25 g/day for
70 adults, of whom one third should be soluble fiber. Fiber incorporation into edible
71 coating formulations may help to meet the daily intakes lagging far below the
72 recommended dietary allowances. Apple dietary fiber, as those obtained from most
73 fruit and vegetable products, possesses a higher soluble portion and better
74 antioxidant properties than fibers from cereal sources (Marín, Soler-Rivas, Benavente-
75 Garcíentala, Castillo, & Pérez-Alvarez, 2007; O'Shea, Arendt, & Gallagher, 2012).

76 Both PL and edible coatings have been applied to fresh-cut produce with the
77 objectives of reducing the incidence of foodborne pathogens, extending the produce
78 shelf-life, and reducing food quality losses along the distribution chain (Oms-Oliu et
79 al., 2010a; Ramos-Villarroel et al., 2011b). Gellan gum-based edible coatings have
80 been shown to be effective in maintaining the fresh-like quality attributes of fresh-cut
81 fruits such as apples, melons, and pears (Oms-Oliu et al., 2008; Pérez-Gago, Alonso,
82 Mateos, & del Rio, 2005; Rojas-Graü et al., 2008). As well, the ability of PL treatments
83 to inactivate microorganisms on fresh-cut fruit surfaces has been demonstrated in
84 several published studies (Gómez, Salvatori, García-Loredo, & Alzamora, 2012a;

Izquier & Gómez-Lopez, 2011; Oms-Oliu et al., 2010b; Ramos-Villarroel et al., 2014). However, so far the combined effect of PL treatments and the use of edible coatings to inhibit microbial growth and to extend the shelf-life of fresh-cut fruits has not been evaluated. Furthermore, the addition of prebiotics for the promotion of health-related properties in such products has been scarcely studied. The main objective of this research was to evaluate the combined application of PL treatments with gellan-gum edible coatings incorporating apple fiber on the quality of fresh-cut apples.

2. Materials and methods

2.1. Materials

'Golden delicious' apples were purchased in a local wholesale distributor (Lleida, Spain) at commercial maturity and stored at 4±1 °C until processing. Food grade gellan gum (Kelcogel®, CPKelco, Chicago, IL, USA) was used as the carbohydrate film-forming biopolymer in coating formulations. Glycerol (Merck, Whitehouse Station, NJ, USA) was added to the coatings as plasticizer. Calcium chloride (Sigma-Aldrich Chemic, Steinhein, Germany) was used to induce crosslinking between the polymer chains. Ascorbic acid (Sigma-Aldrich Chemic, Steinhein, Germany) was added to prevent oxidation of the fruit surface. Dietary fiber concentrate from apple was kindly supplied by the factory Indulleida S. L. (Alguaire, Lleida, Spain). This apple dietary fiber concentrate was the result of drying the washed apple bagasse remaining after apple juice extraction.

106 *2.2. Preparation of film forming and crosslinking solutions*

107 Film-forming solutions were prepared by dissolving gellan (5 g/L water) powders in
108 distilled water and heating at 70 °C while stirring until the solution became clear.
109 Gellan solutions were prepared with and without apple fiber addition (2 g/L). Glycerol
110 was incorporated to the gellan solutions at a concentration of 0.6 g/100 mL. On the
111 other hand, a crosslinking solution was prepared by adding calcium chloride (20 g/L)
112 to an aqueous solution containing 10 g/L ascorbic acid. The concentrations of all
113 ingredients used in these formulations were set up according to previous studies
114 (Rojas-Graü et al., 2008).

115

116 *2.3. Fruit coating*

117 Apples were gently washed, rinsed and dried prior to the cutting operations.
118 Subsequently, each fruit was peeled, cored and diced into 1 cm-thick cubes. A
119 maximum of four fruits were processed at the same time to avoid oxidation before
120 treatments. Apple dices were first dipped for 2 min into a gellan gum film-forming
121 solution, either with or without added apple fiber. The excess of coating solution was
122 allowed to drip off for 1 min before submerging the fruit pieces for 2 min into the
123 crosslinking dip containing ascorbic acid and calcium chloride. Control samples were
124 dipped only into the crosslinking solution. Ten apple cubes (ca. 60 g) were placed into
125 polypropylene trays of 500 cm^3 (Mcp Performance Plastic LTD, Kibbutz Hamaapil,
126 Israel), which were wrap-sealed with a 64 μm-thick polypropylene film with a
127 permeability to oxygen of 110 cm^3 O_2 m^{-2} bar^{-1} day^{-1} at 23 °C and 0% RH (Tecnopack
128 SRL, Mortara, Italy) using a horizontal thermosealing machine (IlpraFoodpack Basic

V/G, Ilpra, Vigenovo, Italy). Trays were heat-sealed and stored at 4±1 °C during less than 30 min prior to PL-processing.

2.4. Pulsed light treatment

The trays containing gellan gum-coated apple cubes were exposed to PL treatments delivered by a XeMaticA-2L device (SteriBeam Systems GmbH, Germany). The system is equipped with two lamps situated at 8.5 cm above and below a quartz sample holder. Experiments were carried out at a charging voltage of 2.5 kV. Each lamp delivered 30 pulses of duration of 0.3 ms with an emitted fluence of 0.4 J/cm^2 at the sample level, thus resulting in an accumulated energy of 12 J/cm^2. The emitted spectrum wavelengths (λ) ranged from 180 to 1100 nm with 15–20% of the light in the UV region. Energy calculations were carried out according to the calibration of the equipment with a standard light source estimated by photodiode readings and following manufacturer's directions. Furthermore, transparency of the polypropylene film in the UV region was found to be above a 97% of the total emitted energy. Reduction of light transmission was negligible for visible wavelengths and increased for shorter wavelengths. However, only a 15% of the incident energy corresponding to wavelengths between 200 and 320 nm was blocked by the packaging material. Furthermore, spectroscopic measurements of the the film-forming solution were carried out to optically characterize the gellan gum coating. The transmittance of the coating was calculated considering a film thickness of 155.75 mm, as reported in previous studies (Rojas-Graü et al., 2007).

151 Temperature increase during the treatments was prevented by coupling a lab vacuum
152 air extractor device to the treatment chamber. Temperature was measured with a
153 thermocouple attached to the package surface and never exceeded 30 °C.
154 Measurements were also taken at the surface of unpackaged fruit prices over the PL
155 treatment to guarantee that abusive temperatures were not reached. Untreated
156 coated apple cubes and PL-treated uncoated apple cubes were used as reference
157 treatments. Immediately after processing, the samples were stored at 4 °C in the
158 absence of light. Analyses were carried out periodically through 14 days for randomly
159 sampled pairs of trays.
160
161 *2.5. Microbiological analysis*
162 Mesophilic aerobic, psychrophilic and yeast and mold counts on fresh-cut apples
163 subjected to the different treatments were evaluated throughout storage. A portion of
164 10 g of apple, obtained from eight different apple pieces, was aseptically removed
165 from each tray and transferred into sterile plastic bags. Samples were diluted with
166 90 mL of saline peptone water (0.1 g peptone/100 mL water, Biokar Diagnostics,
167 Beauvais, France) and homogenized for 1 min in a stomacher blender (IUL
168 Instruments, Barcelona, Spain). Serial dilutions were made and then pour-plated onto
169 plate count agar (PCA) and chloramphenicol glucose agar (GCA) (Biokar Diagnostics,
170 Beauvais, France). Plates were incubated for 48 h at 30 °C to determine mesophilic, 5-
171 7 days at 5 °C for psychrophilic counts and 3-5 days at 25 °C for yeast and mold counts
172 (Alvarez et al., 2013). Colonies were counted and the results expressed as CFU/g of

apples. Analyses were carried out periodically during 14 days in randomly sampled pairs of trays. Two replicate counts were performed for each tray.

2.6. Antioxidant capacity

The antioxidant capacity of the fruit samples was evaluated using the method described by Odriozola-Serrano, Soliva-Fortuny, and Martín-Belloso (2008), which determines the free radical-scavenging effect of a sample extract on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The DPPH assay provides an estimate of the overall antioxidant capacity of a sample, since it is not specific to any particular antioxidant compound. Apple cubes were crushed and centrifuged at 10.000g for 15 min at 4 °C (Centrifuge Medigifer; Select, Barcelona, Spain). The supernatant was collected and filtered. Thereafter, 3.9 mL of methanolic DPPH solution (0.025 $g \cdot L^{-1}$) were added to 100 μL of the clarified extract. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorbance at 515 nm was read with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) against a blank of methanol without DPPH. Antioxidant capacity was calculated as the percentage inhibition of the DPPH radical with respect to the initial amount in a blank DPPH solution with 100 μL of water.

2.7. Color measurement

Cut apple surface color was measured with a Minolta chroma meter (Model CR-400, Minolta, Tokyo, Japan). The equipment was set up for illuminant D75 and 10° observer angle and calibrated using a standard white reflector plate (Y=94.00,

196 x=0.3158, y=0.3322). Five replicates were evaluated for each tray and three measures
197 of the CIE L*, a* and b* values were read per replicate by changing the position of the
198 fruit pieces. Color modification was evaluated through changes in lightness (L*) and
199 hue (h^*). Hue was calculated from a^* (red-green) and b* (blue-yelow) chromatic values
200 with the following expression: $h^* = \arctan(b^*/a^*)$.
201
202 *2.8. Firmness measurements*
203 Apple firmness was evaluated using a TA-XT2 Texture Analyzer (Stable Micro Systems
204 Ltd., England, UK) by measuring the maximum penetration force required for a 4 mm
205 diameter probe to penetrate into apple cubes of 1 cm height to a depth of 5 mm at a
206 rate of 5 mm/s. Ten apple cubes, randomly withdrawn from each pair of trays, were
207 placed perpendicular to the probe so as to penetrate the center of the fruit pieces.
208
209 *2.9. Sensory acceptability*
210 Sensory acceptability of treated and untreated apple cubes was determined by judges
211 who regularly consume apples. For the hedonic tests, ten individuals aged between 20
212 and 30 year old who like and eat apple frequently were recruited among the research
213 personnel of the Department of Food Technology, University of Lleida, Spain and
214 specifically trained to evaluate color, firmness, taste, and overall preference.
215 Evaluations were performed immediately after sample withdrawal from refrigerated
216 packages. The order of the samples was randomized for each judge. They were asked
217 to evaluate each of the samples attributes on non-structured linear scales with anchor
218 points at each end, where 0 indicated extreme dislike and 5 indicated extreme like.

219 The judges' average response was calculated for each attribute. The limit of
220 acceptance was three; hence a score below 3 for any of the evaluated attributes was
221 deemed to indicate end of shelf-life from a sensory point of view (Alvarez et al., 2013).

222 *2.10. Statistical analysis*

223 Data were analyzed using the SAS software (version 9.0, SAS Inst. Inc., Cary, NC, USA).
224 Differences between means were determined using the LSD (least significant
225 difference) test. PROC GLM (general linear model procedure) was used for the
226 variance analysis (ANOVA). Differences were determined by the Tukey–Kramer
227 multiple comparison test ($p < 0.05$). PROC UNIVARIATE was used to validate the
228 ANOVA assumptions. Each processing condition was assayed in duplicate. Each
229 duplicate belong to a separate experimental run. Analytical determinations for each
230 sample were assayed in triplicate.
231

3. Results and Discussion

3.1. Microbial counts

Figure 1 shows the growth of naturally-occurring microorganisms on either coated or uncoated apple cubes exposed to PL treatments. The proliferation of mesophilic aerobic bacteria on fresh-cut apples subjected to the different treatments is displayed in Figure 1A. Mesophilic microorganisms provide an estimate of total viable populations and are indicative of the endogenous microbiota and the contamination undergone by the material (Ponce, Roura, & Fritz, 2002). Just after processing, the initial mesophilic counts on untreated and coated samples not subjected to PL treatment were in the range of 3.7 to 4.0 log CFU/g. The application of PL significantly reduced the initial mesophilic counts (3.0 log CFU/g) regardless the coating application. PL exerted a significant ($p < 0.05$) inactivating effect on the initial mesophilic counts of both uncoated and coated apple cubes. Nevertheless, scarce differences ($p < 0.05$) were observed between the mesophilic counts treated and untreated fresh-cut apples throughout storage. Gomez-López et al. (2007) reported that shielding of microorganisms by rough apple surface and microorganism internalization in apple tissue pores may greatly influence the inactivation patterns. Aerobic counts increased by ca. 4.0 log CFU/g on untreated apple cubes, while microbial loads on treated fruit increased by 2.0-3.0 log CFU/g throughout 2 weeks, regardless the applied treatment. Hence, untreated control apple cubes exhibited significantly higher mesophilic aerobic microbial counts ($p < 0.05$) at 14 d of storage than apple pieces subjected to PL treatments and/or coated with gellan gum. At that

255 point, the greatest inhibition of microbial growth (>2.0 log CFU/g) was found for
256 coated PL-treated apple cubes without incorporation of fiber.

257 The counts of psycrotrophic aerobic bacteria on fresh-cut apples as affected by the
258 different treatments are shown in Figure 1B. Psycrotrophic aerobes represent an
259 important group of microorganisms in fresh-cut products. Although they usually
260 constitute a small percentage of the initial microbiota, they could survive and
261 eventually predominate under chill temperatures recommended for the storage of
262 these commodities (Ponce et al., 2002). Consistently with this statement, the initial
263 counts were low as compared with the significantly higher mesophilic aerobic counts.
264 In addition, significant differences ($p < 0.05$) between the counts of untreated and
265 treated apple pieces were not evidenced during the first 10 days of storage.
266 Nevertheless, counts of psychrophiles on untreated apple pieces increased by about
267 2.5 log CFU/g through 14 days, whereas for any of the evaluated treatments, the
268 increase during the same period was in the range of 1.0 log CFU/g. Interestingly,
269 uncoated PL-treated fresh-cut apples were the only samples to exhibit significantly
270 lower counts ($p < 0.05$) than the untreated fruit throughout the whole storage period.
271 This fact suggests that gellan coating layers could hinder microbial inactivation by
272 pulsed light, which was confirmed by the spectrometric readings. Transmittance
273 values in the UV-A, UV-B and UV-C regions were calculated to be 99.3%, 99.0% and
274 73.0%, respectively, thus indicating that the coating could block not all but a
275 significant part of the incident UV-C radiation.

276 Regarding the effect of the PL treatment, our results are in accordance to those
277 reported by other authors. Luksiene, Buchovec, Paskeviciute, and Viskelis (2012)
278 reported inactivation levels of naturally distributed mesophilic bacteria in different
279 PL-treated fruit and vegetables such as plums, cauliflowers, sweet peppers and
280 strawberries between 1.0 to 1.3 log CFU/g, thus indicating the feasibility of such
281 technology to reduce contamination in food products with surface irregularities. As
282 well, Gómez-López, Devlieghere, Bonduelle, and Debevere (2005) reported significant
283 reductions (1.0 to 2.0 log CFU/g) in mesophilic bacteria counts after treating
284 minimally processed vegetables (spinach, carrot, cabbage) by PL. Similar results were
285 reported by Aguiló-Aguayo, Charles, Renard, Page, and Carlin (2013), working with
286 PL-treated tomatoes stored during 15 days. As well, Oms-Oliu et al. (2010a)
287 investigated the effects of PL treatments on microbial quality of fresh-cut mushrooms
288 and recommended the application fluencies of up to 12 J/cm^2 in combination with the
289 use of antibrowning agents for extending the microbiological shelf-life of fresh-cut
290 mushrooms without affecting their quality and antioxidant properties. However, our
291 results seem to point out a certain antagonistic effect of the combined use of PL
292 treatments and gellan gum edible coatings regardless the addition of dietary fiber.
293 This could probably be attributed to a protective influence of the gellan coating layer
294 towards microorganisms growing on the surface of the cut fruit.

295 The counts of yeast and mold counts on fresh-cut apples are displayed in Figure 1C.
296 Yeast and molds act towards the fruit tissues sometimes as strict plant parasites and
297 sometimes as latent parasites, depending on the plant resistance, the virulence of the
298 strain, the competing microbiota, and the ambient conditions. They may present a

299 dramatic change in their growth rate after harvest, when the plant resistance is
300 diminished, and lead to rapid spoilage (Ponce et al., 2002). Initial yeast and mold
301 counts were in the range of 3.0 to 4.0 log CFU/g. In addition, most treatments resulted
302 into similar or even higher counts than those found in the untreated product. Only
303 uncoated apples treated with PL underwent a reduction in their initial counts. These
304 lower values were consistent through storage. Combinations of PL treatment and
305 edible coatings, with or without incorporated apple fiber, generally resulted into
306 scarce but significant ($p < 0.05$) reductions of the mould and yeast counts with respect
307 to their reference treatments without PL exposure. Our results regarding the effect of
308 PL treatment are consistent with those of Aguiló-Aguayo et al. (2013), who reported
309 that PL treatments caused a significant reduction (approximately 1 order log) in yeast
310 and mold counts on tomatoes kept during 15 days. Other authors have reported
311 significantly lower initial microbial counts on apple slices compared to those
312 presented in this work (Gómez et al., 2012; Ignat, Manzocco, Maifreni, Bartolomeoli, &
313 Nicoli, 2014; Rojas-Graü et al., 2007). In particular, Ignat et al. (2014) reported low
314 counts of mesophilic bacteria (2.2 log CFU/g) and yeast and mold counts below the
315 detection limits (50 CFU/g). Hence, differences when comparing their results with
316 those presented in this work could be, at least in part, originated by the different
317 initial microbial loads reported in each study.

318

319 *3.2. Antioxidant activity*

320 The antioxidant potential status of a vegetable tissue is determined by the type and
321 amount of bioactive compounds present in the product. Figure 2 shows the changes in

322 the DPPH radical-scavenging activity of fresh-cut apples subjected to PL treatments
323 and the application of gellan gum-based edible coatings. No significant differences ($p < 0.05$)
324 were observed among the initial antioxidant potential of fresh-cut apple
325 samples regardless the applied treatment. A dramatic loss of antioxidant potential
326 was observed in both untreated and untreated fruit pieces during the first week of
327 storage. However, apples coated with incorporation of apple fiber exhibited less
328 evident signs of oxidation through storage. Hence, after one week, PL-treated apple
329 cubes with added fiber had lost a 68% of their initial antioxidant value whilst samples
330 only exposed to the PL-treatment exhibited a decrease of 83%, which was similar to
331 that observed for the untreated fruit (81%). These results are in accordance with
332 those reported by Oms-Oliu et al. (2010a), who did not find significant differences
333 between the antioxidant activity of untreated and PL- treated mushrooms (4.8 and 12
334 J/cm2), stored at 4 °C during 15 days. In contrast, gellan-coated apple cubes not
335 exposed to PL exhibited significantly higher antioxidant activities throughout the first
336 week of storage. The addition of fiber was also found to exert a beneficial effect. These
337 results are in accordance to those previously reported by Moreira et al. (2015, under
338 review), stating that a gellan gum coating enriched with apple fiber was effective to
339 maintain the antioxidant capacity of fresh-cut fruit. Accordingly, Robles-Sanchez et al.
340 (2013) reported that a gellan gum-based edible coating effectively increased the
341 antioxidant capacity of fresh-cut mangoes. Also, in recent years, studies have been
342 conducted to demonstrate the functional properties of dietary fibers derived from
343 orange and apple, highlighting their antioxidant properties (Figuerola, Hurtado,
344 Estévez, Chiffelle, & Asenjo, 2005; Marin et al., 2007; MoraesCrizel, Jablonski, Oliveira,

345 Rios, & Rech, 2013). Because of this fact, although apple fiber addition did not result
346 into an immediate increase in the antioxidant potential of fresh-cut apples, its
347 incorporation to the edible coatings formulation could be beneficial for the
348 preservation the antioxidant activity potential of the fruit.
349
350 *3.3. Color*
351 Lightness (L*) is the most indicative parameter associated with the enzymatic
352 browning of fruit and vegetables. Color parameters, L* and hue (h°), of cut apples as
353 affected by gellan gum-coatings and PL treatments are displayed in Table 1. Both
354 untreated and PL-treated fresh-cut pieces exhibited slightly but significantly higher
355 lightness (L*) values than gellan gum-coated apple pieces. These differences were
356 transitory and almost disappeared during the subsequent 48 h. From then on,
357 differences between L* values of gellan gum-coated PL-treated and untreated apple
358 cubes were not observed or were really scarce. Lightness values of apple pieces stored
359 for 14 days were similar to those of the just processed products regardless the applied
360 treatment. Similarly, no significant differences ($p < 0.05$) between the h° values of
361 apple cubes subjected to the different treatments were detected and no major changes
362 could be observed throughout storage ($p < 0.05$). As all samples were dipped into an
363 antibrowning solution containing ascorbic acid and calcium chloride, it seems that the
364 application of other treatments was compatible with this commercial practice, at least
365 in what pertains to color preservation. On the other hand, no signs of browning were
366 detected after PL application. Gómez et al. (2012a,b) reported that exposure of cut
367 apples to PL increased surface browning throughout storage as compared with

368 untreated samples. Our results show that the use of ascorbic acid at 1% before PL
369 application minimized browning through refrigerated storage of apple cubes. This is
370 in agreement with the results published by Gómez-López et al. (2005); and Oms-Oliu
371 et al. (2010c).
372
373 *3.4. Firmness*
374 Figure 3 depicts the changes in firmness of fresh-cut apples as affected by the
375 application of gellan-gum coatings, the incorporation of apple fiber, PL treatments,
376 and storage time.
377 Fruits are likely to soften mainly due to hydrolysis of the pectic acids found in the cell
378 walls, with a consequent loss of fluids (Tapia, Rojas-Graü, Carmona, Rodríguez, Soliva-
379 Fortuny, & Martin-Belloso, 2008). The protective effects of calcium chloride
380 treatments against texture loss in fresh-cut apples have been widely reported (Gómez
381 et al., 2012; Lee et al., 2003; Soliva-Fortuny et al., 2001). In the present work, firmness
382 was maintained as a consequence of the applied treatment and along storage
383 regardless of the applied treatment. This fact was as well observed for gellan gum-
384 coated apples, where calcium chloride is used as a cross-linking agent of the polymer
385 matrix. This is in line with the results obtained by other researchers, which underline
386 the beneficial effects of calcium salts toward fruit firmness maintenance when these
387 are incorporated into edible coating formulations (Olivas and Barbosa-Cánovas, 2007;
388 Rojas-Graü et al., 2008). No further deleterious or beneficial effects could be
389 attributed to the exposure to PL or to the fiber incorporation to the edible coating
390 formulations.

391

392 *3.5. Sensory quality*

393 Figure 4 shows the changes in five relevant sensory attributes of fresh-cut apples as
394 affected by PL treatments, edible coatings and time under refrigerated storage. The
395 taste of PL-treated samples was determined only during the first week of storage due
396 to microbiological criteria. The rest of the tested parameters in all treated samples
397 were always above the rejection limit for up to 14 days. It is important to highlight
398 that the addition of fiber itself did not entail a decrease in the sensory scores. The
399 presence of off-odors limited the overall acceptability of the treated fruits. Thus, the
400 combination of coatings and PL-treatments led to the lowest scores for aroma
401 especially beyond the first storage week. The scores for this attribute fell below the
402 threshold of acceptability beyond the first week of storage (day 10). Gómez-López et
403 al. (2005) reported a distinctive off-odor, described as "plastic", appearing right after
404 the application of PL treatments. However, this immediate effect was not so evident in
405 the current case, probably as a consequence of the differences in color, topography
406 and surface/volume ratio among products.

407

408 **4. Conclusions**

409 Pulsed light (PL) is an emerging technology which has considerable potential as an
410 alternative to thermal and chemical methods for rapid and effective inactivation of
411 microorganisms on food surfaces. The application of gellan coatings with PL

412 treatments may be useful to extend the shelf-life of fresh-cut apple. This study
413 provides new data about the effect of these techniques to decontaminate fresh-cut
414 fruits. Indeed, new information on the possible benefits and drawbacks of their
415 combined application are highlighted. In this regard, it is important to point out that
416 the use of edible coatings could act as a limiting factor for the surface decontamination
417 by PL treatments. However, the combination of both treatments has been shown to
418 favor the preservation of the antioxidant value of fresh-cut apples. The application of
419 PL-treatments before the coating formation might avoid this problem. However, it
420 does not allow the application of PL once the product is inside the package. On the
421 other hand, the incorporation of fiber to the coatings was not found to have any
422 negative implication on the quality of fresh-cut apples, thus becoming an interesting
423 alternative for increasing the prebiotic benefits of fresh-cut commodities.

424

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430

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538

539 **Figure Captions**

540

541 **Figure 1.** Changes in the native-occurring microbiota of fresh-cut apples as affected
542 by PL treatments, the application of gellan gum-based edible coatings enriched with
543 apple fiber and storage at 4ºC: (A) total mesophilic bacteria; (B) psychrophilic
544 bacteria; (C) yeast and molds counts. Bars indicate standard deviations. **PL**: pulsed
545 light treated **G**: gellan gum-coated; **GF**: gellan gum-coated with apple fiber. Results are
546 the mean of two independent experiments counted in duplicate.

547 **Figure 2.** Changes in the DPPH radical-scavenging activity of fresh-cut apples as
548 affected by PL treatments, the application of gellan gum-based edible coatings
549 enriched with apple fiber and storage at 4ºC. Bars indicate standard deviations. **PL**:
550 pulsed light treated **G**: gellan gum-coated; **GF**: gellan gum-coated with apple fiber.
551 Results are the mean of two independent experiments assayed in triplicate.

552

553 **Figure 3.** Changes in the firmness of fresh-cut apples as affected by PL treatments, the
554 application of gellan gum-based edible coatings enriched with apple fiber and storage
555 at 4ºC. Bars indicate standard deviations. **PL**: pulsed light treated **G**: gellan gum-
556 coated; **GF**: gellan gum-coated with apple fiber. Results are the mean of two
557 independent experiments assayed in triplicate.

558 **Figure 4.** Changes in the sensory scores of fresh-cut apples as affected by PL
559 treatments, the application of gellan gum-based edible coatings enriched with apple
560 fiber and storage at 4ºC. Bars indicate standard deviations. **PL**: pulsed light treatment;

561 **G**: gellan gum-coated; **GF**: gellan gum-coated with apple fiber. Results are the mean of

562 two independent experiments assayed in triplicate.

Table 1.

Changes in the color attributes of fresh-cut apples as affected by PL treatments, the application of gellan gum-based edible coatings enriched with apple fiber, and storage at 4ºC.

Storage (days)	time	0	2	4	7	10	14
L*							
Fresh		81.12±0.46^{aA}	80.41±0.83^{abA}	80.31±0.78^{aA}	80.84±0.34^{aA}	81.34±0.63^{aA}	81.38±0.40^{aA}
LP		79.70±0.68^{abA}	81.63±0.88^{aA}	80.38±0.57^{aA}	80.47±0.70^{aA}	79.58±0.82^{aA}	79.31±0.44^{abA}
G		76.46±1.04^{bcA}	76.93±0.83^{bA}	78.01±0.70^{aA}	79.89±0.42^{abA}	80.01±0.74^{aA}	78.48±1.20^{abA}
G+LP		75.01±0.89^{cB}	77.17±0.88^{bAB}	78.38±0.73^{aAB}	78.00±0.69^{bAB}	79.21±0.76^{aA}	76.62±1.26^{bAB}
GF		75.61±0.72^{cA}	76.93±1.39^{bA}	78.14±0.87^{aA}	79.13±0.66^{abA}	79.38±0.71^{aA}	78.24±1.08^{abA}
GF+LP		73.07±1.17^{cB}	79.98±1.13^{abA}	79.25±0.53^{aA}	80.26±0.27^{abA}	79.27±0.56^{aA}	76.29±1.03^{bAB}
h°							
Fresh		104.7±0.9^{aA}	103.4±0.6^{aAB}	103.2±0.5^{abAB}	103.9±0.4^{abAB}	102.3±0.5^{aAB}	101.0±0.5^{abB}
LP		104.3±0.7^{aA}	103.5±0.4^{aA}	105.3±1.1^{abA}	105.4±0.6^{abA}	100.6±0.5^{aB}	99.0±0.6^{bcB}
G		103.2±0.6^{aAB}	103.1±0.8^{aAB}	104.3±0.4^{abA}	104.5±0.7^{abA}	102.8±0.8^{aAB}	99.7±0.9^{bcB}
G+LP		105.5±1.3^{aA}	103.3±1.0^{aA}	105.6±0.7^{aA}	106.2±0.6^{aA}	103.8±0.8^{aA}	103.0±0.6^{aA}
GF		104.6±0.6^{aA}	100.9±0.6^{aBC}	104.4±0.8^{abA}	103.2±0.5^{bAB}	100.4±0.7^{aBC}	98.2±0.6^{cC}
GF+LP		102.8±0.7^{aAB}	101.3±0.6^{aAB}	102.6±0.7^{bAB}	103.2±0.8^{bA}	100.4±0.5^{aBC}	98.2±0.6^{cC}

Data is shown as means ± standard deviations. Mean values with different lower case letters in the same column indicate significant differences ($p<0.05$) between treatments. Mean values with different capital letters in the same row indicate significant differences ($p<0.05$) with respect to storage time. **LP**: light pulses treatment; **G**: gellan edible coating; **GF**: gellan edible coating with apple fiber.

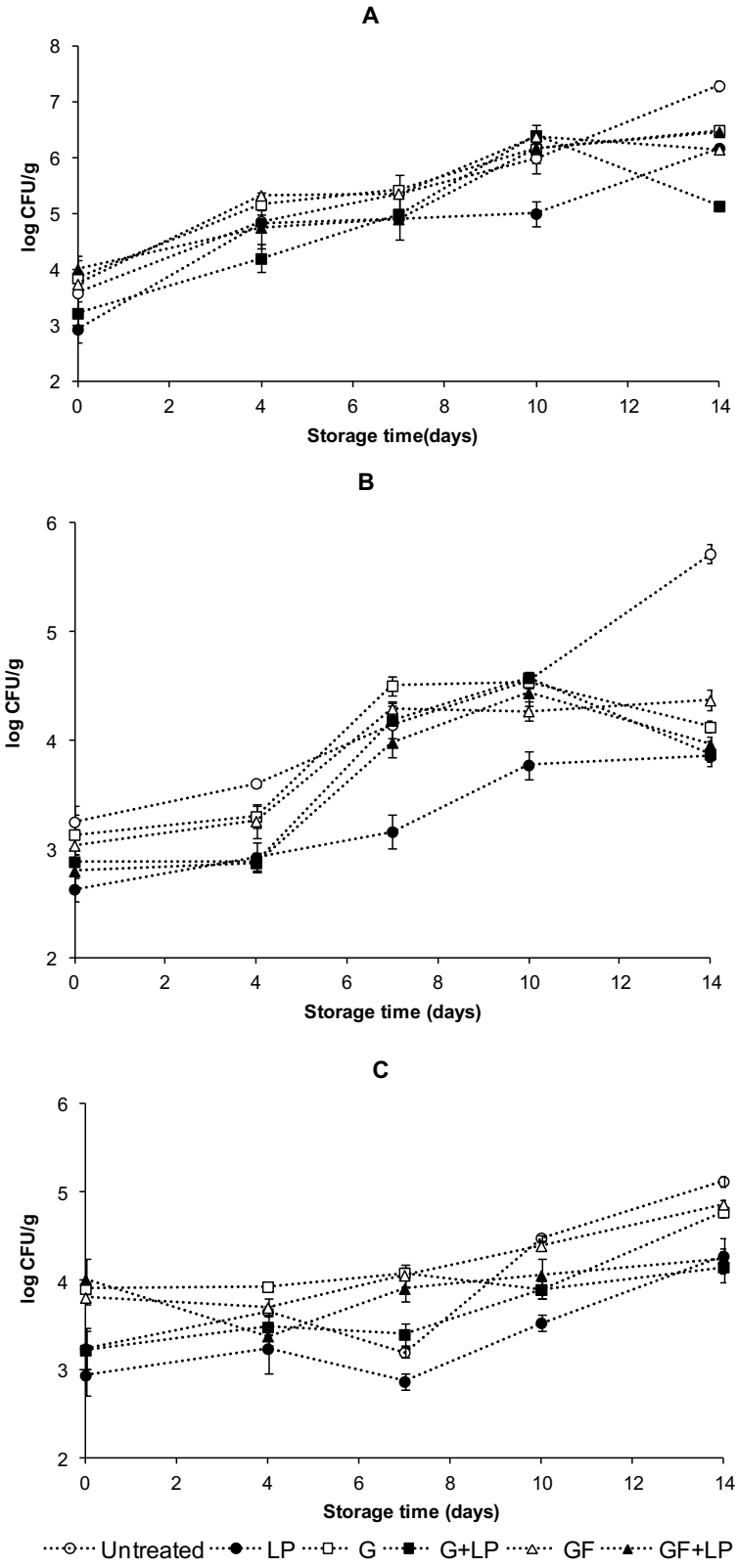
A
log CFU/g
Storage time(days)
B
log CFU/g
Storage time (days)
C
log CFU/g
Storage time (days)
Untreated
LP
G
G+LP
GF
GF+LP

Figure 1.

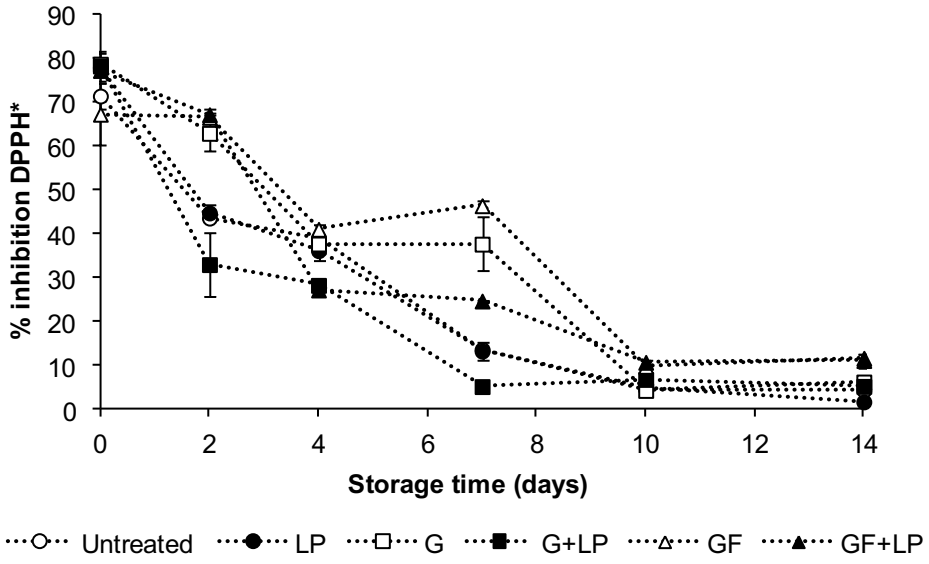
90
80
70
60
50
40
30
20
10
0
% inhibition DPPH*
0
2
4
6
8
10
12
14
Storage time (days)
Untreated
LP
G
G+LP
GF
GF+LP

Figure 2.

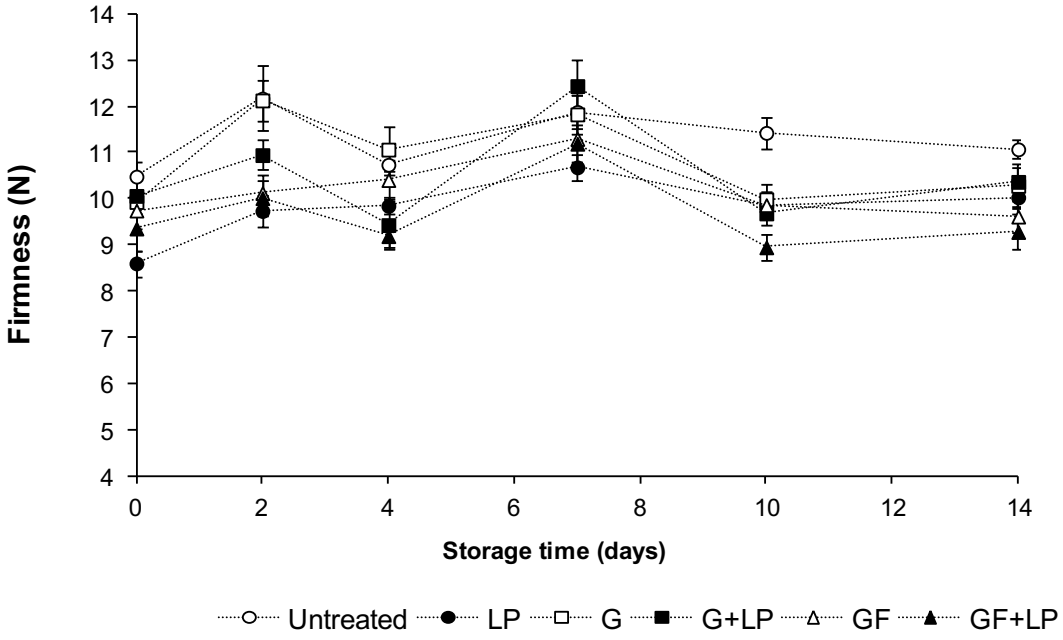
Firmness (N)
14
13
12
11
10
9
8
7
6
5
4
0
2
4
6
8
10
12
14
Storage time (days)
Untreated
LP
G
G+LP
GF
GF+LP

Figure 3.

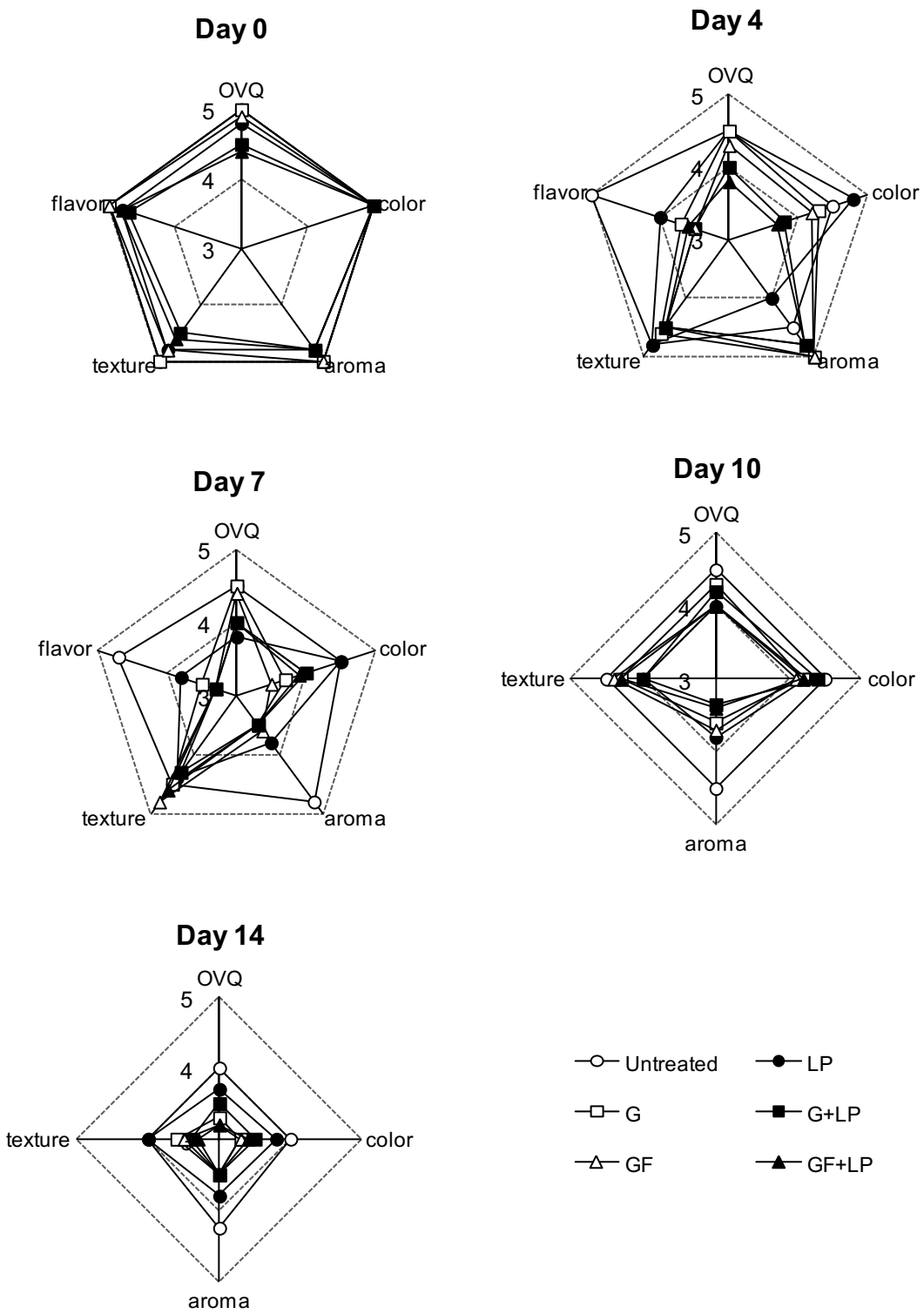
Day 0
OVQ
5
4
3
flavor
color
texture
aroma
Day 4
OVQ
5
4
3
flavor
color
texture
aroma
Day 7
OVQ
5
4
3
flavor
color
texture
aroma
Day 10
OVQ
5
4
3
texture
color
aroma
Day 14
OVQ
5
4
texture
color
aroma
Untreated
LP
G
G+LP
GF
GF+LP

Figure 4.