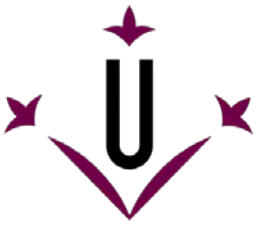

Impact of pulsed light treatments and storage time on the texture quality of fresh-cut tomatoes

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Abstract

The effect of pulsed light (PL) treatments at fluences of 4, 6 or 8 J·cm^{-2} on microbial growth, weight loss, pectinmethyl esterase (PME) and polygalacturonase (PG) activities of fresh-cut tomatoes was evaluated through 20 days of storage at 5 ºC. Additionally, a pair-wise comparison test was assayed to determine whether potential consumers could detect differences between untreated and PL-treated samples.

Microbial counts of PL-treated tomato slices were up to 2 log CFU g lower than those on untreated samples over storage. Fresh-cut tomatoes exhibited slight firmness decrements, changes on the pectinolytic enzymes and increased weight losses over the storage. However, sensory evaluation did not reveal significant differences over at least 10 days. In summary, PL-treatments showed to be effective to reduce the microbial growth with a low impact on the physical quality of fresh-cut tomatoes.

Industrial relevance: PL-treatments are proposed as a non-thermal strategy to increase the safety of fresh-cut commodities. In spite of their non-thermal nature, these treatments may have a photothermal effect, which could be deleterious to the product quality and shelf-life. This study contributes to the understanding of PL and its impact on the physical quality of fresh-cut tomatoes, thus helping to identify the range of conditions that can be industrially applied without causing major texture damage on the treated product.

Keywords

fresh-cut tomatoes; pulsed light; texture; pectin methylesterase; polygalacturonase; sensory evaluation

38 **1. Introduction**

39 Tomato (*Lycopersicum esculentum*) is one of the most demanded vegetables worldwide.
40 Tomato consumption is considered as an indicator of good nutritional habits and healthy
41 lifestyle due to its remarkable contents in folates, vitamin C and vitamin E (Gahler et al., 2003).
42 Moreover, it is a good source of other natural antioxidants especially carotenoids and phenolic
43 compounds (Navia et al., 2006 and Odriozola-Serrano et al., 2008).

44 The increasing consumer's demand for fresh-cut tomatoes to be used in salads and other
45 ready to eat products has promoted the development of technologies that contribute to undertake
46 their industrial production. Because quality and marketability of fresh-cut tomatoes deteriorates
47 rapidly after cutting (Artés et al., 1999), proper processing and packaging conditions are
48 fundamental to avoid the deleterious consequences of processing (Aguiló-Aguayo et al., 2013;
49 Francis and O'Bierne, 2005). Tomato ripening is usually accompanied by the degradation of the
50 middle lamella and loss of cell adhesion. Ripening process in plants has been related to the
51 depolymerization of pectic components and/or action of hydrolytic enzymes such as
52 polygalacturonase (PG;EC 3.2.1.15) and pectin methylesterase (PME;EC 3.1.1.11) leading to
53 the loss of integrity of the cell walls (Chisari et al., 2011). PG and PME are considered as the
54 primary hydrolysis enzymes involved in tomato softening. However, softening of fresh-cut
55 fruits and vegetables is not only influenced by enzymatic action. Mechanical processes such as
56 slicing, shredding or dicing operations can also cause dramatic losses in firmness of fruit tissues
57 (Soliva-Fortuny and Martín-Belloso, 2003) activating, accelerating or promoting
58 physicochemical phenomena such as dehydration (Oms-Oliu et al., 2010), ethylene production
59 (Rugkong at el., 2010), ripeness and changes in turgor and crispness (Toivonen and Brunell,
60 2008; Soliva-Fortuny et al., 2004).

61 Thermal food processing methods ensure microbiological safety. However, these
62 treatments can also modify the sensory properties of fresh-cut produce. As a result, non-thermal
63 technologies may be an alternative in order to preserve the nutritional content and quality of
64 fresh-cut commodities demanded by consumers. Pulsed light *(PL)* treatments have emerged as a
65 non-thermal method for microbial decontamination of food surfaces. The main advantage of

66 applying this technology to fresh-cut fruits concerns the reduction of microbial loads without
67 significantly affecting the physical characteristics of the living tissues (Ramos-Villarroel et al.,
68 2012). Microbial cell death may be attained through the generation of photochemical and
69 photothermal effects. Photochemical damage is related to the induction of DNA strand breaks
70 and formation of pyrimidine dimers by UV wavelengths. Nevertheless, several studies have
71 described changes in the antioxidant properties, enzymatic browning and nutritional
72 characteristics of fresh-cut produce subjected to PL treatments (Gómez-López et al., 2005;
73 Oms-Oliu et al., 2010). As well, texture changes after exposure to PL may also occur, and this
74 phenomenon has been attributed to disruption of cell wall membranes caused by PL-fluences,
75 probably as a consequence of undesired thermal effects of the treatment (Aguiló-Aguayo et al.,
76 2013; Charles et al., 2008). Therefore, although food researchers have investigated the
77 possibility of exploiting pulsed light technology to inactivate enzymes in fruits and vegetables,
78 still controversial results are available in literature (Manzocco, et al., 2009).
79 Hence, the aim of this work was to investigate the effects of PL treatments applied with
80 decontamination purposes on the activity of pectinolytic enzymes (pectinmethyl esterase and
81 polygalacturonase) and to evaluate their relationship with texture modifications occurring in
82 fresh-cut tomato over chilled storage.

83 **2. Materials and Methods**

84 *2.1 Raw materials and processing*

85 Tomatoes (*Lycopersicum e esculentum* Mill. cv. Daniela), were purchased in a local
86 wholesale distributor (Lleida, Spain) at commercial maturity and refrigerated at 5 ± 1 ºC before
87 processing.
88 Fresh whole tomatoes were sanitized for 2 min in chlorinated water (100 ppm free
89 chlorine L^{-1}) at 5 ± 1 ºC, rinsed with tap water and gently dried by hand. Tomato fruits were
90 then cut into 5 mm-thick slices using an electric slicer (Food Slicer-6128: Toastmaster Corp,
91 Elgin, USA). Tomato slices (ca. 100g) were weighed in polypropylene trays (350 cm^3, 5025 RM

92 PTT-ATS Packaging S.r.l. VE, Italia), which were thermo sealed using an ILPRA Food Pack
93 Basic V/6 packaging machine (ILPRA Systems, CP, Vigevono, Italia). The O_2 and CO_2
94 permeances of the sealing film were 5.2419 x 10^{-13} mol O_2 m^{-2} s^{-1} Pa^{-1} and 2.3825 x 10^{-12} mol
95 CO_2 m^{-2} s^{-1} Pa^{-1} at 23ºC and 0 % RH, respectively (ILPRA Systems España, S.L. Mataró,
96 Spain). The packages were stored at 5 °C in darkness prior to PL application.
97 A physico-chemical characterization of tomato slices was carried out before and after
98 PL processing and through 20 days storage (Table 1). pH (Crison 2001 pH-meter; Crison
99 instruments S. A., Barcelona, Spain), tritable acidity and soluble solids content (Atago R-X-
100 1000 refractometer; Atago Company Ltd., Japan) were assayed.

101 *2.2 Pulsed light treatments*

102 Pulsed light (PL) treatments were performed using an automatic laboratory flash lamp
103 system (Steribeam Xe-Matic-2L-A, Kehl, Germany). The emitted spectrum ranged from 200 to
104 1100 nm. The duration of each pulse was 0.3 ms with a fluence of 0.4 J cm^{-2} per pulse emitted
105 from each one of two xenon lamps situated above and below the sample holder, respectively.
106 Samples were treated with 10, 15 or 20 pulses to evaluate the effect of different treatment doses.
107 Hence, the fluences applied were 4, 6 and 8 J cm^{-2} respectively. According to previous studies
108 (Aron Maftei, et al., 2014) the film transparency was 97%. A set of untreated samples was kept
109 as reference. Finally, samples were stored at 4 °C for 20 days in darkness until random
110 withdrawal for analysis.

111 *2.3 Microbiological stability*

112 In order to evaluate the sanitizing effect of PL treatments, microbiological analyses were
113 carried out prior to analytical and sensory evaluations. Psychrophilic bacteria and yeast and
114 mold counts were carried out over 20 days of storage. Samples of 10 g tomato were
115 homogenized for 2 min with 90 ml of 0.1% sterile peptone solution with a Stomacher Lab
116 blender 400 (Seward Medical, London, UK). Serial dilutions of fruit homogenates were made

and plated onto plate-count agar (PCA) (Biokar Diagnostics, Beauvais, France) and incubated for 10 days at 5 ± 1 ºC for psychrophilic bacteria counts. For yeast and mold counts, serial dilutions of tomato homogenate were spread on agar plates with chloramphenicol glucose (CGA) (Biokar Diagnostics, Beauvais, France) and incubated in the dark at 25 ± 1 ºC for 3-5 days. Analyses were carried out every 5 days in randomly sampled pairs of trays. Three replicate counts were performed for each tray.

2.4 Texture evaluation

The texture of the samples was evaluated using a TA-XT2 texturometer (Stable Micro Systems Ltd., Surrey, England, UK) equipped with a 5 kg load cell. A resistance test was used to discriminate texture differences among cross sectional sliced tomato pieces. Uniaxial compression test was assayed to measure the tomato slices resistance to uniaxial single type forces using a 50 mm diameter aluminum cylindrical probe (P/50), with an test speed of 5 mm/s, a final target at 25% strain, plus 10 s holding time at the maximum strain.

Force-displacement-time data were recorded using Texture Exponent 32 software (Stable Micro Systems LTD. Surrey England). The firmness was measured as the maximum force required for shearing 5 mm-thick tomato slices. At least 4 repetitions from 2 replicate packages were evaluated at each sampling time and results were expressed as firmness in N.

2.5 Weight loss

Tomato slices are highly susceptible to weight loss, mainly attributed to transpiration, and then leading to softening (Mencarelli & Salveit, 1988). With the aim of not interrupting the storage conditions, containers with fresh-cut tomatoes were pierced (2 mm) and then juice was drained using a sterile micropipette at each sampling time. Trays with tomato slices were weighed just after processing (W_0) and throughout the storage period (W_1) for weight loss determination. Drilled trays were sealed again with polypropylene film and stored. The last method was conducted in duplicate and three replicate analyses were carried out for each

142 sample in order to obtain the mean value. Weight losses were expressed as percentage (%)
143 weight loss relative to the initial weight.

144 *2.6 Enzymatic determinations*

145 *2.6.1 Sample preparation*

146 To obtain the enzyme extracts for PME and PG activity determinations, 25 g of sliced
147 tomatoes were ground with an Ultra Turrax T25 (IKA® WERKE, Germany) equipped with a
148 S25N-G25G probe and filtered throughout a 2-mm diameter steel sieve. Then, tomatoes juice
149 was collected and immediately analyzed.

150 *2.6.2 Determination of pectin methylesterase (PME) activity*

151 PME activity was measured using the method described by Kimball (1991). Pectin from
152 citrus fruit (67-71% esterified), sodium chloride and NaOH were purchased from Acros
153 Organics (NJ, U.S.A.), Rectapur (Fontenay, France) and Panreac Química (Barcelona, Spain),
154 respectively. Namely, 5 milliliters of tomato juice sample were mixed with 20 ml of 1% citrus
155 pectin solution in 2 N NaCl. After reaching a temperature of 30 ºC, the pH of the mixture was
156 adjusted to 7.7 with 2 N NaOH using a pH-meter (C-2001; Crison Instruments S.A., Alella,
157 Barcelona, Spain). When the pH was stabilized, 1 ml of 0.05 N NaOH was added and the time
158 required for the pH to return to 7.7 was measured. PME activity (A) was calculated through Eq.
159 1 and expressed in pectin esterase units (PEU) per ml of juice. Then, 1 unit of PME activity was
160 defined as the amount of the enzyme that liberates 1.0 micro equivalent of acid per minute
161 under the assay conditions.

162 $$A\left(\frac{PME}{mL}\right) = \frac{\left(NaOH \cdot V_{NaOH} \cdot 10^2\right)}{V_{juice} \cdot t'} \qquad \text{(Eq. 1)}$$

where [NaOH] is the NaOH concentration (0.05 N), V_{NaOH} is the volume of NaOH solution (0.05 N) V_{juice} is the volume of juice (5 ml) and t' is the time in minutes required for the solution to reach a pH of 7.7 after the addition of NaOH.

2.6.3 Determination of polygalacturonase (PG) activity

PG activity was determined using the method described by Aguiló-Aguayo et al. (2009). A sample of 2.5 g of tomato juice was transferred to a 5 ml centrifuge tube and centrifuged at 8,000 *g* for 15 min at 4 ºC. The supernatant was discarded and the pellet was dispersed into cold (4 ºC) distilled water at 1:1 ratio (w/v). Then, the mixture was adjusted at pH 3.0 with 0.1 M HCl. After that, the sample was centrifuged at 9,000 *g* for 15 min at 4 ºC. The supernatant was again discarded and the pellet was dissolved into a 1.2 M NaCl solution in a ratio of 1:1 (w/v). After stirring during 1 hour at 4 ºC, the mixture was centrifuged at 18,200 g for 10 min at the same temperature. The resulting supernatant was assayed for PG activity.

The PG activity assay was based on the release of reducing groups produced by PG and measured by spectrophotometry (Gross, 1982). Therefore, 100 µL of the extracted enzyme solution were mixed and incubated with 300 µL of 0.2% polygalacturonic acid at 35 ºC for 10 min. To stop the reaction, 2 ml of 0.1 M borate buffer (pH 9.0) and 400 µL of 1% cyanoacetamide solution were added and the homogenate was boiled for 10 min. After cooling down, the absorbance was measured at 276 nm at 22 ºC. A blank sample was determined in the same way without the addition of the enzymatic extract. A standard curve was built with α-D-galacturonic acid as reducing sugar. Hence, 1 unit (U) of PG activity was defined as the amount of enzyme that releases 1 µmol of galacturonic acid per min under the assay conditions.

2.6.4 Relative residual activity

Residual PME (RA_{PME}) and PG (RA_{PG}) were calculated through Eq. 2, were A_o denotes the enzyme activity of untreated tomato slices and A_t is the enzyme activity on the PL-treated samples.

188 $$RA(\%) = \frac{A_E}{A_0} \cdot 100$$ (Eq. 2)

189 *2.7 Sensory testing*

190 The texture-sensory evaluation was carried out in a sensory analysis room with
191 individual booths. Pair-wise ranking tests were carried out just after processing and after 5 and
192 10 days of refrigerated storage. Sensory tests were not conducted beyond that point in view of
193 the results of the microbial analysis. To evaluate sensory quality in fresh-cut tomato, samples
194 were coded using random numbers (to avoid bias) and a set of six sample pairs were served on a
195 white dish to a panel composed of twelve members (20-55 years of age). Judges were then
196 instructed to select and record the inclination for a sample of every pair based on their own
197 preference about the texture of tomato slices. Evaluations were performed immediately after the
198 tomato slices were removed from refrigeration.

199 *2.8 Statistical analysis*

200 Statistical analysis was performed using Statgraphics plus v. 5.1 Windows package
201 (Statistical graphics Co., Rockville, MD). Each processing condition was assayed in triplicate at
202 each sampling time and three replicate analyses were carried out for each trail to obtain the
203 mean value (n= 9). Analysis of variance (ANOVA) was carried out to compare sample mean
204 values. Data were analyzed using multifactor analysis of variance and a LSD multiple range test
205 was applied to determine differences among means with a significance level of 0.05.

206 The results of the sensory analysis were evaluated by a Friedman-type statistical
207 analysis. The first step of this analysis was to obtain the rank sum by addition of preferences
208 rank for each sample to twice the sum of the preferences frequencies. Then, the Friedman's test
209 (T) (Eq. 3) was used to determine significant differences between samples.

$$T = \frac{4}{pt}\sum_{i-1}^{t} R^2 - \left(9p[t-1]^2\right)$$ (Eq. 3)

where p is the number of times the design is repeated; t is the number of compared treatments; R_i is the rank sum for each treatment; and $\sum R^2$ is the sum of all R's squared from R_1 to R_t.

Hence, T values obtained to each tomato sample were compared to the critical value of χ^2 with (t-1) degrees of freedom. Additionally, the HSD value (honestly significant difference) was determined to compare two rank sums (α=0.05). Finally, Pearson correlation coefficients were calculated to obtain the statistical correlations between firmness and related parameters (weight loss, PG and PME activities and the texture sensory rank-sums).

3. Results

3.1 Microbiological stability

The effects of pulsed light (PL) treatments on the inactivation of psychrophilic bacteria (PB) and moulds and yeasts (MY) growing on fresh-cut tomatoes are shown in Figure 1. The initial PB counts on tomato slices (3.1±0.1 Log CFU g^{-1}) were not substantially affected by PL treatments (Figure 1-A). Conversely, initial MY loads (4.9±0.1 Log CFU g^{-1}) on fresh-cut tomatoes were significantly reduced (p<0.05) just after exposure to fluences of 6 and 8 J cm^{-2} (4.2 and 3.8 Log CFU g^{-1}, respectively) (Figure 1-B).

Psychrophilic bacteria and molds and yeasts counts significantly increased (p<0.05) on fresh-cut tomatoes through storage time. Regarding the effect of PL-treatments, tomato slices subjected to PL exhibited lower microbial counts than those on untreated at each sampling time (p<0.05), being the higher the PL-fluence applied, the lower the increase in microbial counts over storage. At the end of storage, PB counts on untreated samples increase up to 7.0±0.2 Log (CFU g^{-1}), while those on samples subjected to PL-treatments were downgraded up to 0.7-1.8 Log (CFU g^{-1}), with respect those on untreated samples. On the other hand, the MY loads on fresh-cut tomatoes reached 8.0±0.2 Log (CFU g^{-1}), being the samples exposed to PL-fluences of 8 J cm^{-2} which exhibited the lower increases (up to 0.5 Log CFU g^{-1}) with respect to those on untreated tomato slices.

235 *3.2 Firmness*

236 Changes in firmness of fresh-cut tomato over chilled storage as affected by PL
237 treatments are presented in Table 1. PL treatments did not lead to firmness modifications of
238 fresh-cut tomatoes immediately after processing (p<0.05). However, firmness values
239 significantly decreased (p<0.05) throughout the storage period regardless the treatment applied,
240 being the higher the PL-fluence applied, the faster the firmness decrements. Then, untreated
241 tomato slices exhibited reductions of 63% of their initial firmness over 20 days of storage,
242 whereas those subjected to fluences of 4 and 6 J cm^{-2} exhibited greater firmness losses (72 and
243 74%, respectively). In contrast, firmness of fresh-cut tomatoes exposed to fluences of 8 J cm^{-2}
244 only lost a 55.7% of their initial value throughout storage.

245 *3.3 Relative weight loss*

246 As shown in Figure 2, fresh-cut tomatoes underwent slight weight changes just after PL
247 processing. Tomato slices exposed to 8 J cm^{-2} exhibited the highest weight loss (1.6%), which
248 was 4-fold and twice greater than that observed for fresh-cut tomatoes treated with 4 and 6 J cm^{-2}
249 , respectively. At that point, weight loss values were found to be directly related with the
250 amount of incident energy received by fruit samples. Moreover, PL exposure of fresh-cut
251 tomatoes led to consistently higher weight loss values through storage irrespective of the
252 applied conditions (p<0.05). For all untreated and PL-treated tomato slices, increased weight
253 loss was especially evident during the days following processing (ca. day=4), whereas the rate
254 of increase significantly dropped beyond the first week of storage. Then, differences between
255 weight loss values for untreated and PL-treated tomato slices during the resting storage period
256 were maintained over time (p<0.05). Hence, weight loss values on fresh-cut tomatoes subjected
257 to PL and stored for 20 days ranged from 9 to 10 %, while a lower but not negligible value was
258 observed for untreated samples (7%).

259 *3.4 Pectin methylesterase and polygalacturonase activities*

Changes in pectin methylesterase (PME) and polygalacturonase (PG) activities in untreated and PL treated fresh-cut tomatoes as affected by PL fluence are shown in Figures 3 and 4, respectively. PL processing did not cause dramatic changes in the initial PME activity on fresh-cut tomatoes. The PME activity of untreated and PL-treated fresh-cut tomatoes significantly decreased (p<0.05) over storage time. However, tomato slices subjected to PL treatments kept higher PME activity values than untreated samples over storage (p<0.05). Indeed, tomato samples exposed to highest PL-treatments (8 J cm^{-2}) maintained greater PME activity than on those exposed to 4 and 6 J cm^{-2}.

Regarding PG, PL treatments did not appear to have a significant effect (p<0.05) on the activity of this pectinolytic enzyme. PG activity dropped sharply (ca. 60%) during the few days following processing regardless the applied treatment. Residual PG activity values were kept without substantial changes beyond the initial depletion (p<0.05).

3.5 Sensory analysis

Texture acceptability scores for untreated and PL-treated fresh-cut tomatoes are shown in Table 3. Significant differences (p<0.05) between untreated and PL-treated fresh-cut tomatoes were not immediately observed after processing. After 5 days of storage, untreated tomato slices were preferred to those treated with fluencies of 6, 8 and 4 J cm^{-2}, following this order. However, over a 10-day period differences in sensory scores among samples appeared to be less significant. Remarkably, at that point judges preferred tomato samples exposed to higher fluences, although differences in acceptability values for untreated and PL-treated tomato slices were scarcely significant (p<0.05). Some judges reported certain undesirable quality attributes such as mealiness and dryness in several samples in random way.

3.6 Correlation analysis

Table 4 shows correlation coefficients between firmness, microbial growth, weight loss and enzymatic activities. Increases in microbial counts were inversely correlated with firmness

285 values (R^2≤-0.92). Besides, a good correlation was observed between firmness and weight loss
286 values (R^2≤-0.91), meaning that weight loss values and firmness reduction were directly related.
287 Regarding the enzymatic activities, correlation between PME activity and firmness was also
288 high (R^2≤-0.93). Although PME and PG activities exhibited different patterns over the storage,
289 PG activity influenced on the firmness change over the first 2 days of storage. Eventually,
290 significant correlations between firmness and PG activity were not observed regardless the
291 applied treatment.

292 **4. Discussion**

293 Firmness is a key component determining the fresh-like quality of tomato slices.
294 However, microbial growth triggered by minimal processing can lead to texture modification of
295 fresh-cut commodities (O'Beirne, 2006; Francis & O'Beirne, 1998; Barth et al., 2009). In this
296 study, PL treatments have demonstrated to be an effective alternative to maintain lower
297 microbial counts on fresh-cut tomatoes along the storage (figure 1). Microbial reductions were
298 similar to those previously reported for microbial inactivation on tomato slices exposed to PL-
299 fluences of 4, 6 and 8 J cm^{-2} (Valdivia-Nájar et al., 2017). Although the effect of PL treatments
300 can be different depending of the structure and physicochemical characteristics on every
301 commodity, some studies and have related the effectiveness of PL on the microbial growth to
302 the amount of PL-fluence applied, initial level of contamination and temperature conditions
303 (Gleeson & O'Beirne, 2005; Ramos-Villarroel et al., 2013, 2012a, 2012b; Gómez, et al., 2011).
304 Previous reports indicate that the mode of action of PL is generally related to structural
305 changes and cell wall alterations provoked by photochemical (Gómez-López et al., 2007 and
306 Manzocco et al., 2009) photophysical (Ramos-Villarroel et al., 2013) and photothermal (Oms-
307 Oliu et al., 2010) effects, which may lead to quality changes of fresh-cut products. As a matter
308 of fact, fresh-cut tomatoes did not evidence important physical alterations just after slicing or
309 exposure to pulsed light treatments (Table 1). However, slight changes in firmness of fresh-cut
310 tomatoes subjected to PL were detected during the storage period. In this way, the highest the

311 PL-fluence applied (8 J cm^{-2}) the highest the observed changes (Table 2). Similarly, Aguiló-
312 Aguayo et al. (2013) observed physical alterations on whole tomatoes subjected to lower PL-
313 fluences (2.68-5.36 J cm^{-2}) and stored for 15 days at room temperature. Nevertheless, the extent
314 of the changes was in the same order or magnitude than those occurring in the untreated fruits.
315 Some authors reported that minimal processing (Ahmed et al., 2011; Gil et al., 2002; Sandhya et
316 al., 2010) as well as PL treatments (Rico et al, (2007) are capable of increasing the stress and
317 respiration rate and possibly induced a lignification-like process, causing structural changes as
318 water-soaked areas, dehydration and cellular decompartmentalitzation, and thus leading to
319 weight loss of the fresh-cut commodities. In fact, a linear dependency between PL-fluence and
320 the increase in the respiration rate of fresh-cut tomatoes stored under chilled conditions was
321 previously described (Valdivia-Nájar et al., 2017). Therefore, decreased firmness on tomato
322 slices could be related to those triggered physicochemical changes. Similarly, Gómez et al.
323 (2011) observed an increase in the weight loss of sliced apples after a treatment with UV-C light
324 and related it to decreased rigidity and turgidity, as a consequence of membrane breakage and
325 vacuole burst provoked by pulsed light treatments.
326 Moreover, physicochemical changes facilitate the contact between cell wall enzymes
327 and their substrates trigger the enzymatic hydrolysis of cell wall pectic substances (Alandes et
328 al., 2006), prompting water losses and thus altering the firmness. Some authors mentioned that
329 changes on the PME and PG activities cause textural changes along the storage of tomato
330 (Duvetter et al., 2009). Van Djik et al. (2006). In fact, the results of the present study show a
331 rapid decrease in PG activity (Figure 4) during the first 2 days of storage, while PME activity
332 decreased progressively over storage (Figure 3). These results coincide with those reported by
333 Wei et al. (2011), who indicated that PME and PG activities in sweet tomato cherry presented
334 dramatic changes over 30 days of storage at low temperature. Moreover, imbalances of the PG
335 and PME activities could be the cause of symptoms such as firmness decline and manifestation
336 of undesirable characteristics. Mealiness development in fresh-cut commodities has been
337 associated to imbalances in the pectinolytic enzyme activities. It is widely assumed that low PG
338 activities, together with high PME activities, promote this disorder (Artés et al, 1996; Lurie et

339 al, 2003; Brumell et al., 2004), which is in line with our results. Then, mealiness reported
340 through sensory tests could be well related to the different trends of PME and PG activities
341 observed along the storage. Furthermore, some authors have related mealiness and firmness
342 decrease in fresh-cut tomatoes to the loss of turgor and subsequent development of water-
343 soaked areas caused by chilling injury (Hong & Gross, 2000; Rugkong et al., 2010 and Natalini
344 et al., 2011) with coincides with our findings.

345 From our results it can be stated that firmness changes may be triggered in tomato slices
346 when PL treatment are applied. These effects are related to changes in cell wall integrity, thus
347 allowing microbial growth and triggering weight losses and enzymatic activation over the
348 storage. Remarkably, consumers could not detect differences among the untreated samples and
349 those subjected to any PL-fluence, along 10 days of storage (Table 3). In general, PL-treatments
350 of 8 J cm^{-2} allowed reducing microbial loads without drastically affecting the texture of fresh-
351 cut tomatoes. The results of the present study contribute to the development of novel
352 applications of PL technology, exploring its ability to reduce microbial loads and its effects on
353 the physical and sensorial quality of tomato slices.

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Tables

Table 1. Changes on the physicochemical properties of fresh-cut tomatoes subjected to PL-treatments and stored at 5 ºC during 20 days.

	Storage time (day)	PL- treatment (fluence)			
		Untreated	4 J cm^2	6 J cm^2	8 J cm^2
Soluble solids (ºBrix)					
	0	4.00 ^{aA}	4.25 ^{aA}	4.25 ^{aA}	4.50 ^{aB}
	5	4.71 ^{aA}	5.07 ^{aB}	4.46 ^{aA}	4.75 ^{aA}
	10	4.81 ^{aA}	5.39 ^{bB}	4.92 ^{aB}	4.67 ^{aA}
	15	5.40 ^{bA}	5.37 ^{bA}	5.61 ^{aA}	5.36 ^{bA}
	20	5.58 ^{bA}	5.50 ^{bA}	5.75 ^{aB}	6.00 ^{bB}
pH					
	0	4.59^{aA}	4.54 ^{aA}	4.45 ^{aB}	4.35 ^{aB}
	5	4.73 ^{aA}	4.73 ^{bA}	4.73 ^{bA}	4.73 ^{bB}
	10	4.65 ^{aA}	4.65 ^{bA}	4.65 ^{bA}	4.66 ^{bA}
	15	4.68 ^{aA}	4.62 ^{bB}	4.78 ^{bB}	4.93 ^{cC}
	20	4.75 ^{bB}	4.60 ^{aA}	4.66 ^{bB}	4.62 ^{bA}
Tritratable acidity					
	0	0.31 ^{aB}	0.39 ^{cC}	0.34 ^{bB}	0.33 ^{bAB}
	5	0.33 ^{bB}	0.31 ^{aB}	0.29 ^{aA}	0.30 ^{bA}
	10	0.34 ^{bC}	0.28 ^{aB}	0.29 ^{aB}	0.26 ^{aA}
	15	0.35 ^{cB}	0.29 ^{aA}	0.27 ^{aA}	0.28 ^{aA}
	20	0.25 ^{aA}	0.33 ^{bB}	0.27 ^{aA}	0.27 ^{aA}

Values are expressed as mean ± standard deviation.
^{abc}Different lower case letter in the same column for each sample indicate significant differences among storage time ($p<0.05$).
^{ABC}Different capital letters in the same row for the each sample indicate significant differences among treatments.
Values ± SD.

Table 2. Firmness of fresh-cut tomatoes subjected to pulsed light treatments and stored through 20 days at 5 ºC.

	Storage time (day)	PL-treatment (fluence)			
		Untreated	**4 J cm^{-2}**	**6 J cm^{-2}**	**8 J cm^{-2}**
Firmness values					
	0	20.7±10.9^{c}	17.8±10.1^{c}	18.5±10.3^{c}	19.9±8.9^{e}
	2	16.9±2.1^{abc}	16.8±10.9^{bc}	16.1±7.9^{bc}	17.3±8.6^{cde}
	4	15.0±10.5^{abc}	16.0±3.7^{bc}	15.6±5.3^{bc}	15.2±7.4^{bcde}
	8	13.6±9.1^{abc}	12.5±4.3^{abc}	12.3±5.6^{abc}	10.0±2.0^{abc}
	12	13.3±5.3^{abc}	10.9±3.3^{ab}	12.0±1.9^{abc}	9.9±3.8^{abc}
	16	10.8±6.4^{ab}	8.7±1.7^{a}	11.2±5.5^{ab}	9.0±4.7^{ab}
	20	7.7±2.4^{a}	7.9±1.5^{a}	7.9±3.9^{a}	7.3±1.0^{a}

^{abc}Different lower case letter in the same column for each sample indicate significant differences among storage time ($p<0.05$).
Values ± SD.

Table 3. Rank sums obtained through the sensory evaluation of texture of fresh-cut tomatoes after PL treatments and stored at 5 ºC for 10 days.

PL-treatment (fluence)	Storage time (day)		
	0	**5**	**10**
Rank Sums			
Untreated	54	60	52
4 J cm^{-2}	56	48	54
6 J cm^{-2}	51	56	53
8 J cm^{-2}	55	52	57

Values ± SD.
Friedman's T=10

Table 4. Correlation coefficients among the firmness values, microbial growth, weight loss and PG-PME activities on fresh-cut tomatoes subjected to different PL-fluences and stored at 5 ºC for 20 days.

	PL treatment (fluence)			
	Untreated	**4 J cm^{-2}**	**6 J cm^{-2}**	**8 J cm^{-2}**
Firmness	-	-	-	-
PB	-0.950	-0.956	-0.939	-0.981
MY	-0.937	-0.965	-0.918	-0.966
WL	-0.960	-0.906	-0.931	-0.915
PG	0.886	0.758	0.860	0.719
PME	-0.948	-0.929	-0.983	-0.967

PB: psychrophilic bacteria; MY: moulds and yeast; WL: weight loss (%); PG: polygalacturonase activity (%) and PME: pectinmethyl esterase activity (%).
P-value <0.05 at the 95 % confidence level.

Figure captions

Figure 1. Effect of pulsed light (PL) treatments on microbial load: (A) psychrophilic bacteria and (B) moulds and yeast through 20 days of chilling storage (5 ºC) of fresh-cut tomatoes. PL-treatments: Untreated (♦); 4 J cm^{-2} (□); 6 J cm^{-2} (Δ) and 8 J cm^{-2} (○). Points are the means of three repetitions from two replicate packages ±SD.

Figure 2. Changes on relative weight loss of fresh-cut tomatoes treated with pulsed light (PL) and stored throughout 20 days at 5 ºC. PL-treatments: Untreated (♦); 4 J cm^{-2} (□); 6 J cm^{-2} (Δ) and 8 J cm^{-2} (○). Points are the means of three repetitions from two replicate packages ±SD.

Figure 3. Effects of PL treatments on the residual pectin methylesterase activity (PME) of fresh-cut tomatoes throughout storage at 5 ºC for 20 days. PL-treatments: Untreated (♦); 4 J cm^{-2} (□); 6 J cm^{-2} (Δ) and 8 J cm^{-2} (○). Points are the means of three repetitions from two replicate packages ±SD.

Figure 4. Effects of PL treatments on the residual polygalacturonase activity (PG) of fresh-cut tomatoes throughout 20 days of storage at 5 ºC. PL-treatments: Untreated (♦); 4 J cm^{-2} (□); 6 J cm^{-2} (Δ) and 8 J cm^{-2} (○). Points are the means of three repetitions from two replicate packages ±SD.

Figure 1

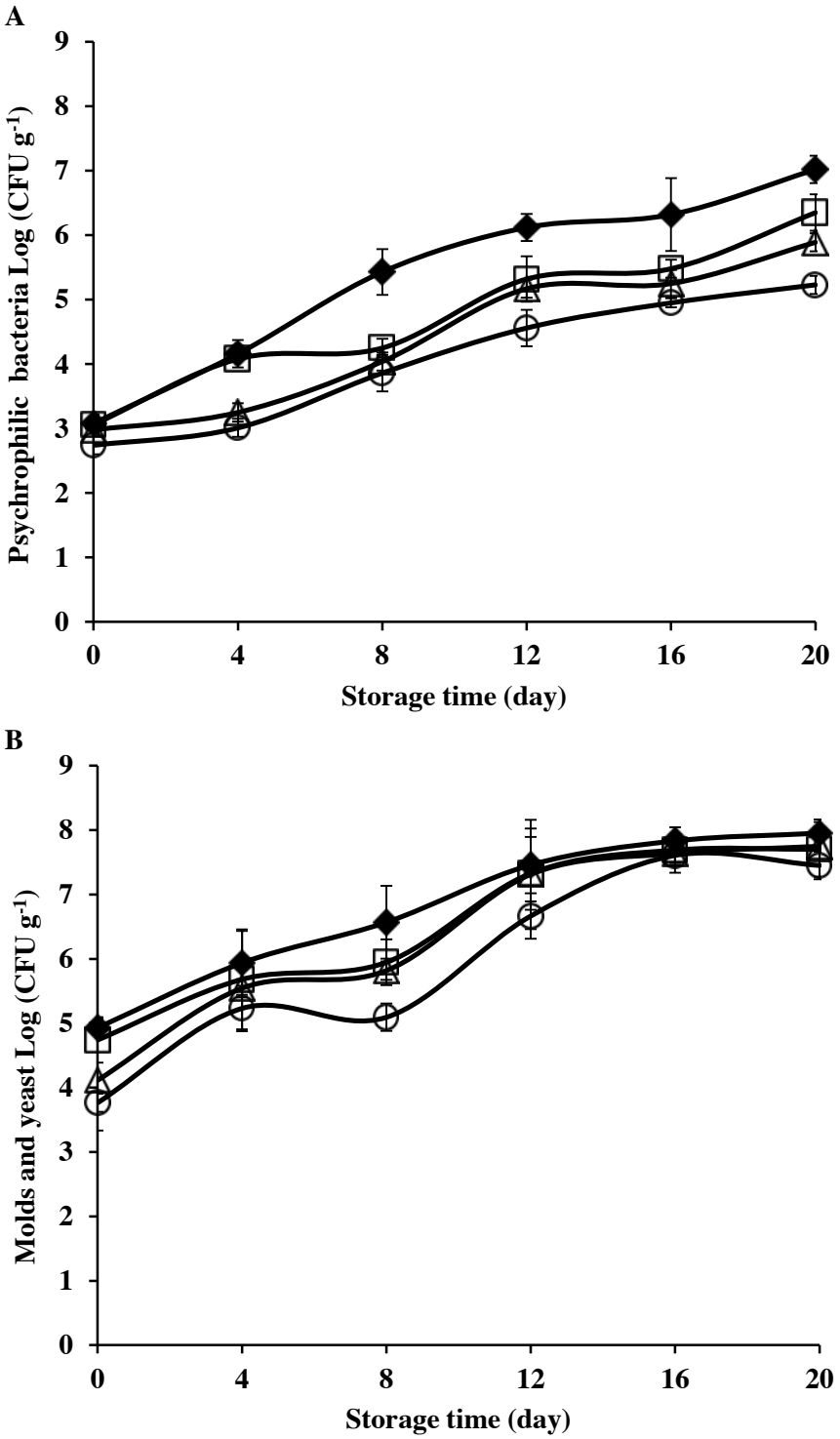
A
Psychrophilic bacteria Log (CFU g-1)
0
1
2
3
4
5
6
7
8
9
0
4
8
12
16
20
Storage time (day)
B
Molds and yeast Log (CFU g-1)
0
1
2
3
4
5
6
7
8
9
0
4
8
12
16
20
Storage time (day)

Figure 2