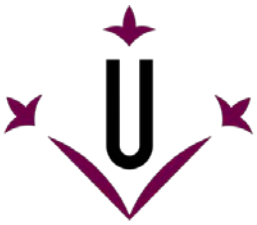

Impact of pulsed light treatments on antioxidant characteristics and quality attributes of fresh-cut apples

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ABSTRACT

The effects of pulsed light (PL) treatments combined with a quality-stabilizing dip on the quality and antioxidant attributes of fresh-cut 'Golden delicious' apples was studied. Apple wedges were dipped into a solution of 1% w/v N-acetylcysteine and 0.5% w/v $CaCl_2$ and flashed with broad-spectrum light with an overall radiant exposure of 4, 8, 12 and 16 $J \cdot cm^{-2}$. General microbial counts, color, firmness, phenolic compounds and vitamin C contents were evaluated over 15 days at 5 ºC. More pronounced reductions of the naturally-occurring microbiota were observed as the applied PL-dose increased. The quality-stabilizing pre-treatment effectively prevented browning phenomena on the cut-tissue surface. In addition, browning and oxidation were not promoted in PL flashed samples. Indeed, the initial contents in phenolic compounds and vitamin C were even better maintained than in untreated samples. Treatments of 8 and 16 J cm^{-2} were most effective for maintaining the quality and antioxidant characteristics.

Keywords: Pulsed light, fresh-cut apples, antioxidant properties, shelf-life, quality

1. Introduction

Current well-being culture is promoting natural food products as the most desirable items of a healthy diet. Fruit and vegetables are the paradigms within this trend due to their amounts of highly desirable nutrients as well as to their low fat content.

Consequently, food researchers and stakeholders are looking for ways to follow this trend by developing new products such as ready-to-eat fruits, which preserve the fresh-like properties of the raw materials and, at the same time, are convenient and appealing to consumers. As processing operations cause injuries to fruit tissues that result in a reduction of their shelf-life, industry is looking for gentle technological processes that minimize microbial safety threads in processed fruits while keeping under control the typical quality losses of a living product. Chemical compounds such as antioxidants, texture stabilizers, and antimicrobials, either from natural or synthetic origin, have been broadly used for such targets (Martín-Diana, Rico, Frías, Barat, Henehan, & Barry-Ryan, 2007; Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2008; Soliva-Fortuny, Ricart-Coll, & Martín-Belloso, 2005). On minimally processed fruits and vegetables, these treatments have been applied alone or incorporated into edible coating layers (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martín-Belloso, 2009; Valencia-Chamorro, Palou, del Rio, & Pérez-Gago, 2011; Vargas, Pastor, Chiralt, Mc Clements, & González-Martínez, 2008). Their industrial application may be sometimes limited by regulations or, most frequently, by the awareness of consumers to food additives. However, certain antioxidant treatments including dips into ascorbic acid or naturally occurring thiol-compounds are commercially used to delay the development of signs of browning and discoloration on the cut surface of fresh-cut produce. Furthermore, calcium salts have been widely used as firming agents in the fruits and vegetables industry for both whole and fresh-cut commodities (Martín-Diana

et al., 2007). On the other hand, calcium treatments have been widely applied in combination with ascorbic acid and thiol-compounds such as cysteine, N-acetylcysteine, and reduced glutathione to prevent enzymatic browning and maintain firmness of fruits (Rojas-Graü, Sobrino-López, Tapia, & Martín-Belloso, 2006; Rojas-Graü et al., 2008; Soliva-Fortuny, Grigelmo-Miguel, Odriozola-Serrano, Gorinstein, & Martín-Belloso, 2001; Soliva-Fortuny, Oms-Oliu, & Martín-Belloso, 2002). Since calcium chloride may impart flavor, the use of other calcium salts such as calcium propionate, lactate, and ascorbate has been recently suggested (Aguayo, Requejo-Jackman, Stanley, & Woolf, 2010; Alandes, Hernando, Quiles, Pérez-Munuera, & Lluch, 2006; Barbagallo, Chisari, & Caputa, 2012; Quiles, Hernando, Pérez-Munuera, & Lluch, 2007).

An innovative approach for this type of highly valuable products is the use of physical technologies. Pulsed light (PL) is a non-thermal emerging alternative for the superficial decontamination of surfaces by application of light pulses of short duration, on the order of milliseconds and high frequency. The intensity of the light pulses as well as their wide range of wavelengths start a cascade of photo-thermal and photo-chemical processes on the surface tissue of the fruit (Gómez-López, Ragaert, Debevere, & Devlieghere, 2007; Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010b). Previous studies demonstrated the applicability of PL treatments for the decontamination of fresh-cut products such as watermelon, different apple cultivars, avocado, or mushrooms (Gómez, Salvatori, García-Loredo, & Alzamora, 2012; Ignat, Manzocco, Maifreni, Bartolomeoli, & Nicoli, 2014; Oms-Oliu, Aguiló-Aguayo, Martín-Belloso, & Soliva-Fortuny, 2010a; Ramos-Villarroel, Aron-Maftei, Martín-Belloso, & Soliva-Fortuny, 2012; Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny, 2011). However, it is well known that light can have a negative effect on the quality of the fresh-cut products, leading to the degradation of various compounds, such

as those significantly contributing to the antioxidant properties of fruit. Little information about this topic has been published and only a few works have evaluated the effects of PL treatments on antioxidant compounds of whole (Aguiló-Aguayo, Charles, Renard, Page, & Carlin, 2013; Rodov, Vinokur & Horev, 2012) and fresh-cut (Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013; Oms-Oliu et al., 2010a; Zhan, Li, Hu, Pang, & Fan, 2012) fruit and vegetables. In this context, the present work was aimed to study the effect of different pulsed light doses in combination with the use of a quality-stabilizing solution on different quality aspects of fresh-cut 'Golden delicious' apples with a stress on their antioxidant content throughout storage.

2. Materials and methods

2.1. Processing of fresh-cut apples

'Golden delicious' apples were purchased in a local supplier in Lleida (Spain) at commercial maturity, and stored at 5 ± 1 °C prior to processing. The fruits were washed and sanitized by immersion into a 200 μL L^{-1} sodium hypochlorite solution for 1 min; then rinsed, and dried with paper cloth prior to cutting. Apples were peeled with a sharp stainless-steel knife, cored and cut into 10 wedges with a hand-operated apple corer/slicer. After that, apple pieces were dipped for 1 min into a quality-stabilizing solution containing 1% w/v N-acetylcysteine and 0.5% w/v $CaCl_2$ in a solid/liquid ratio of 1:2, as per the commercial practice. Once the excess of solution was blotted off by draining for 5 min, five apple wedges with a weight of approximately 14 g each (ca. 70 g) were placed separately in transparent polypropylene trays which were thermosealed using a packaging machine

104 (ILPRA Food Pack Basic V/G, ILPRA Systems, Vigevano, PV, Italy). The sealing film had
105 64 μm-thick and an oxygen permeability of 110 cm^3 O_2 m^{-2} bar^{-1} day^{-1} at 23 °C and 0% RH
106 (Tecnopack SRL, Mortara, Italy). The film transparency was more than a 97% to the
107 incident UV-radiation and almost a 100% to the visible radiation, whereas, the packaging
108 transparency was a 85% of the incident energy corresponding to wavelengths between 200
109 and 320 nm, which is why the fresh-cut apple pieces were exposed to the PL-treatments
110 once inside the package. Untreated apple samples were prepared with and without
111 immersion into the quality-stabilizing solution to be used as a reference. Once processed,
112 fresh-cut apples were immediately stored for 15 days at 5 ± 1 °C in darkness. Twelve
113 replicates of each one of the assayed treatment conditions were prepared to be randomly
114 withdrawn every 3 days for analysis. Fresh tissues were used for microbiological and
115 quality determinations whereas a portion of 25 g was immediately freeze-dried and stored
116 at -40 ºC until extraction and determination of antioxidant compounds.
117
118 *2.2. Pulsed light treatments*
119 Pulsed light (PL) treatments were carried out using a pulsed UV system Model XeMaticA-
120 2L (360° sample illumination) (SteriBeam Systems GmbH, Kehl, Germany) with two air
121 cooled Xenon lamps situated 8.5 cm far above and below a quartz sample shelf (Fig. 1).
122 The emitted spectrum wavelengths ranged from 180 to 1100 nm with 15–20% of the light
123 in the UV region. Each pulse lasted 0.3 ms and the energy deposition per pulse delivered
124 by each lamp at the sample level was 0.4 $J \cdot cm^{-2}$ per pulse. Each package was individually
125 treated with 10, 20, 30 or 40 light flashes, corresponding to doses of 4, 8, 12, and 16 $J \cdot cm^{-2}$
126 per side, respectively. PL-doses were obtained by measuring the amount of energy received
127 by a photodiode detector placed at the sample holder. The photodiode was connected to an

128 oscilloscope and the recorded signal was transformed into radiance values using a
129 calibration with a standard light source as per the instruction of the manufacturer. In
130 addition, a Makrolon® filter was used to evaluate the amount of radiation in the UV range.
131 Broad-range and UV-range radiations emitted by top and bottom lamps were not much
132 differentially blocked by the packaging materials. Photodiode readings revealed differences
133 of less than 5% in the fluences at the sample level after passing through the package foils.
134 In concomitance with the dose increase, the temperature of fruit surface may gradually rise
135 on the treated surfaces as well as inside the treatment chamber. Just after the highest PL-
136 treatment (16 $J \cdot cm^{-2}$), the highest temperature recording at the sample shelf level was 42.4 ±
137 1.0 °C.
138
139 *2.3. Microbiological quality evaluation*
140 Approximately 10 g of fresh-cut apple wedges were homogenized for 1 min with 90 mL of
141 saline peptone water (0.1% w/v peptone + 0.85% w/v NaCl) with a stomacher blender
142 under sterile conditions (IUL Instruments, Barcelona, Spain). Serial dilutions of the
143 homogenates were poured in plate count agar (PCA) at 30±1°C for 72±3 h and 7±1 °C for 7
144 days for mesophilic and psychrophilic aerobic bacteria counts, respectively (ISO 4833,
145 1991) and chloramphenicol glucose agar (CGA) at 25±1 °C for 5 days for molds and yeasts
146 counts (ISO 7954, 1988). Peptone and agar media were purchased from Biokar Diagnostics
147 (Beauvais, France). Two packages were taken at each sampling time to perform the
148 analysis and two replicate counts were carried out for each one. Results were expressed as
149 colony forming units (CFU) per gram of fresh apple piece.
150
151 *2.4. Color determination*

152 Cut apple surface color values were directly measured with a colorimeter (ChromaMeter
153 Model CR-400, Konica Minolta Sensing Inc., Osaka, Japan) using a D65 illuminant and an
154 observation angle of 10°. The instrument was calibrated using a standard white reflector
155 plate. Three readings of L* (lightness), a* (green-red chromaticity) and b* (blue-yellow
156 chromaticity) coordinates were recorded from each apple piece. At least 5 samples from 2
157 replicate packages were evaluated for each treatment and sampling time.
158
159 *2.5. Firmness measurements*
160 Apple firmness was evaluated with a TA-XT2 Texture Analyzer (Stable Micro Systems
161 Ltd., Surrey, U.K.) by measuring the force required for a 4-mm-dia probe to penetrate apple
162 slices of 20 mm height to a depth of 10 mm at a rate of 5 mm s^{-1} and automatic return.
163 Samples were placed perpendicular to the probe, so that they could be penetrated in their
164 geometric center. Bags were randomly withdrawn each 3 days and their content was
165 directly analyzed. At least 10 repetitions obtained from 2 replicate packages were evaluated
166 for each treatment at each sampling time. Results were expressed as the maximum
167 penetration force in N.
168
169 *2.6. Evaluation of antioxidant properties*
170
171 *2.6.1. Preparation of methanolic extracts*
172 One gram of freeze-dried samples was extracted twice with 5 mL of 80% v/v methanol
173 using an Ultra-Turrax homogenizer (IKA Model T 25 Digital, Germany) (2 min, 10,200
174 rpm, 0 °C). The mixture was centrifuged at 16,546 x *g* for 10 min at 5 °C (AVANTI J-26

175 XP centrifuge, Beckman Coulter Inc., Fullerton, CA, USA). The supernatant was recovered
176 and made up to 10 mL with 80% v/v methanol. The resulting solution constituted the
177 methanolic extract.
178
179 *2.6.2. Determination of antioxidant capacity*
180 The antioxidant capacity was studied through the evaluation of the free radical-scavenging
181 effect of the extracts on a solution of 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radical (Fluka
182 Chemie, Switzerland), according to the procedure proposed by de Ancos, Sgroppo, Plaza,
183 & Cano (2002). Aliquots of 0.03 mL of the methanolic extract were mixed with 3.9 mL of
184 methanolic $DPPH^{\cdot}$ solution (0.025 g L^{-1}) and 0.07 mL of distilled water. The homogenate
185 was shaken vigorously and kept in darkness for 30 min. The absorbance was measured with
186 a CECIL CE 1021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 515 nm
187 against a blank of methanol without $DPPH^{\cdot}$. Results were expressed as percentage of
188 inhibition of the radical $DPPH^{\cdot}$, that is the decrease in absorbance with respect to the
189 control value ($DPPH^{\cdot}$ initial absorption value).
190
191 *2.6.3. Determination of total phenolic content*
192 Total phenolic content was determined according to the Folin-Ciocalteu procedure
193 (Singleton, Orthofer, & Lamuela-Raventós, 1999) with some modifications. One hundred
194 microliters of methanolic extract were added at 4.5 mL of water and 400 µL of Folin-
195 Ciocalteau reagent (Sigma Chemical, St. Louis, MO, USA). After 3 min, 1 mL of saturated
196 solution of Na_2CO_3 was added, and the reaction mixture was incubated for 1 h at ambient
197 temperature. The absorbance was measured at 760 nm. The total phenolic content was
198 calculated using gallic acid (Sigma Chemical, St. Louis, MO, USA) as standard in the 0-9

199 μg mL^{-1} concentrations range. Results were expressed as milligrams of gallic acid in 100 g
200 of fresh weight.
201
202 *2.6.4. Determination of total flavonoids*
203 Total flavonoids were spectrofotometrically determined by using a modification of the
204 technique described by Kim, Jeong, & Lee (2003). A test tube was added with 1900 μL of
205 distilled water and 400 μL of the methanolic extract. Other compounds were added
206 sequentially: initially (zero time) a volume of 80 μL of 5% (w/v) $NaNO_2$; after 5 min, 80
207 μL of 10% (w/v) $AlCl_3$ and finally, after additional 6 min, 540 μL of 1 mol L^{-1} NaOH.
208 Solutions were stirred in a vortex and then absorbance at 510 nm was measured. Catechin
209 (Sigma-Aldrich, St. Louis, MO, USA) was used as standard in the 0-27 μg mL^{-1}
210 concentration range. Results were expressed as mg in 100 g of fresh weight.
211
212 *2.6.5. Determination of vitamin C*
213 Vitamin C content of fresh-cut apples was analyzed by HPLC. The extraction procedure
214 and chromatographic conditions were based on a previous study carried out by Odriozola-
215 Serrano, Hernandez-Jover, & Martín-Belloso (2007). Lyophilized samples of 0.2 g were
216 extracted twice with 5 mL of a solution containing 45 g L^{-1} metaphosphoric acid and 7.2 g
217 L^{-1} DL-1,4-dithiotreitol, using an Ultra-Turrax (IKA Model T 25 Digital, Germany)
218 homogenizer (2 min, 10,200 rpm, 0 °C). The mixture was centrifuged at 4,020 x *g* for 15
219 min (Hettich ZENTRIFUGEN EBA 21, Andreas Hettich GmbH & co. KG, Tuttlingen,
220 Germany). The supernatant was recovered and made up to 10 mL. An aliquot of 20 μL was
221 injected into the HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 mm)

222 stainless steel column (250 mm x 4.6 mm). The mobile phase was a 0.01% v/v sulfuric acid
223 solution adjusted to pH = 2.6. The flow rate was fixed at 1.0 mL/min at room temperature.
224 Detection was performed with a 2996 Photodiode Array Detector (Waters, Milford, MA)
225 set at 245 nm. Vitamin C content was calculated using ascorbic acid (Scharlau Chemie
226 S.A., Barcelona, Spain) as standard in the 0-15 μg mL^{-1} concentration range. Results were
227 expressed as mg of vitamin C per 100 g of fresh weight.
228
229 *2.7. Data analysis*
230 Statistical analysis was performed using the InfoStat/L v. 2012I software package (Di
231 Rienzo, Casanoves, Balzarini, Gonzalez, Tablada, & Robledo, 2012). Three independent
232 trials were performed according to a factorial design. The factors were the treatment (with
233 or without), and the storage time. Means and standard deviation of the three trials were
234 depicted. Data were analyzed by means of an analysis of variance, and the means were
235 compared by the least significant difference (LSD) test at $P < 0.05$.
236
237 **3. Results and discussion**
238
239 *3.1. Microbiological stability*
240 As displayed in Fig. 2, PL treatments exerted a significant antimicrobial effect on the
241 indigenous microbial load of fresh-cut apple wedges. The quality-stabilizing dip seemed to
242 favor the growth of microorganism on the cut product surface in a first instance, probably
243 due to the presence of available water required for spoilage microorganisms. The
244 application of PL helped to control this initial increase. Just after the treatment, both the
245 mesophilic and psychrophilic microbial counts on apple wedges exposed to a fluence of 4

246 $J \cdot cm^{-2}$ per side were reduced by 0.75 log units. Energy dosages of 8, 12 and 16 $J \cdot cm^{-2}$ led to
247 reductions of up to 1.55 log units (Fig. 2a and 2b). Interestingly, treatments with a fluence
248 greater than 8 $J \cdot cm^{-2}$ did not appear to have an additional antimicrobial effect on the
249 naturally-occurring aerobes, as the initial counts were not further reduced. A slightly
250 different pattern was observed for mold and yeast counts (Fig. 2c) which were more
251 noticeably reduced under the most intense treatment conditions. In this regard, fluence
252 values of 16 $J \cdot cm^{-2}$ caused 2.3 log reductions of the initial molds and yeasts loads. Gómez
253 et al. (2012) found reductions in the same order of magnitude for mesophilic
254 microorganisms and molds and yeasts just after apply higher doses on Granny Smith cut
255 apple.
256 Energy dosage has been previously identified as a critical factor determining the
257 antimicrobial effect of a PL treatment (Gómez et al., 2012; Oms-Oliu et al., 2010a). It is
258 known that PL can led to microbial cell death through the generation of photochemical and
259 photothermal damage. In the first case, inactivation is related to the induction of DNA
260 strand breaks and formation of pyrimidine dimers by UV radiation. In addition, heating is a
261 localized phenomenon which may end with the generation of thermal stress (Demirci &
262 Krishnamurthy, 2011). In our study, signs of abusive temperatures during processing were
263 not recorded at the fruit surface, which supports the hypothesis that most of the inactivation
264 was attained by non-thermal mechanisms.
265 Although some PL treatments were reported to have a similar impact on the initial
266 microbiota of fresh-cut apple wedges, differences among fruit samples treated with
267 different fluence values became evident throughout storage at 5 ºC. The growth inhibition
268 of the naturally-occurring microorganisms was found to be dependent on the intensity of
269 the PL treatment. In this regard, the effect was more pronounced for higher applied doses.

Hence, although inactivation values were recorded for mesophilic aerobic bacteria exposed to 8 and 16 $J \cdot cm^{-2}$ were similar, the subsequent growth through 15 days of refrigerated storage significantly differed, leading to increases of 2.7 and 1.4 log CFU g^{-1}, respectively (Fig. 2a).

These results may be an indicator of the occurrence of sub-lethal injuries under certain treatment conditions. Two main mechanisms could explain these observations. On the one hand, and as previously reported (Lasagabaster & Martínez de Marañón, 2014), cold storage might prevent injured cells from repairing sub-lethal damage, which was presumably higher under the most intense treatments, thus limiting cell recovery and subsequent growth upon storage. On the other hand, some injured microorganisms possess an enzyme-mediated repair mechanism, known as photoreactivation, which typically occurs when photoliases catalyze the reversion of pyrimidine dymers to their monomeric form (Weber, 2005). Photoreactivation occurs when UV-injured cells are exposed to UV-A (320-400 nm) nm and blue light (400-500 nm). Since, in the present experiment, fresh-cut apples were stored under darkness conditions, this latter mechanism did not likely underlie the differences in microbial growth through storage.

3.2. Color parameters

Immersion into an N-acetylcysteine solution did not significantly impact the fresh-like appearance of fresh-cut apple wedges. Differences among the initial lightness (L*) values of apple pieces immersed or not immersed into the quality-stabilizing solution were not observed regardless the exposure to pulsed light (Fig. 3a). Nevertheless, a slight increase in a* values, thus becoming less negative, could be appreciated in apple tissues not immersed into the N-acetylcysteine dip immediately after cutting (Fig. 3b). In addition, this increase

294 approached positive a* values (less green but redder) along the first week of storage. This
295 trend, which was accompanied by a marked decrease in L* values during the same period
296 of time, evidences the susceptibility to enzymatic browning of the cut apple tissue as well
297 as the need for the antioxidant dip. Furthermore, b* values (data not shown) were not
298 affected by the treatment and did not change over storage. The N-acetylcysteine dip
299 prevented color changes on cut apple surface as a consequence of PL exposure and storage
300 time. As expected, and also confirmed by preliminary tests (data not shown), PL treatments
301 applied to fresh-cut apple tissues may promote the development of browning, especially
302 when high energy doses are delivered. Gómez et al. (2012) reported that apple discs
303 subjected to PL fluencies higher than 2.4 $J \cdot cm^{-2}$ without any further antibrowning treatment
304 turned darker (lower L* values) and less green (higher a* values) than untreated ones,
305 which was hypothesized to be caused by photothermal damage. Similarly, Ignat et al.
306 (2014) reported browning promotion in apple slices treated at 15.75 $J \cdot cm^{-2}$ and attributed
307 the color changes to the increase in enzyme-substrate interactions caused by cell damage.
308 Our results indicate that these undesirable effects of PL treatments on the visual appearance
309 of fresh-cut produce may be effectively prevented for energy fluencies of at least as much
310 as 16 $J \cdot cm^{-2}$ per side. This is in line with the results reported by Oms-Oliu et al. (2010a)
311 and Ramos-Villarroel et al. (2011) in fresh-cut avocadoes and mushrooms treated under
312 similar conditions.
313
314 *3.3. Firmness*
315 As shown in Fig. 4, the firmness of PL-treated apples did not majorly change as a
316 consequence of processing. Differences among firmness values of apple wedges subjected
317 to PL did not significant differ among treatments. Only apple pieces exposed to 16 $J \cdot cm^{-2}$

318 exhibited significantly higher firmness values of 11.2 N, but these values declined
319 subsequently. The immersion into the quality-stabilizing dip, which contained 0.5% (w/v)
320 $CaCl_2$, did not result into significantly firmer fruit. Indeed, untreated fruit pieces better kept
321 their initial firmness values throughout storage. A clear pattern governing the effects of PL
322 treatments on firmness cannot be noticed. However, the overall firmness values at
323 prolonged storage significantly differed among apple pieces processed under different
324 conditions. Hence, over 15 days of storage the firmness of fresh-cut apples flashed with 4
325 $J \cdot cm^{-2}$ almost doubled that of those flashed with 12 $J \cdot cm^{-2}$. The slight declines in firmness
326 values through storage are in agreement with the results obtained by Ramos-Villarroel et al.
327 (2011), who proposed the use calcium lactate (1%) to help to maintain firmness of cut
328 avocadoes. The slight changes in firmness could be a consequence of the modulation of
329 pectinolytic enzyme activities as a consequence of the applied treatments. Pectic enzymes,
330 namely pectin methylesterase and polygalacturonase, from several plant sources exhibit
331 activity increases at specific temperatures or as a consequence of the exposure to stress
332 conditions (Jurick et al. 2009; Denès et al. 2000; Castaldo et al. 1989). Hence, the
333 modulation of pectinolytic activities under certain conditions as well as the consequences of
334 a stress-induced response produced in the fruit after the application of the most intense
335 treatments could explain the poor correlation between dose and effect.
336
337 *3.4. Antioxidant properties*
338 As seen in Fig. 5, the $DPPH^{\cdot}$ radical scavenging capacity of apple samples was dramatically
339 increased by the immersion into an N-acetylcysteine solution. Nevertheless, pulsed light
340 treatments did not cause a decrease in the antioxidant potential of fresh-cut apples.
341 Considering the effects of energy fluence on the antioxidant activity of dipped apple pieces,

342 major differences among treatments were not remarkable just after processing and over the
343 first 9 days of storage. However, fresh-cut apples exposed to the highest energy doses (8,
344 12 and 16 $J \cdot cm^{-2}$) better retained their initial antioxidant capacity values until the end of the
345 evaluated storage period (Fig. 5).
346 The effect of PL treatments on the phenolic and vitamin C contents of apple pieces can be
347 observed in Fig. 6 and 7. As can be seen, antioxidant capacity values correlated well with
348 the content in phenolic compounds throughout storage. Vitamin C contents were as well
349 correlated with the total antioxidant potential, although to a lesser extent. This is due to the
350 fact that the overall antioxidant potential in apple is mainly a consequence of the presence
351 of phenolic compounds rather than to vitamin C, as reported by Lee, Kim, Kim, Lee, & Lee
352 (2003) for several apple cultivars.
353 An analysis of the phenolic content as affected by the assayed treatments reveals that the
354 use of the quality-stabilizing dip stood as the most significant factor ($p<0.0001$) influencing
355 the content in total phenolics (Fig. 6a). This is probably caused by an interference of N-
356 acetylcysteine with the Folin-Ciocalteau reagent. Some sulfur and nitrogenous compounds,
357 together with ascorbic acid, sugars, Fe(II), and a long list of organic compounds have been
358 reported to interfere the Folin-Ciocalteu assay for the determination of phenolics (Prior,
359 Wu, & Schaich, 2005). Therefore, although the method proposed by Singleton et al. (1999)
360 is widely used as an indicator of the phenolic content, values should be interpreted as a
361 measurement of the total reducing capacity of the apple pieces. This also explains the good
362 correlation of the total phenolic content with the antioxidant capacity values estimated by
363 the $DPPH^{\cdot}$ assay. However, the N-acetylcysteine did not appear to interfere in the
364 determination of total flavonoids, as the contents in samples not immersed and immersed
365 into the antioxidant dip were in the same order of magnitude (Fig. 6b). In addition, flashed

366 fresh-cut apples exhibited higher total phenolic compounds throughout storage than apple
367 pieces only immersed into the quality-stabilizing solution. Consistently, PL-treated cut
368 apples better kept the highest flavonoids contents during storage. This is in agreement with
369 the results reported by Rodov et al. (2012), who reported higher values of phenolics content
370 in fig fruits irradiated by PL than in the untreated fruits. This may be explained by the
371 presence of stress-induced phenylpropanoids promoted by the exposure to PL treatments.
372 Among flavonoids, anthocyanins and flavones have been reported to increase in plants in
373 response to high visible light levels, as it is thought that these compounds help attenuate the
374 amount of light reaching the photosynthetic cells. As well, UV irradiation has been shown
375 to induce the synthesis of some types of flavonoids in different plant species, as a mean of
376 protection against UV-B damage (Dixon & Paiva, 1995). Despite our results are in line
377 with these findings, a correlation between the amount of incident energy and the production
378 of flavonoids could not be found.
379 Whether this stress-induced response is caused or not by thermal means is another question
380 that remains unanswered. According to Murugesan, Orsat, & Lefsrud (2012), the
381 temperature increase on the surface of elderberry fruits during PL-treatments (7 to 33 $J \cdot cm^{-2}$)
382 may lead to the synthesis of phenolic compounds by the fruits. However, Oms-Oliu et al.
383 (2010a) reported that it is thermal damage itself which limits this stress-induced response,
384 as high fluence treatements (28 J cm^{-2}) significantly reduced the antioxidant capacity and
385 the total phenolic content in fresh-cut mushrooms. Although temperature on the surface of
386 apple wedges was not recorded during the PL-treatment, it was monitored over the whole
387 treatment with a thermocouple. Hence, after exposure to the highest energy dose, a
388 temperature of 42.4 ± 1.0 ºC may suggest that abusive heating did not occur on the surface
389 of apple slices.

390 As ascorbic acid is largely thermo-labile, a focus on vitamin C levels may cast light on the
391 underlying phenomena. As can be observed in Fig. 7, the vitamin C content of dipped fruit
392 pieces was between 10-70% above that observed in undipped samples, probably as a
393 consequence of the reducing effect of N-acetylcysteine which helped protecting ascorbic
394 acid from oxidation. The vitamin C contents in PL-treated samples were maintained during
395 at least two weeks and were in line with those found in untreated fresh-cut apples or even
396 slightly above. These results are in contrast with those reported by Oms-Oliu et al. (2010a),
397 who found a continuous vitamin C decrease during the storage of PL-treated fresh-cut
398 mushrooms, with a more pronounced effect for higher energy doses. In the present case, the
399 high ascorbic acid contents seem to point out that heating was not behind the observed
400 effects, so that differences in the antioxidant potential, as well as in microbial counts, were
401 more likely to be caused by photochemical rather than to thermal processes.
402
403 **4. Conclusions**
404 Pulsed light treatments stand as a feasible alternative for extending the microbiological
405 shelf life of fresh-cut apples at 5°C, while maintaining their quality and antioxidant
406 attributes. The growth inhibition of the native microbiota was generally dependent on the
407 energy fluence delivered at the time of processing. A negative impact on the antioxidant
408 potential of flashed fresh-cut apples was not observed. Instead, experimental results suggest
409 the promotion of the phenylpropanoid metabolism by nonthermal means, as vitamin C
410 levels were preserved throughout refrigerated storage.
411 Taken together, our results suggest that PL-treatments at 8 and 16 $J \cdot cm^{-2}$ combined with
412 immersion into the quality-stabilizing solution help to maintain a better quality and
413 antioxidant characteristics of the product for 15 days at 5 °C. Although a dose of 12 $J \cdot cm^{-2}$

affected most parameters studied in similar way to the other treatments applied, this treatment did not allow maintaining the original firmness. Since regulations of certain countries, for instance those issued by the U.S. Food and Drug Administration, stipulate that the total cumulative treatment shall not exceed 12 $J \cdot cm^{-2}$, a treatment of 8 $J \cdot cm^{-2}$ in combination with the immersion into a quality-stabilizing solution may be selected to extend the microbiological shelf life of fresh-cut apples without dramatically affecting their texture. Future research works should be aimed at evaluating the efficacy of the PL-treatments regarding against pathogenic microorganisms. Furthermore, to the best of the authors' knowledge no dramatic changes regarding the sensory quality of the treated fruit were caused by the assayed treatments. However, in-depth studies should evaluate the sensory implications of such treatments for the sake of transferring the generated knowledge to the productive sector.

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554 ## Figure captions

555

556 **Fig. 1** Schematics of the PL-treatment chamber.

557

558 **Fig. 2** Mesophilic (A) and psychrophilic (B) aerobic bacteria, and yeasts and molds (C)
559 counts (expressed as log CFU g^{-1}) of fresh-cut apples exposed to light pulses (expressed as
560 J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% w/v N-acetylcysteine + 0.5% w/v $CaCl_2$
561 (LSD at $p<0.05$; $LSD_A = 0.31$; $LSD_B = 0.40$; $LSD_C = 0.28$)

562

563 **Fig. 3** L* (A) and a* (B) values of fresh-cut apples exposed to light pulses (expressed as J
564 cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% w/v N-acetylcysteine + 0.5% w/v $CaCl_2$
565 (LSD at $p<0.05$; $LSD_A = 2.29$; $LSD_B = 0.78$)

566

567 **Fig. 4** Firmness values (expressed in N) of fresh-cut apples exposed to light pulses
568 (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% w/v N-acetylcysteine + 0.5%
569 w/v $CaCl_2$ ($LSD_{0.05} = 0.18$)

570

571 **Fig. 5** Antioxidant capacity (expressed as % $DPPH^{\cdot}$ in g^{-1}) of fresh-cut apples exposed to
572 light pulses (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% w/v N-
573 acetylcysteine + 0.5% w/v $CaCl_2$ ($LSD_{0.05} = 4.93$)

574

575 **Fig. 6** Total phenols (A) and flavonoids (B) contents (expressed as mg gallic acid 100 g^{-1}
576 and mg catechin 100 g^{-1}, respectively) of fresh-cut apples exposed to light pulses

577 (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% w/v N-acetylcysteine + 0.5%
578 w/v $CaCl_2$ (LSD at $p<0.05$; $LSD_A = 6.17$; $LSD_B = 2.02$)
579
580 **Fig. 7** Vitamin C content (expressed as mg ascorbic acid 100 g^{-1}) of fresh-cut apples
581 exposed to light pulses (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% w/v
582 N-acetylcysteine + 0.5% w/v $CaCl_2$ ($LSD_{0.05} = 0.18$)
583

Figure 1

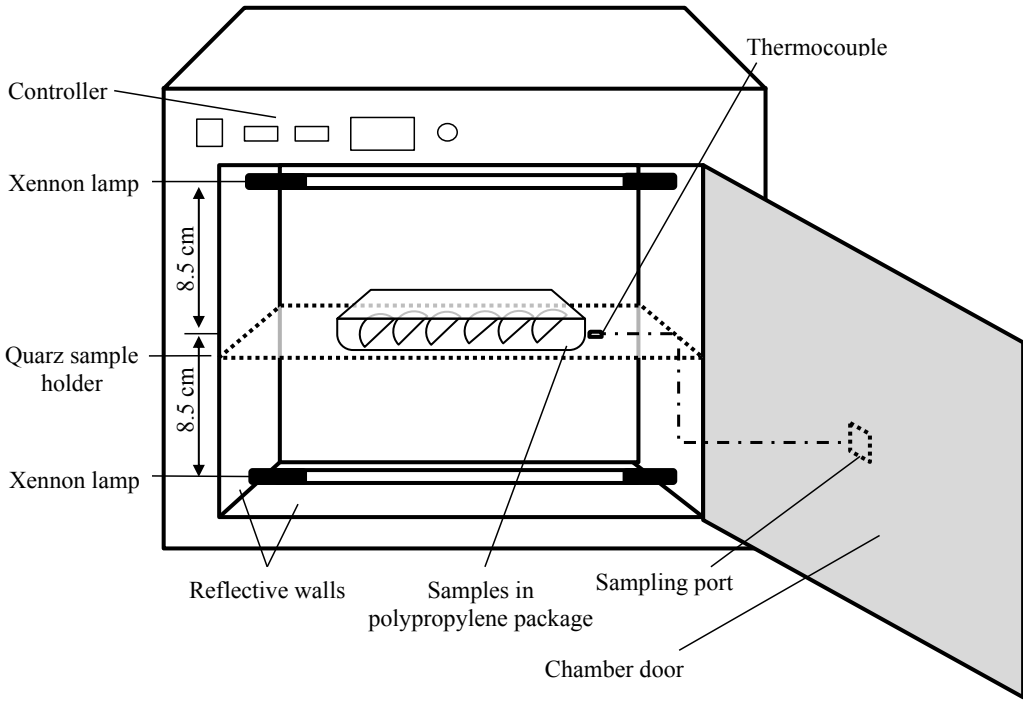
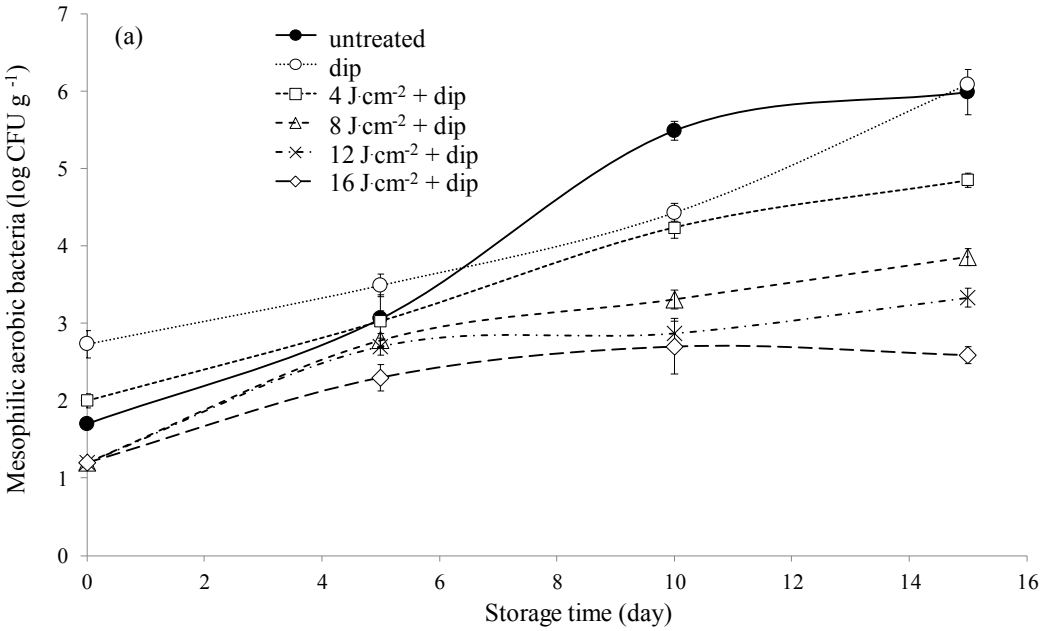
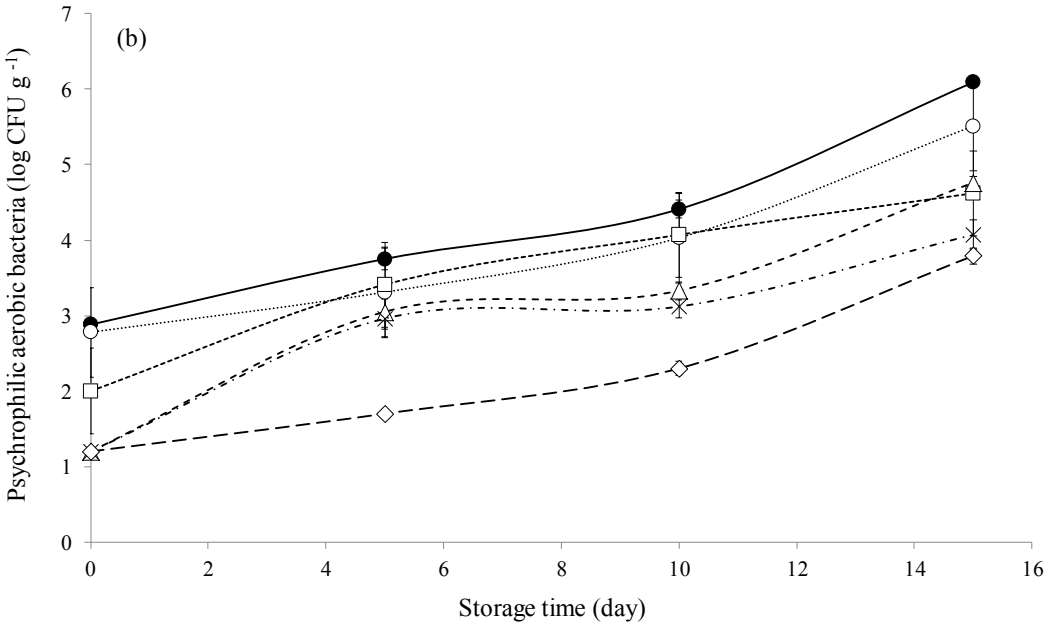

Thermocouple
Controller
Xennon lamp
8.5 cm
Quarz sample holder
8.5 cm
Xennon lamp
Reflective walls
Samples in polypropylene package
Sampling port
Chamber door

Figure 2


(a)
untreated
dip
4 J·cm-2 + dip
8 J·cm-2 + dip
12 J·cm-2 + dip
16 J·cm-2 + dip
Mesophilic aerobic bacteria (log CFU g-1)
Storage time (day)


(b)
Psychrophilic aerobic bacteria (log CFU g-1)
Storage time (day)

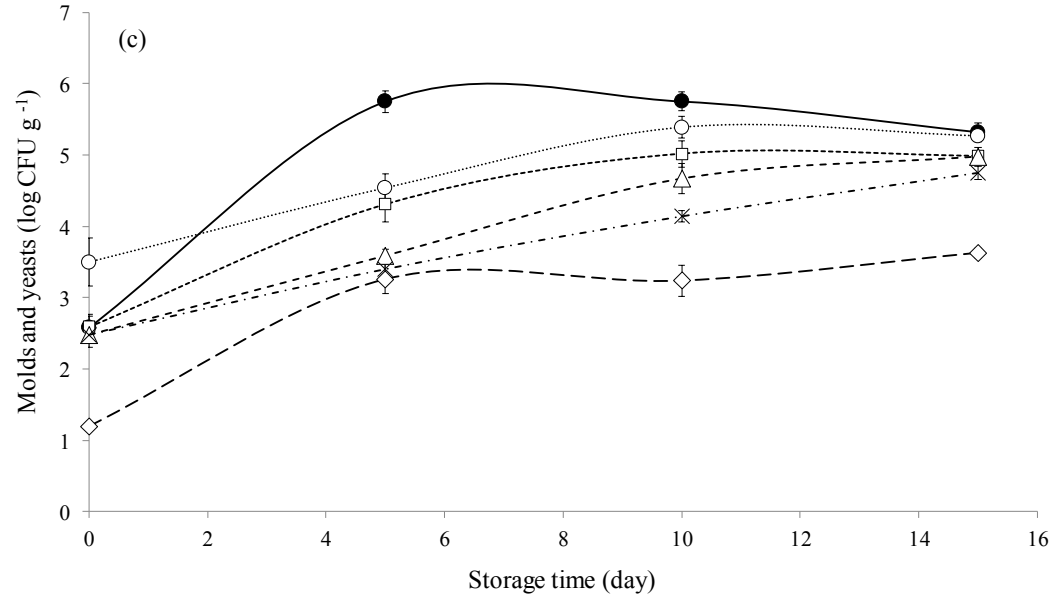

(c)
Molds and yeasts (log CFU g-1)
Storage time (day)

Figure 3

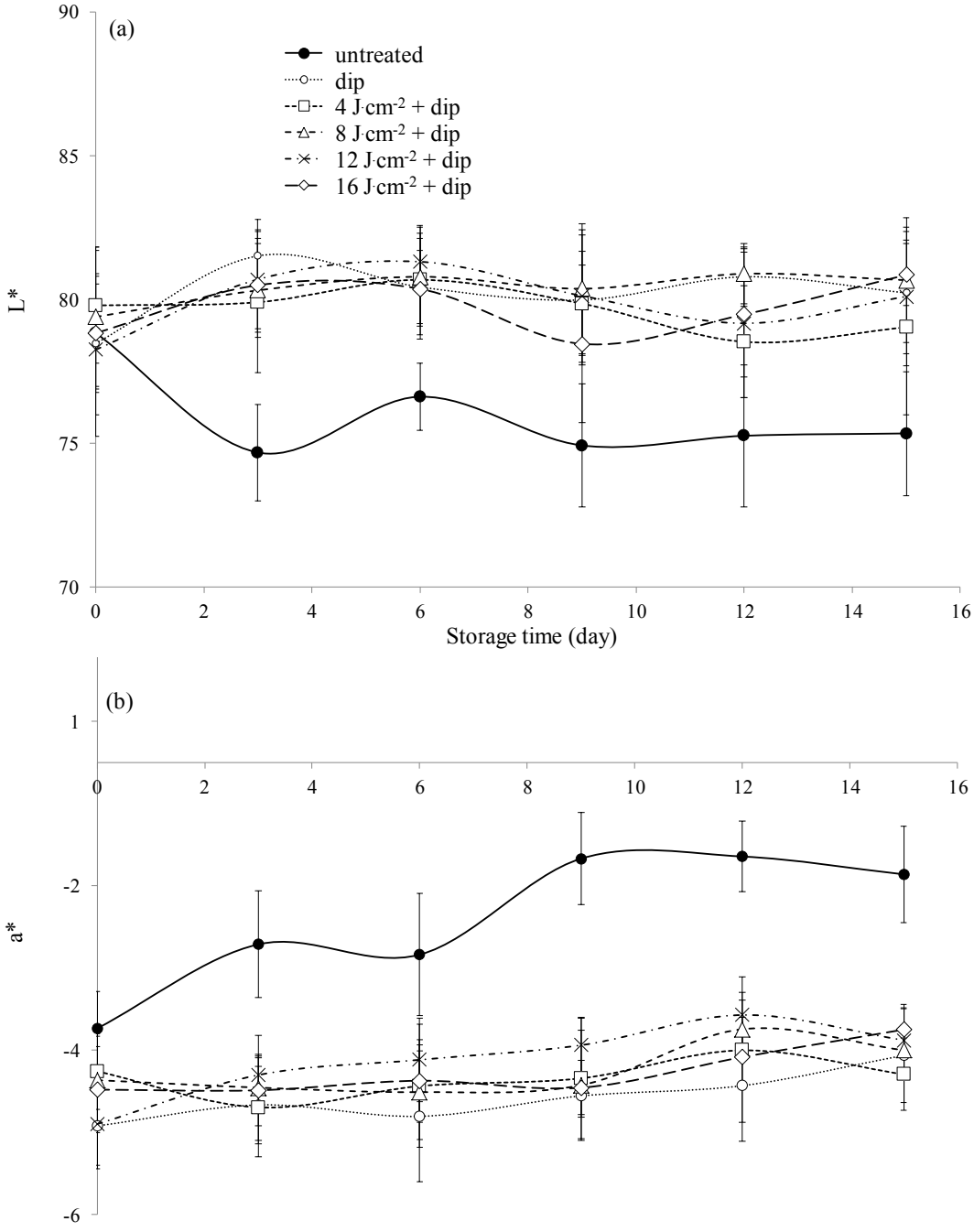

(a)
untreated
dip
4 J·cm-2 + dip
8 J·cm-2 + dip
12 J·cm-2 + dip
16 J·cm-2 + dip
L*
90
85
80
75
70
0
2
4
6
8
10
12
14
16
Storage time (day)
(b)
a*
1
-2
-4
-6

Figure 4

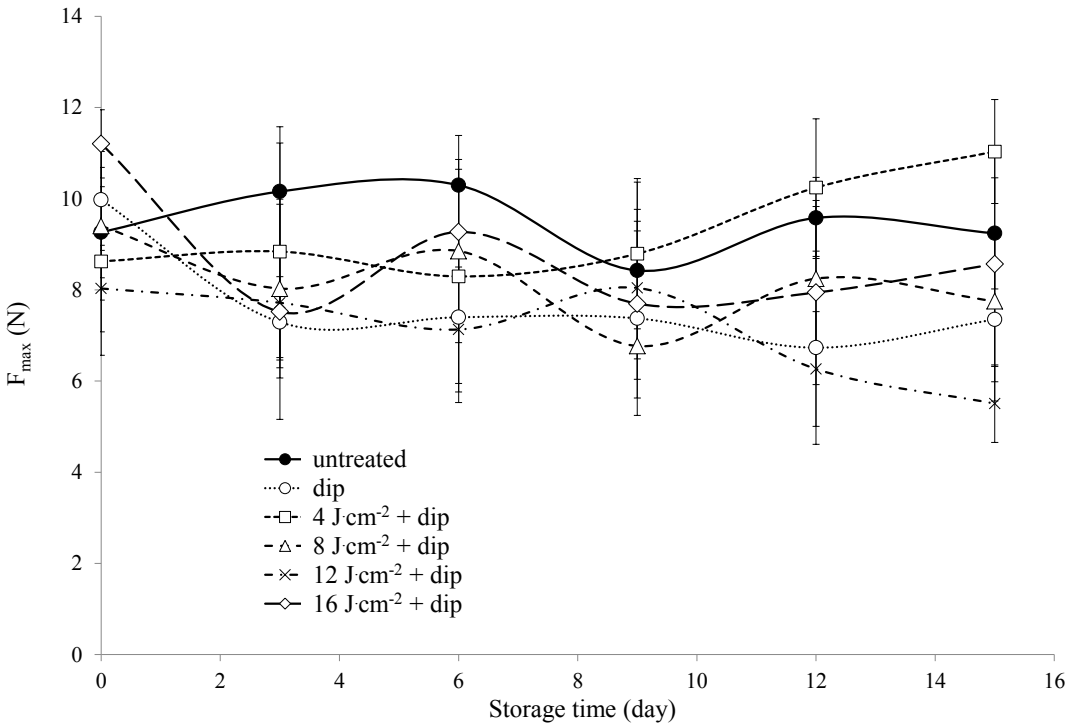

14
12
10
8
6
4
2
0
F_{max} (N)
0
2
4
6
8
10
12
14
16
Storage time (day)
untreated
dip
4 J·cm^{-2} + dip
8 J·cm^{-2} + dip
12 J·cm^{-2} + dip
16 J·cm^{-2} + dip

Figure 5

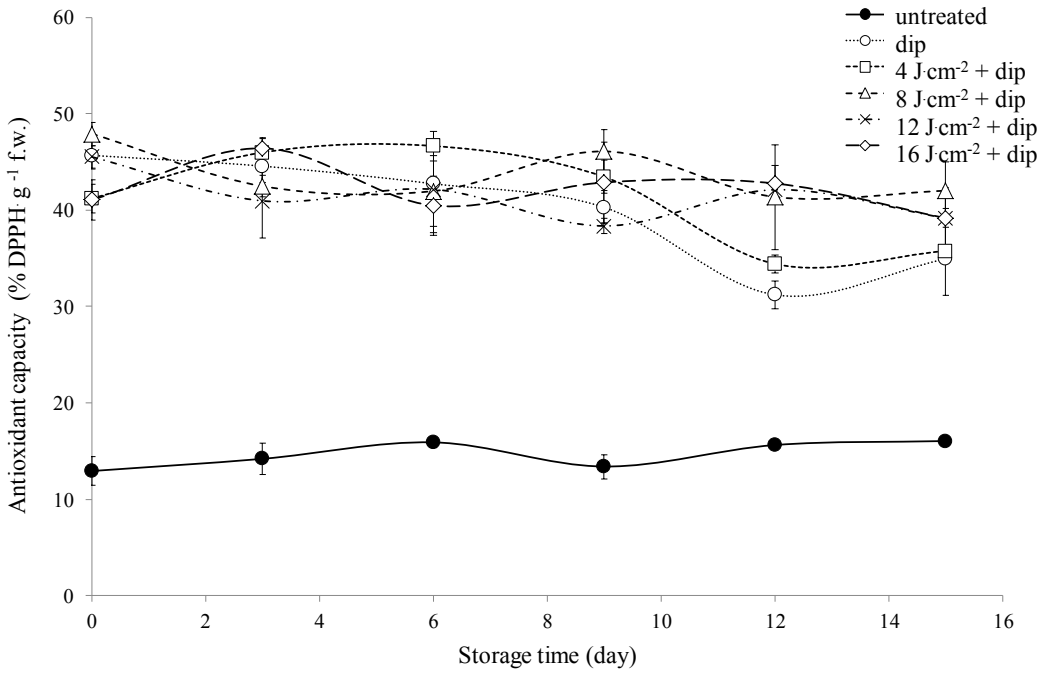

untreated
dip
4 J·cm-2 + dip
8 J·cm-2 + dip
12 J·cm-2 + dip
16 J·cm-2 + dip
Antioxidant capacity (% DPPH· g -1 f.w.)
Storage time (day)
0
10
20
30
40
50
60
0
2
4
6
8
10
12
14
16

Figure 6

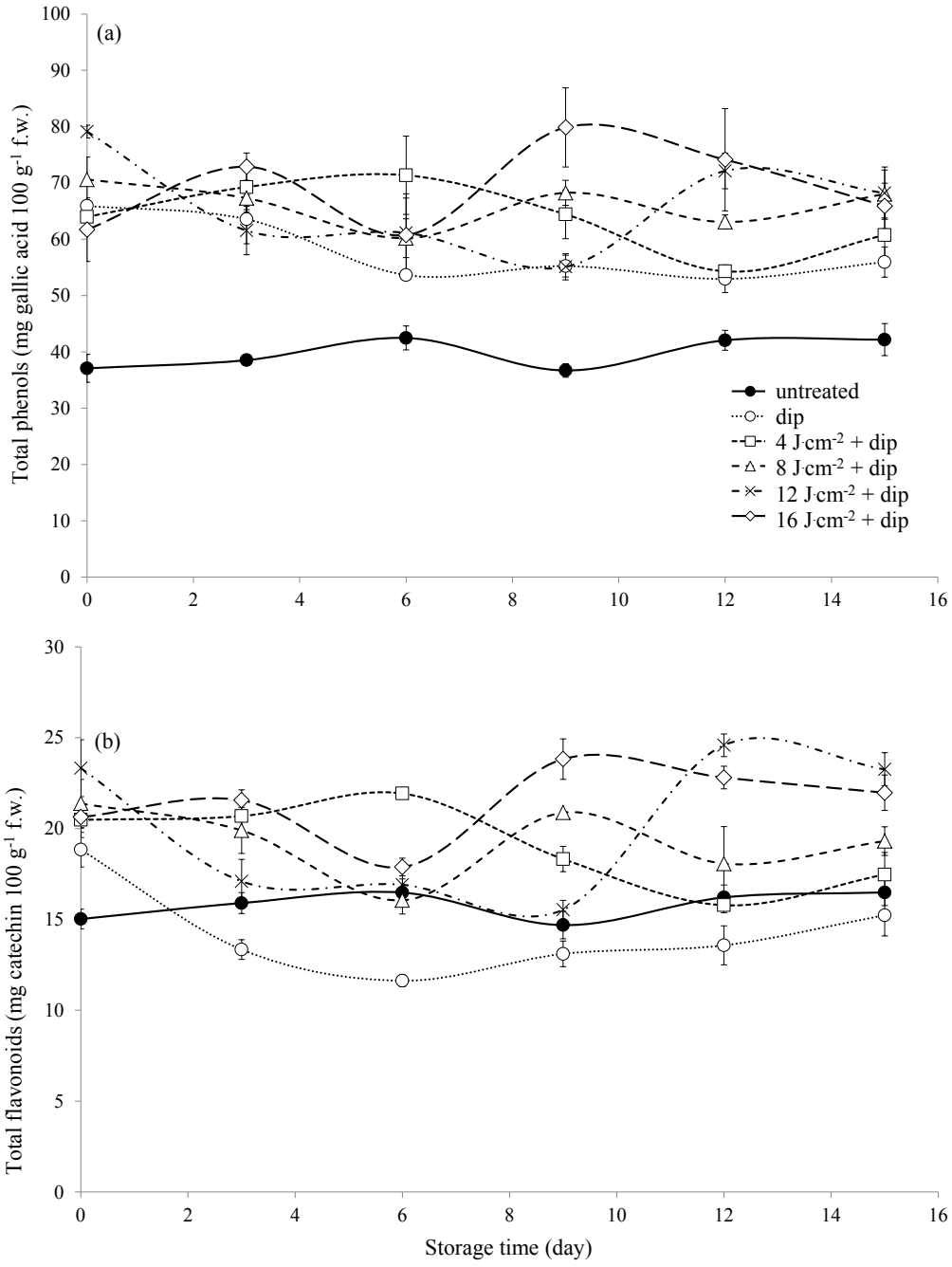
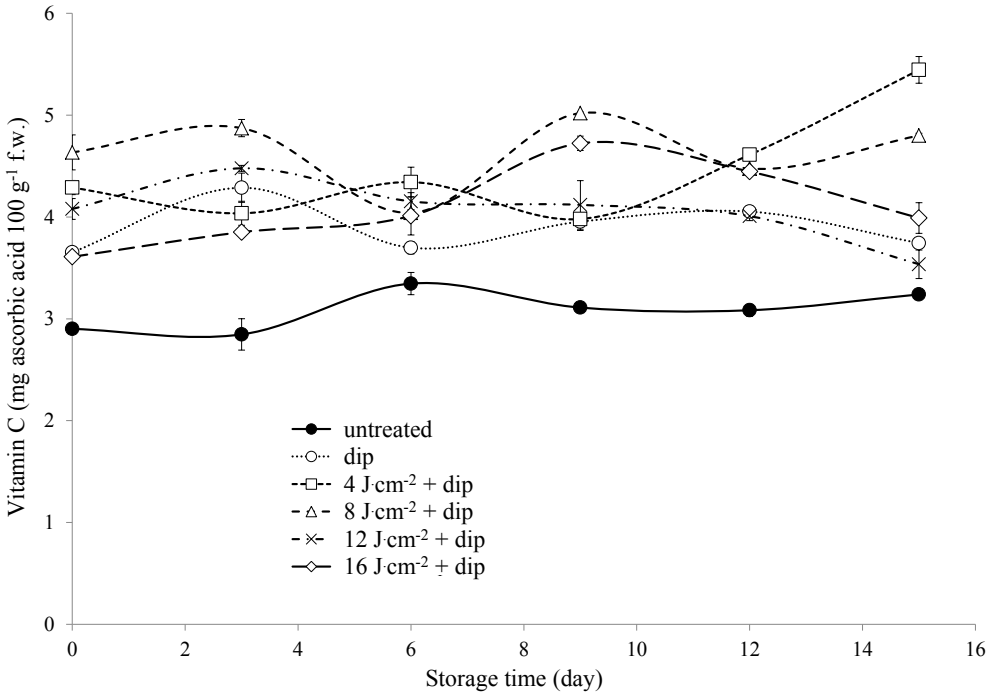

(a)
Total phenols (mg gallic acid 100 g-1 f.w.)
untreated
dip
4 J·cm-2 + dip
8 J·cm-2 + dip
12 J·cm-2 + dip
16 J·cm-2 + dip
(b)
Total flavonoids (mg catechin 100 g-1 f.w.)
Storage time (day)

Figure 7


Vitamin C (mg ascorbic acid 100 g-1 f.w.)
Storage time (day)
0
1
2
3
4
5
6
0
2
4
6
8
10
12
14
16
untreated
dip
4 J·cm-2 + dip
8 J·cm-2 + dip
12 J·cm-2 + dip
16 J·cm-2 + dip