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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, colour, firmness, phenolic compounds and vitamin C contents were evaluated over 15 days at 5 °C. 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.

Industrial relevance: Pulsed light technology is an emerging technique with good prospects for the decontamination of foods and food contact surfaces. Application of pulse light treatments for increasing safety and extending microbial shelf life of fresh-cut produce seems feasible. However, their effects on the quality and antioxidant characteristics of fruit need to be evaluated for successfully applying the technology at an industrial level.

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 et al., 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 Río, & 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 flavour, 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).

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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 (ILPRA Food Pack Basic V/G, ILPRA Systems, Vigevano, PV, Italy). The sealing film had 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 (Tecnopack SRL, Mortara, Italy). The film transparency was more than a 97% to the incident UV-radiation and almost a 100% to the visible radiation, whereas, the packaging transparency was a 85% of the incident energy corresponding to wavelengths between 200 and 320 nm, which is why the fresh-cut apple pieces were exposed to the PL-treatments once inside the package. Untreated apple samples were prepared with and without immersion into the quality-stabilizing solution to be used as a reference. Once processed, fresh-cut apples were immediately stored for 15 days at 5 ± 1 °C in darkness. Twelve replicates of each one of the assayed treatment conditions were prepared to be randomly withdrawn every 3 days for analysis. Fresh tissues were used for microbiological and quality determinations whereas a portion of 25 g was immediately freeze-dried and stored at −40 °C until extraction and determination of antioxidant compounds.

2.2. Pulsed light treatments

Pulsed light (PL) treatments were carried out using a pulsed UV system Model XeMaticA-2 L (360° sample illumination) (SteriBeam Systems GmbH, Kehl, Germany) with two air cooled Xenon lamps situated 8.5 cm far above and below a quartz sample shelf (Fig. 1). The emitted spectrum wavelengths ranged from 180 to 1100 nm with 15–20% of the light in the UV region. Each pulse lasted 0.3 ms and the energy deposition per pulse delivered by each lamp at the sample level was 0.4 J·cm^{-2} per pulse. Each package was individually treated with 10, 20, 30 or 40 light flashes, corresponding to doses of 4, 8, 12, and 16 J·cm^{-2} per side, respectively. PL-doses were obtained by measuring the amount of energy received by a photodiode detector placed at the sample holder. The photodiode was connected to an oscilloscope and the recorded signal was transformed into radiance values using a calibration with a standard light source as per the instruction of the manufacturer. In addition, a Makrolon® filter was used to evaluate the amount of radiation in the UV range. Broad-range and UV-range radiations emitted by top and bottom lamps were not much differentially blocked by the packaging materials. Photodiode readings revealed differences of less than 5% in the fluences at the sample level after passing through the package foils. In concomitance with the dose increase, the temperature of fruit surface may gradually rise on the treated surfaces as well as inside the treatment chamber. Just after the highest PL-treatment (16 J·cm^{-2}), the highest temperature recording at the sample shelf level was 42.4 ± 1.0 °C.

2.3. Microbiological quality evaluation

Approximately 10 g of fresh-cut apple wedges were homogenized for 1 min with 90 mL of saline peptone water (0.1% *w/v* peptone + 0.85% *w/v* NaCl) with a stomacher blender under sterile conditions (IUL Instruments, Barcelona, Spain). Serial dilutions of the homogenates were poured in plate count agar (PCA) at 30 ± 1 °C for 72 ± 3 h and 7 ± 1 °C for 7 days for mesophilic and psychrophilic aerobic bacteria counts, respectively (ISO 4833, 1991) and chloramphenicol glucose agar (CGA) at 25 ± 1 °C for 5 days for molds and yeasts counts (ISO 7954, 1988). Peptone and agar media were purchased from Biokar Diagnostics (Beauvais, France). Two packages were taken at each sampling time to perform the analysis and two replicate counts were carried out for each one. Results were expressed as colony forming units (CFU) per gram of fresh apple piece.

2.4. Colour determination

Cut apple surface colour values were directly measured with a colorimeter (ChromaMeter Model CR-400, Konica Minolta Sensing Inc.,

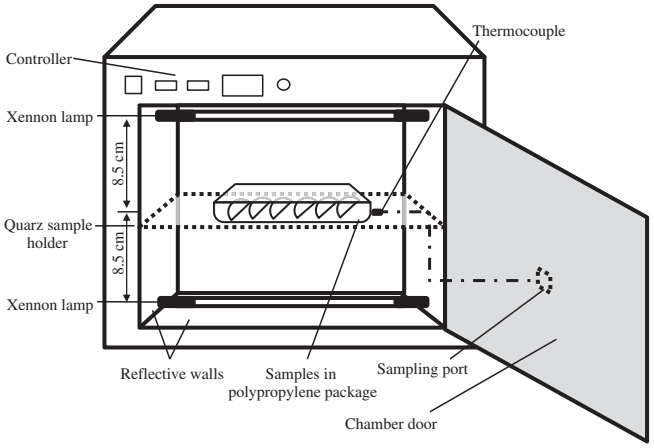

Fig. 1. Schematics of the PL-treatment chamber.

Osaka, Japan) using a D65 illuminant and an observation angle of 10°. The instrument was calibrated using a standard white reflector plate. Three readings of L* (lightness), a* (green-red chromaticity) and b* (blue-yellow chromaticity) coordinates were recorded from each apple piece. At least 5 samples from 2 replicate packages were evaluated for each treatment and sampling time.

2.5. Firmness measurements

Apple firmness was evaluated with a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, U.K.) by measuring the force required for a 4-mm-dia probe to penetrate apple slices of 20 mm height to a depth of 10 mm at a rate of 5 mm s^{-1} and automatic return. Samples were placed perpendicular to the probe, so that they could be penetrated in their geometric center. Bags were randomly withdrawn each 3 days and their content was directly analyzed. At least 10 repetitions obtained from 2 replicate packages were evaluated for each treatment at each sampling time. Results were expressed as the maximum penetration force in N.

2.6. Evaluation of antioxidant properties

2.6.1. Preparation of methanolic extracts

One gram of freeze-dried samples was extracted twice with 5 mL of 80% *v*/*v* methanol using an Ultra-Turrax homogenizer (IKA Model T 25 Digital, Germany) (2 min, 10,200 rpm, 0 °C). The mixture was centrifuged at 16,546 x *g* for 10 min at 5 °C (AVANTI J-26 XP centrifuge, Beckman Coulter Inc., Fullerton, CA, USA). The supernatant was recovered and made up to 10 mL with 80% *v*/*v* methanol. The resulting solution constituted the methanolic extract.

2.6.2. Determination of antioxidant capacity

The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect of the extracts on a solution of 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radical (Fluka Chemie, Switzerland), according to the procedure proposed by de Ancos, Sgroppo, Plaza, & Cano (2002). Aliquots of 0.03 mL of the methanolic extract were mixed with 3.9 mL of methanolic $DPPH^{\cdot}$ solution (0.025 g L^{-1}) and 0.07 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorbance was measured with a CECIL CE 1021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 515 nm against a blank of methanol without $DPPH^{\cdot}$ Results were expressed as percentage of inhibition of the radical $DPPH^{\cdot}$, that is the decrease in absorbance with respect to the control value ($DPPH^{\cdot}$ initial absorption value).

2.6.3. Determination of total phenolic content

Total phenolic content was determined according to the Folin-Ciocalteu procedure (Singleton, Orthofer, & Lamuela-Raventós, 1999) with some modifications. One hundred microliters of methanolic extract were added at 4.5 mL of water and 400 μL of Folin- Ciocalteau reagent (Sigma Chemical, St. Louis, MO, USA). After 3 min, 1 mL of saturated solution of Na_2CO_3 was added, and the reaction mixture was incubated for 1 h at ambient temperature. The absorbance was measured at 760 nm. The total phenolic content was calculated using gallic acid (Sigma Chemical, St. Louis, MO, USA) as standard in the 0–9 μg mL^{-1} concentrations range. Results were expressed as milligrams of gallic acid in 100 g of fresh weight.

2.6.4. Determination of total flavonoids

Total flavonoids were spectrofotometrically determined by using a modification of the technique described by Kim, Jeong, & Lee (2003). A test tube was added with 1900 μL of distilled water and 400 μL of the methanolic extract. Other compounds were added sequentially: initially (zero time) a volume of 80 μL of 5% (*w*/*v*) $NaNO_2$; after 5 min, 80 μL of 10% (*w*/*v*) $AlCl_3$ and finally, after additional 6 min, 540 μL of 1 mol L^{-1} NaOH. Solutions were stirred in a vortex and then absorbance at 510 nm was measured. Catechin (Sigma-Aldrich, St. Louis, MO, USA) was used as standard in the 0–27 μg mL^{-1} concentration range. Results were expressed as mg in 100 g of fresh weight.

2.6.5. Determination of vitamin C

Vitamin C content of fresh-cut apples was analyzed by HPLC. The extraction procedure and chromatographic conditions were based on a previous study carried out by Odriozola-Serrano, Hernandez-Jover, & Martín-Belloso (2007). Lyophilized samples of 0.2 g were extracted twice with 5 mL of a solution containing 45 g L^{-1} metaphosphoric acid and 7.2 g L^{-1} DL-1,4-dithiotreitol, using an Ultra-Turrax (IKA Model T 25 Digital, Germany) homogenizer (2 min, 10,200 rpm, 0 °C). The mixture was centrifuged at 4020 x *g* for 15 min (Hettich ZENTRIFUGEN EBA 21, Andreas Hettich GmbH & co. KG, Tuttlingen, Germany). The supernatant was recovered and made up to 10 mL. An aliquot of 20 μL was injected into the HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 mm) stainless steel column (250 mm × 4.6 mm). The mobile phase was a 0.01% *v*/*v* sulfuric acid solution adjusted to pH = 2.6. The flow rate was fixed at 1.0 mL/min at room temperature. Detection was performed with a 2996 Photodiode Array Detector (Waters, Milford, MA) set at 245 nm. Vitamin C content was calculated using ascorbic acid (Scharlau Chemie S.A., Barcelona, Spain) as standard in the 0–15 μg mL^{-1} concentration range. Results were expressed as mg of vitamin C per 100 g of fresh weight.

2.7. Data analysis

Statistical analysis was performed using the InfoStat/L v. 2012I software package (Di Rienzo et al., 2012). Three independent trials were performed according to a factorial design. The factors were the treatment (with or without), and the storage time. Means and standard deviation of the three trials were depicted. Data were analyzed by means of an analysis of variance, and the means were compared by the least significant difference (LSD) test at $P < 0.05$.

3. Results and discussion

3.1. Microbiological stability

As displayed in Fig. 2, PL treatments exerted a significant antimicrobial effect on the indigenous microbial load of fresh-cut apple wedges. The quality-stabilizing dip seemed to favor the growth of microorganism on the cut product surface in a first instance, probably due to the presence of available water required for spoilage microorganisms. The application of PL helped to control this initial increase. Just after the treatment, both the mesophilic and psychrophilic microbial counts on apple wedges exposed to a fluence of 4 J·cm^{-2} per side were reduced by 0.75 log units. Energy dosages of 8, 12 and 16 J·cm^{-2} led to reductions of up to 1.55 log units (Figs. 2a and b). Interestingly, treatments with a fluence greater than 8 J·cm^{-2} did not appear to have an additional antimicrobial effect on the naturally-occurring aerobes, as the initial counts were not further reduced. A slightly different pattern was observed for mold and yeast counts (Fig. 2c) which were more noticeably reduced under the most intense treatment conditions. In this regard, fluence values of 16 J·cm^{-2} caused 2.3 log reductions of the initial molds and yeasts loads. Gómez et al. (2012) found reductions in the same order of magnitude for mesophilic microorganisms and molds and yeasts just after apply higher doses on Granny Smith cut apple.

Energy dosage has been previously identified as a critical factor determining the antimicrobial effect of a PL treatment (Gómez et al., 2012; Oms-Oliu et al., 2010a). It is known that PL can led to microbial cell death through the generation of photochemical and photothermal damage. In the first case, inactivation is related to the induction of DNA strand breaks and formation of pyrimidine dimers by UV radiation. In addition, heating is a localized phenomenon which may end with the

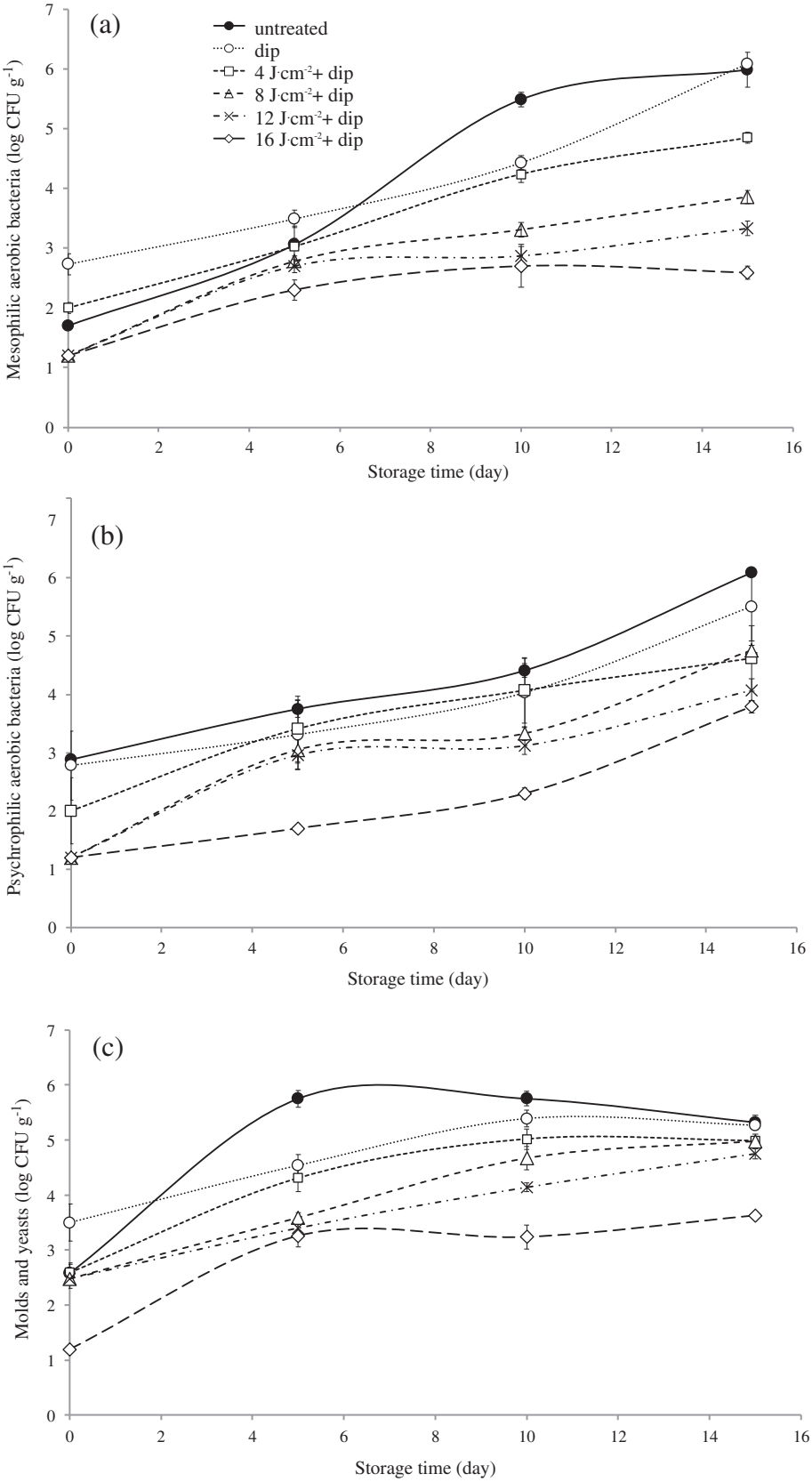

Fig. 2. Mesophilic (a) and psychrophilic (b) aerobic bacteria, and yeasts and molds (c) counts (expressed as log CFU g^{-1}) of fresh-cut apples exposed to light pulses (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% *w*/*v* N-acetylcysteine + 0.5% *w*/*v* $CaCl_2$ (LSD at $p < 0.05$;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

generation of thermal stress (Demirci & Krishnamurthy, 2011). In our study, signs of abusive temperatures during processing were not recorded at the fruit surface, which supports the hypothesis that most of the inactivation was attained by non-thermal mechanisms.

Although some PL treatments were reported to have a similar impact on the initial microbiota of fresh-cut apple wedges, differences among fruit samples treated with different fluence values became evident throughout storage at 5 °C. The growth inhibition of the naturally-occurring microorganisms was found to be dependent on the intensity of 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. Colour 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

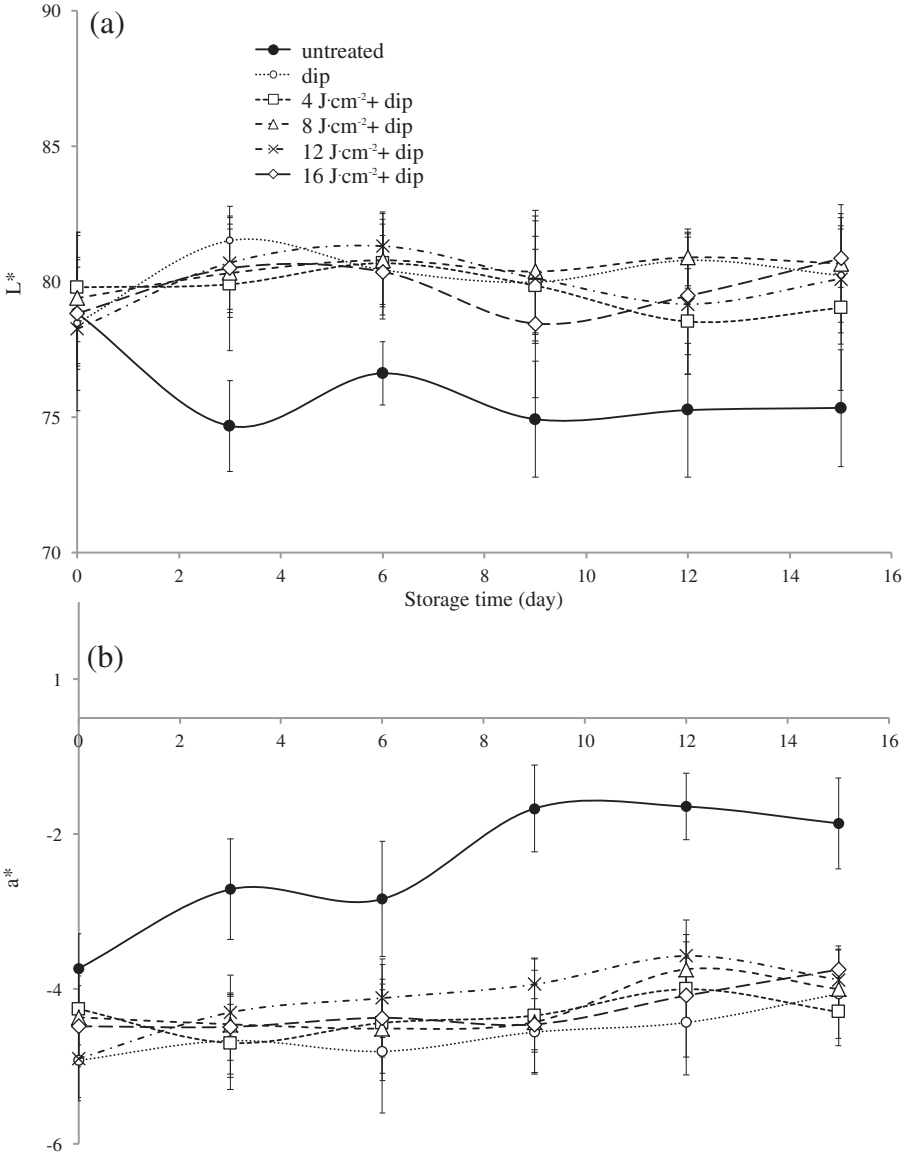

Fig. 3. L* (a) and a* (b) values of fresh-cut apples exposed to light pulses (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% *w/v* N-acetylcysteine + 0.5% *w/v* $CaCl_2$ (LSD at $p < 0.05$; $LSD_A = 2.29$; $LSD_B = 0.78$).

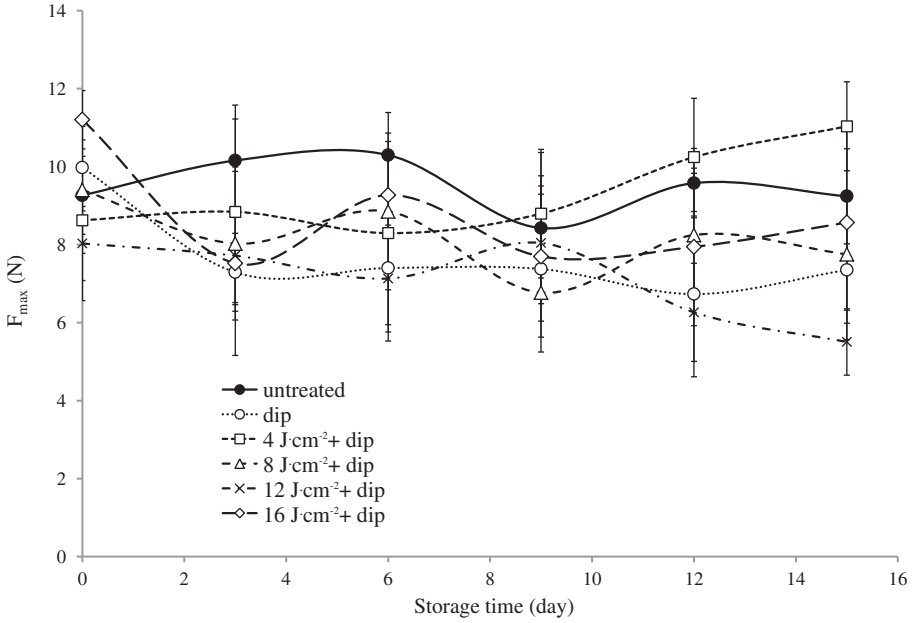

Fig. 4. Firmness values (expressed in N) of fresh-cut apples exposed to light pulses (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% *w/v* N-acetylcysteine + 0.5% *w/v* $CaCl_2$ ($LSD_{0.05} = 0.18$).

appreciated in apple tissues not immersed into the N-acetylcysteine dip immediately after cutting (Fig. 3b). In addition, this increase approached positive a* values (less green but redder) along the first week of storage. This trend, which was accompanied by a marked decrease in L* values during the same period of time, evidences the susceptibility to enzymatic browning of the cut apple tissue as well as the need for the antioxidant dip. Furthermore, b* values (data not shown) were not affected by the treatment and did not change over storage. The N-acetylcysteine dip prevented colour changes on cut apple surface as a consequence of PL exposure and storage time. As expected, and also confirmed by preliminary tests (data not shown), PL treatments applied to fresh-cut apple tissues may promote the development of browning, especially when high energy doses are delivered. Gómez et al. (2012) reported that apple disks subjected to PL fluencies higher than 2.4 J·cm^{-2} without any further antibrowning treatment turned darker (lower L* values) and less green (higher a* values) than untreated ones, which was hypothesized to be caused by photothermal damage. Similarly, Ignat et al. (2014) reported browning promotion in apple slices treated at 15.75 J·cm^{-2} and attributed the colour changes to the increase in enzyme-substrate interactions caused by cell damage. Our results indicate that these undesirable effects of PL treatments on the visual appearance of fresh-cut produce may be effectively prevented for energy fluencies of at least as much as 16 J·cm^{-2} per side. This is in line with the results reported by Oms-Oliu et al. (2010a) and Ramos-Villarroel et al. (2011) in fresh-cut avocadoes and mushrooms treated under similar conditions.

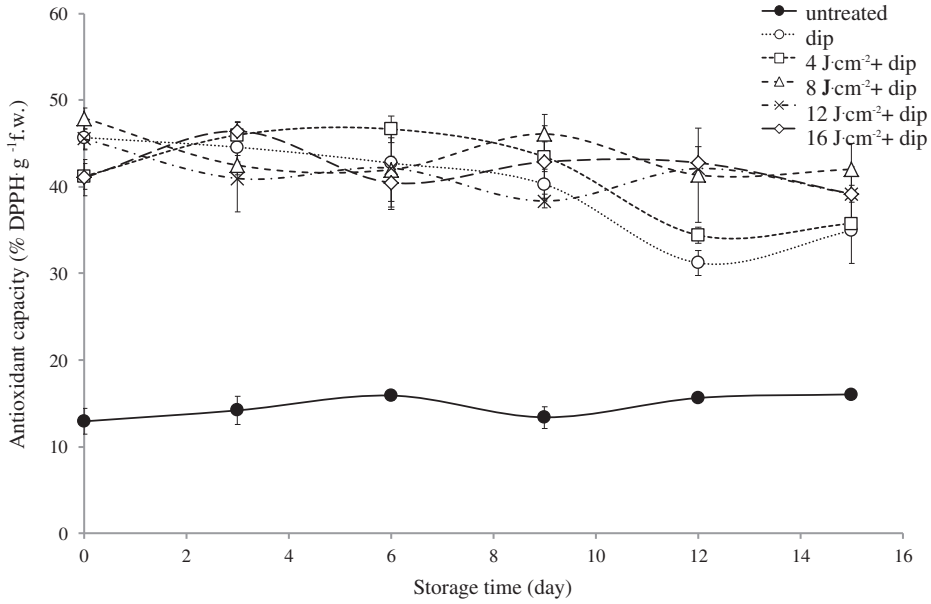

Fig. 5. Antioxidant capacity (expressed as % $DPPH^{\cdot}$ in g^{-1}) of fresh-cut apples exposed to light pulses (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% *w/v* N-acetylcysteine + 0.5% *w/v* $CaCl_2$ ($LSD_{0.05} = 4.93$).

3.3. Firmness

As shown in Fig. 4, the firmness of PL-treated apples did not majorly change as a consequence of processing. Differences among firmness values of apple wedges subjected to PL did not significant differ among treatments. Only apple pieces exposed to 16 J·cm^{-2} exhibited significantly higher firmness values of 11.2 N, but these values declined subsequently. The immersion into the quality-stabilizing dip, which contained 0.5% (*w*/*v*) $CaCl_2$, did not result into significantly firmer fruit. Indeed, untreated fruit pieces better kept their initial firmness values throughout storage. A clear pattern governing the effects of PL treatments on firmness cannot be noticed. However, the overall firmness values at prolonged storage significantly differed among apple pieces processed under different conditions. Hence, over 15 days of storage the firmness of fresh-cut apples flashed with 4 J·cm^{-2} almost doubled that of those flashed with 12 J·cm^{-2}. The slight declines in firmness values through storage are in agreement with the results obtained by Ramos-Villarroel et al. (2011), who proposed the use calcium lactate (1%) to help to maintain firmness of cut avocadoes. The slight changes in firmness could be a consequence of the modulation of pectinolytic enzyme activities as a consequence of the applied treatments. Pectic enzymes, namely pectin methylesterase and polygalacturonase, from several plant sources exhibit activity increases at specific temperatures or as a consequence of the exposure to stress conditions (Jurick et al., 2009; Denès, Baron, & Drilleau, 2000; Castaldo, Quagliuolo, Servillo, Balestrieri, & Giovane, 1989). Hence, the modulation of pectinolytic activities under certain conditions as well as the consequences of a stress-induced response produced in the fruit after the application of the most intense treatments could explain the poor correlation between dose and effect.

3.4. Antioxidant properties

As seen in Fig. 5, the DPPH· radical scavenging capacity of apple samples was dramatically increased by the immersion into an N-

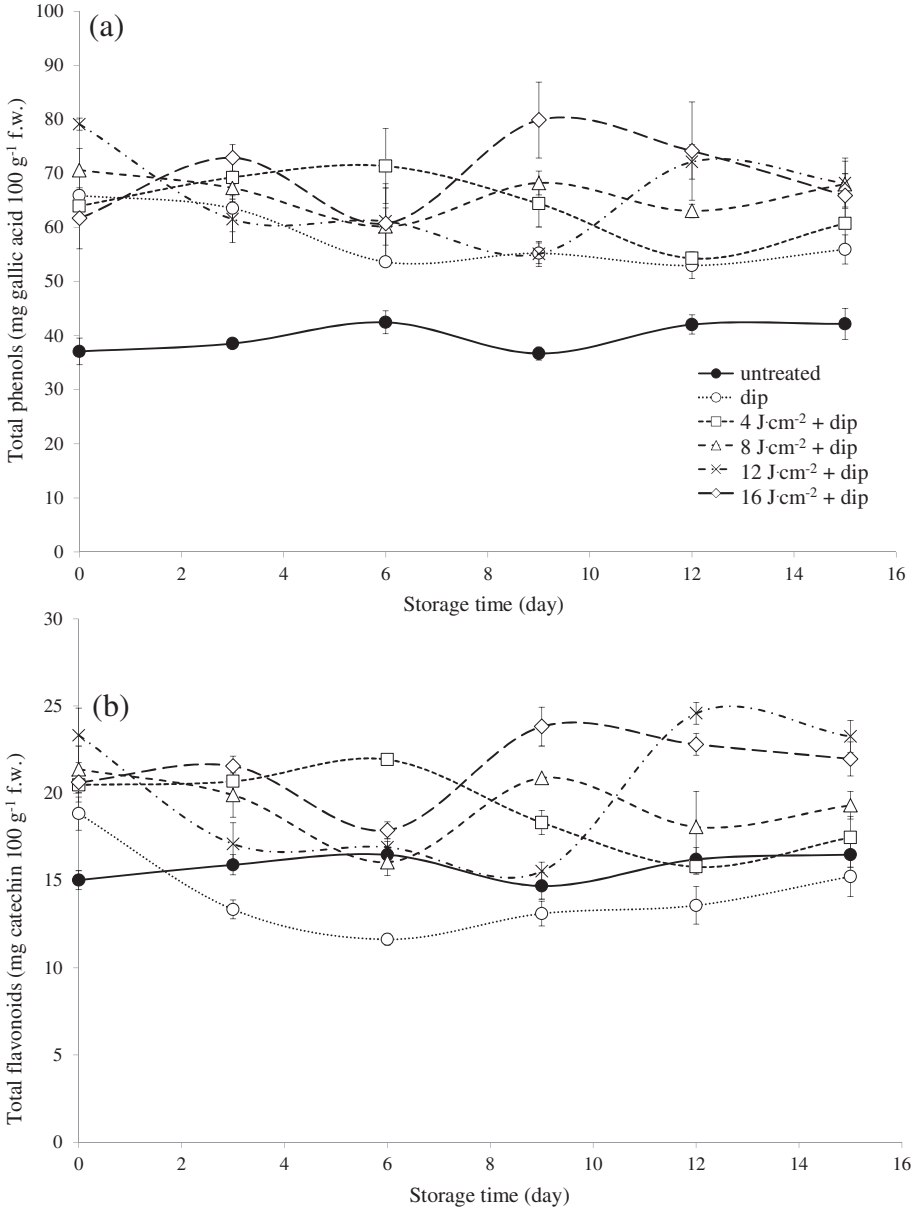

Fig. 6. Total phenols (a) and flavonoids (b) contents (expressed as mg gallic acid 100 g^{-1} and mg catechin 100 g^{-1}, respectively) of fresh-cut apples exposed to light pulses (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% *w*/*v* N-acetylcysteine + 0.5% *w*/*v* $CaCl_2$ (LSD at $p < 0.05$; $LSD_A = 6.17$; $LSD_B = 2.02$).

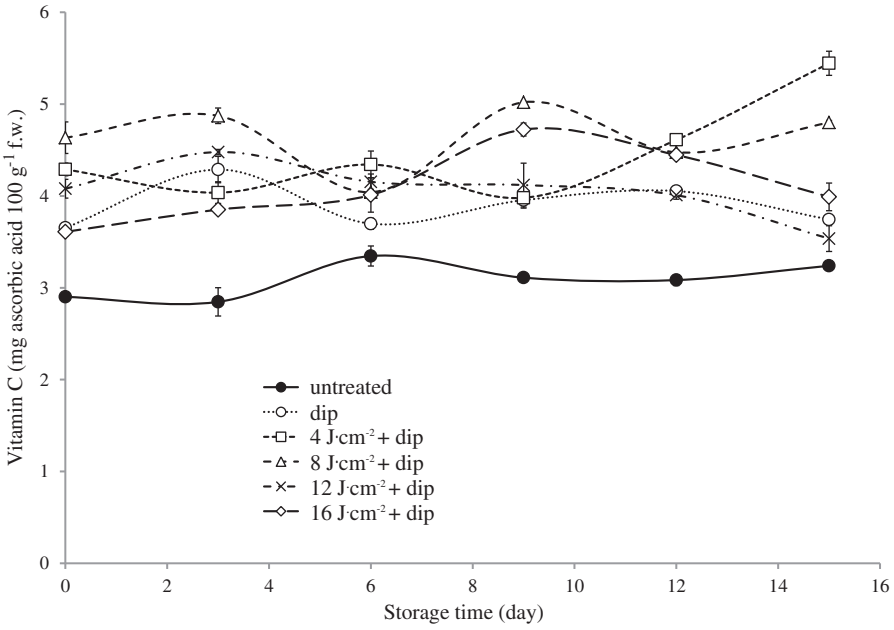

Fig. 7. Vitamin C content (expressed as mg ascorbic acid 100 g^{-1}) of fresh-cut apples exposed to light pulses (expressed as J cm^{-2}) and stored for 15 days at 5 °C. Dip: 1% *w/v* N-acetylcysteine + 0.5% *w/v* $CaCl_2$ ($LSD_{0.05} = 0.18$).

acetylcysteine solution. Nevertheless, pulsed light treatments did not cause a decrease in the antioxidant potential of fresh-cut apples. Considering the effects of energy fluence on the antioxidant activity of dipped apple pieces, major differences among treatments were not remarkable just after processing and over the first 9 days of storage. However, fresh-cut apples exposed to the highest energy doses (8, 12 and 16 J·cm^{-2}) better retained their initial antioxidant capacity values until the end of the evaluated storage period (Fig. 5).

The effect of PL treatments on the phenolic and vitamin C contents of apple pieces can be observed in Figs. 6 and 7. As can be seen, antioxidant capacity values correlated well with the content in phenolic compounds throughout storage. Vitamin C contents were as well correlated with the total antioxidant potential, although to a lesser extent. This is due to the fact that the overall antioxidant potential in apple is mainly a consequence of the presence of phenolic compounds rather than to vitamin C, as reported by Lee, Kim, Kim, Lee, & Lee (2003) for several apple cultivars.

An analysis of the phenolic content as affected by the assayed treatments reveals that the use of the quality-stabilizing dip stood as the most significant factor ($p < 0.0001$) influencing the content in total phenolics (Fig. 6a). This is probably caused by an interference of N-acetylcysteine with the Folin-Ciocalteau reagent. Some sulfur and nitrogenous compounds, together with ascorbic acid, sugars, Fe(II), and a long list of organic compounds have been reported to interfere the Folin-Ciocalteu assay for the determination of phenolics (Prior, Wu, & Schaich, 2005). Therefore, although the method proposed by Singleton et al. (1999) is widely used as an indicator of the phenolic content, values should be interpreted as a measurement of the total reducing capacity of the apple pieces. This also explains the good correlation of the total phenolic content with the antioxidant capacity values estimated by the $DPPH^{\cdot}$ assay. However, the N-acetylcysteine did not appear to interfere in the determination of total flavonoids, as the contents in samples not immersed and immersed into the antioxidant dip were in the same order of magnitude (Fig. 6b). In addition, flashed fresh-cut apples exhibited higher total phenolic compounds throughout storage than apple pieces only immersed into the quality-stabilizing solution. Consistently, PL-treated cut apples better kept the highest flavonoids contents during storage. This is in agreement with the results reported by Rodov et al. (2012), who reported higher values of phenolics content in fig fruits irradiated by PL than in the untreated fruits. This may be explained by the presence of stress-induced phenylpropanoids promoted by the exposure to PL treatments. Among flavonoids, anthocyanins and flavones have been reported to increase in plants in response to high visible light levels, as it is thought that these compounds help attenuate the amount of light reaching the photosynthetic cells. As well, UV irradiation has been shown to induce the synthesis of some types of flavonoids in different plant species, as a mean of protection against UV-B damage (Dixon & Paiva, 1995). Despite our results are in line with these findings, a correlation between the amount of incident energy and the production of flavonoids could not be found.

Whether this stress-induced response is caused or not by thermal means is another question that remains unanswered. According to Murugesan, Orsat, & Lefsrud (2012), the temperature increase on the surface of elderberry fruits during PL-treatments (7 to 33 J·cm^{-2}) may lead to the synthesis of phenolic compounds by the fruits. However, Oms-Oliu et al. (2010a) reported that it is thermal damage itself which limits this stress-induced response, as high fluence treatements (28 J cm^{-2}) significantly reduced the antioxidant capacity and the total phenolic content in fresh-cut mushrooms. Although temperature on the surface of apple wedges was not recorded during the PL-treatment, it was monitored over the whole treatment with a thermocouple. Hence, after exposure to the highest energy dose, a temperature of 42.4 ± 1.0 °C may suggest that abusive heating did not occur on the surface of apple slices.

As ascorbic acid is largely thermo-labile, a focus on vitamin C levels may cast light on the underlying phenomena. As can be observed in Fig. 7, the vitamin C content of dipped fruit pieces was between 10 and 70% above that observed in undipped samples, probably as a consequence of the reducing effect of N-acetylcysteine which helped protecting ascorbic acid from oxidation. The vitamin C contents in PL-treated samples were maintained during at least two weeks and were in line with those found in untreated fresh-cut apples or even slightly above. These results are in contrast with those reported by Oms-Oliu et al. (2010a), who found a continuous vitamin C decrease during the storage of PL-treated fresh-cut mushrooms, with a more pronounced

effect for higher energy doses. In the present case, the high ascorbic acid contents seem to point out that heating was not behind the observed effects, so that differences in the antioxidant potential, as well as in microbial counts, were more likely to be caused by photochemical rather than to thermal processes.

4. Conclusions

Pulsed light treatments stand as a feasible alternative for extending the microbiological shelf life of fresh-cut apples at 5 °C, while maintaining their quality and antioxidant attributes. The growth inhibition of the native microbiota was generally dependent on the energy fluence delivered at the time of processing. A negative impact on the antioxidant potential of flashed fresh-cut apples was not observed. Instead, experimental results suggest the promotion of the phenylpropanoid metabolism by nonthermal means, as vitamin C levels were preserved throughout refrigerated storage.

Taken together, our results suggest that PL-treatments at 8 and 16 $J{\cdot}cm^{-2}$ combined with immersion into the quality-stabilizing solution help to maintain a better quality and 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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References

Aguayo, E., Requejo-Jackman, C., Stanley, R., & Woolf, A. (2010). Effects of calcium ascorbate treatments and storage atmosphere on antioxidant activity and quality of fresh-cut apple slices. *Postharvest Biology and Technology*, *57*(1), 52–60.

Aguiló-Aguayo, I., Charles, F., Renard, C. M., Page, D., & Carlin, F. (2013). Pulsed light effects on surface decontamination, physical qualities and nutritional composition of tomato fruit. *Postharvest Biology and Technology*, *86*, 29–36.

Alandes, L., Hernando, I., Quiles, A., Pérez-Munuera, I., & Lluch, M. A. (2006). Cell wall stability of fresh-cut Fuji apples treated with calcium lactate. *Journal of Food Science*, *71*(9), S615–S620.

de Ancos, B., Sgroppo, S., Plaza, L., & Cano, M. P. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science of Food and Agriculture*, *82*(8), 790–796.

Barbagallo, R. N., Chisari, M., & Caputa, G. (2012). Effects of calcium citrate and ascorbate as inhibitors of browning and softening in minimally processed 'birgah'eggplants. *Postharvest Biology and Technology*, *73*, 107–114.

Castaldo, D., Quagliuolo, L., Servillo, L., Balestrieri, C., & Giovane, A. (1989). Isolation and characterization of pectin methylesterase from apple fruit. *Journal of Food Science*, *54*(3), 653–655.

Charles, F., Vidal, V., Olive, F., Filgueiras, H., & Sallanon, H. (2013). Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. *Innovative Food Science & Emerging Technologies*, *18*, 190–195.

Demirci, A., & Krishnamurthy, K. (2011). Pulsed ultraviolet light. In H. Q. Zhang, G. V. Barbosa-Cánovas, V. M. Balasubramaniam, C. P. Dunne, D. F. Farkas, & J. T. C. Yuan (Eds.), *Nonthermal processing technologies for food* (pp. 249–261). New Delhi, India: Blackwell Publishing Ltd.

Denès, J. M., Baron, A., & Drilleau, J. F. (2000). Purification, properties and heat inactivation of pectin methylesterase from apple (cv golden delicious). *Journal of the Science of Food and Agriculture*, *80*(10), 1503–1509.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2012). InfoStat versión 2012. *Grupo InfoStat, FCA*. Argentina: Universidad Nacional de Córdoba http://www.infostat.com.ar.

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell*, *7*(7), 1085–1097.

Gómez, P. L., Salvatori, D. M., García-Loredo, A., & Alzamora, S. M. (2012). Pulsed light treatment of cut apple: dose effect on color, structure, and microbiological stability. *Food and Bioprocess Technology*, *5*(6), 2311–2322.

Gómez-López, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: a review. *Trends in Food Science & Technology*, *18*(9), 464–473.

Ignat, A., Manzocco, L., Maifreni, M., Bartolomeoli, I., & Nicoli, M. C. (2014). Surface decontamination of fresh-cut apple by pulsed light: effects on structure, colour and sensory properties. *Postharvest Biology and Technology*, *91*, 122–127.

Jurick, W. M., Vico, I., McEvoy, J. L., Whitaker, B. D., Janisiewicz, W., & Conway, W. S. (2009). Isolation, purification, and characterization of a polygalacturonase produced in Penicillium solitum-decayed 'Golden Delicious' apple fruit. *Phytopathology*, *99*(6), 636–641.

Kim, D. O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, *81*, 321–326.

Lasagabaster, A., & Martínez de Marañón, I. (2014). Survival and growth of *listeria innocua* treated by pulsed light technology. Impact of post-treatment temperature and illumination conditions. *Food Microbiology*, *41*, 76–81.

Lee, K. W., Kim, Y. J., Kim, D., Lee, H. J., & Lee, C. Y. (2003). Major phenolics in apple and their contribution to the total.Antioxidant capacity. *Journal of Agricultural and Food Chemistry*, *51*, 6516–6520.

Martín-Diana, A. B., Rico, D., Frías, J. M., Barat, J. M., Henehan, G. T. M., & Barry-Ryan, C. (2007). Calcium for extending the shelf life of fresh whole and minimally processed fruits and vegetables: a review. *Trends in Food Science & Technology*, *18*, 210–218.

Murugesan, R., Orsat, V., & Lefsrud, M. (2012). Effect of pulsed ultraviolet light on the total phenol content of elderberry (*sambucus nigra*) fruit. *Food and Nutrition Sciences*, *3*, 774–783.

Odriozola-Serrano, I., Hernandez-Jover, T., & Martín-Belloso, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, *105*, 1151–1158.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O., & Soliva-Fortuny, R. (2010a). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (*Agaricus bisporus*). *Postharvest Biology and Technology*, *56*(3), 216–222.

Oms-Oliu, G., Martín-Belloso, O., & Soliva-Fortuny, R. (2010b). Pulsed light treatments for food preservation. A review. *Food and Bioprocess Technology*, *3*, 13–23.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*, 4290–4302.

Quiles, A., Hernando, I., Pérez-Munuera, I., & Lluch, M.Á. (2007). Effect of calcium propionate on the microstructure and pectin methy-lesterase activity in the parenchyma of fresh-cut Fuji apples. *Journal of the Science of Food and Agriculture*, *87*(3), 511–519.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. (2012). Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. *Postharvest Biology and Technology*, *69*, 32–39.

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Using antibrowning agents to enhance quality and safety of fresh-cut avocado treated with intense light pulses. *Journal of Food Science*, *76*(9), 528–534.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. *Comprehensive Reviews in Food Science and Food Safety*, *8*(3), 157–180.

Rodov, V., Vinokur, Y., & Horev, B. (2012). Brief postharvest exposure to pulsed light stimulates coloration and anthocyanin accumulation in fig fruit (*Ficus carica* L.). research note. *Postharvest Biology and Technology*, *68*, 43–46.

Rojas-Graü, M. A., Sobrino-López, A., Tapia, M. S., & Martín-Belloso, O. (2006). Browning inhibition in fresh-cut 'Fuji' apple slices by natural antibrowning agents. *Journal of Food Science*, *71*(1), S59–S65.

Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Effect of natural antibrowning agents on color and related enzymes in fresh-cut Fuji apples as an alternative to the use of ascorbic acid. *Journal of Food Science*, *73*(6), 267–272.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteau reagent. *Methods in Enzymology*, *299*, 152–178.

Soliva-Fortuny, R. C., Grigelmo-Miguel, N., Odriozola-Serrano, I., Gorinstein, S., & Martín-Belloso, O. (2001). Browning evaluation of ready-to-eat apples as affected by modified atmosphere packaging. *Journal of Agricultural and Food Chemistry*, *49*(8), 3685–3690.

Soliva-Fortuny, R. C., Oms-Oliu, G., & Martín-Belloso, O. (2002). Effects of ripeness stages on the storage atmosphere, color, and textural properties of minimally processed apple slices. *Journal of Food Science*, *67*(5), 1958–1963.

Soliva-Fortuny, R. C., Ricart-Coll, M., & Martín-Belloso, O. (2005). Sensory quality and internal atmosphere of fresh-cut golden delicious apples. *International Journal of Food Science and Technology*, *40*, 369–375.

Valencia-Chamorro, S., Palou, L., del Río, M. A., & Pérez-Gago, M. B. (2011). Antimicrobial edible films and coatings for fresh and minimally processed fruits and vegetables: a review. *Critical Reviews in Food Science and Nutrition*, *51*(9), 872 Retrieved from http://search.proquest.com/docview/890074940?accountid=15296.

Vargas, M. M., Pastor, C. C., Chiralt, A. A., Mc Clements, D. J., & González-Martínez, C. C. (2008). Recent advances in edible coatings for fresh and minimally processed fruits. *Critical Reviews in Food Science and Nutrition*, *48*(6), 496–511 Retrieved from http://search.proquest.com/docview/69244025?accountid=15296.

Weber, S. (2005). Light-driven enzymatic catalysis of DNA repair: a review of recent biophysical studies on photolyase. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1707*(1), 1–23.

Zhan, L. J., Li, Y., Hu, J. Q., Pang, L. Y., & Fan, H. P. (2012). Browning inhibition and quality preservation of fresh-cut romaine lettuce exposed to high intensity light. *Innovative Food Science & Emerging Technologies*, *14*, 70–76.