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ORIGINAL PAPER

Pulsed Light Treatment of Cut Apple: Dose Effect on Color, Structure, and Microbiological Stability

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Abstract This study investigated the effect of pulsed light (PL) dose on color, microstructure, and microbiological stability of cut apples during 7-day refrigerated storage. Apples were irradiated at two different distances from the lamp (5 or 10 cm) during 2 to 100 s (2.4 to 221.1 J/cm^2). Cut-apple surface exposed to high PL fluencies turned darker (lower L^* values) and less green (higher a^* value) than the control, and this effect was more pronounced as PL dose and/or storage time increased. On the contrary, the application of few flashes (2.4 J/cm^2) allowed maintaining the original color of apples slices along storage. Light microscopy images of treated samples showed degraded walls and broken plasmalemma and tonoplast, which may explain, at least partially, the increase in browning of irradiated apples at high doses. Inactivation patterns of inoculated microorganisms depended on PL dose and the type of microorganism. After 100 s PL treatment at 5 cm, no counts were observed for *Saccharomyces cerevisiae* KE162, while for *Escherichia coli* ATCC 11229 and *Listeria innocua* ATCC 33090, reduction levels were 2.25 and 1.7 logs, respectively. Native microflora population was in general higher in control samples than in 10 and 60 s PL irradiated apples along the whole storage. Although the application of high PL fluencies allowed obtaining greater microbial reductions, they also promoted browning of apple. Application of PL at a dose of 11.9 J/cm^2 could extend the shelf life of cut apple with minimal modification in color.

Keywords Pulsed light · Dose · Cut apple · Structure · Color · Microbiological stability

Introduction

In response to consumer expectations, researchers in the food industry, the academia and government institutions have explored in the last two decades milder fruit preservation techniques with better retention of product flavor, texture, color, and nutrient content than comparable conventional treatments. Consumer trends towards fresh food image on one side and convenience on the other side often conflict. In most cases, fresh quality is negatively affected by the processing procedure. The most crucial challenge is to retain the natural functional properties and the sensory and nutritional quality of fruits with the appropriate shelf life and safety (Alzamora and Salvatori 2006).

Pulsed light (PL) is an emerging preservation technique that is being studied as a feasible alternative to conventional processes. It involves the use of intense and short-duration (1 μs–0.1 s) pulses of broad spectrum light of wavelength

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ranging from UV to near-infrared (200–1,100 nm). Power is magnified by storing electricity in a capacitor over relatively long times (fractions of a second) and releasing it in a short time (millionths of thousandths of a second) (Dunn et al. 1995; Gómez-López et al. 2007). Its use has been approved by the FDA (1996) for the decontamination of food and food surfaces. The significant microbial reduction in very short treatment times, the limited energy cost, and its great flexibility are some of the major benefits claimed for this technique (Oms-Oliu and Martín-Belloso 2010). Moreover, PL systems do not significantly contribute negatively to the environmental impact of the processes where it is included because it has the potential to eliminate microorganisms without the need for chemicals. Furthermore, it does not produce volatile organic compounds and generates only reduced amounts of solid wastes (Pereira and Vicente 2010).

The effect of PL on different groups of microorganisms reported in the literature is contradictory. Some authors did not observe differences in the response of bacterial, yeast, and mold species (Gómez-López et al. 2005a), but others found a decreasing order of sensitivity: Gram-negative bacteria, Gram-positive bacteria, and fungal spores (Anderson et al. 2000). Differences could be attributed to the different equipment and experimental set up used by the different authors (Rajkovic et al. 2010). Furthermore, the mechanisms responsible for microbial inactivation by PL treatments are still in debate. Major contribution to inactivation appears to be provided by the rich UV content from 220 to 290 nm in the UV spectrum which is damaging to most microorganisms, because it alters the microbial DNA through the formation of pyrimidine dimers, pyrimidine adducts, and DNA-protein crosslinks, preventing cells from replicating. In addition to photochemical changes induced by UV, the high peak energy and the photothermal effects caused by visible and near-infrared portions of PL spectrum seem to be involved (FDA 2000; Takeshita et al. 2003; Gómez-López et al. 2007; Woodling and Moraru 2007). Krishnamurthy et al. (2010) also suggested photophysical effects such as cell wall damage, cytoplasmic membrane shrinkage, and cellular content leakage caused by PL when temperature increase during treatment was negligible.

PL technology is of limited efficacy for the in-depth treatment of opaque substrates due to absorption and scattering of light, and therefore is only suitable to control surface microflora (Woodling and Moraru 2005). The ability of PL to inactivate microorganisms on food surfaces has been well documented. Reductions of *Escherichia coli* O157:H7 on alfalfa seeds (Sharma and Demirci 2003), *Aspergillus niger* spores on corn meal (Jun et al. 2003), *Listeria monocytogenes* and *E. coli* O157:H7 on raw salmon fillets (Ozer and Demirci 2006), *Salmonella enterica* and *E. coli* O157:H7 on raspberries and strawberries (Bialka et al. 2008), and *L. monocytogenes* on infant foods (Choi et al. 2010) have been reported, indicating that this technology could be a powerful nonchemical (residue-free) option for decontaminating foods.

Food components absorb light, and food composition appears to affect the efficacy of the decontamination by PL. Proteinaceous or fatty foods have been reported to be inappropriate to be treated by this technique because these components reduce the killing efficiency of PL. For this reason, vegetables and fruits which do not contain high concentrations of protein and fat could be suitable for it (Gómez-López et al. 2005a). In spite of this, the literature on the application of PL technology in fruit and vegetables is still scarce, especially in minimally processed commodities.

The present work was aimed to investigate the effect of PL at different doses on surface color, microstructure, and microorganism behavior (native and inoculated flora) in cut apple slices during 7 days of refrigerated storage.

Materials and Methods

Sample Preparation

Raw apples (*Malus pumila*, Granny Smith var.; $a_w \cong 0.98$; 10.4–12.2°Brix and pH 3.3–3.4) were purchased at a local market and maintained at 4–5 °C until use. Before processing, whole fruit was washed in water, dipped in sodium hypochlorite solution (100-ppm free chlorine, 3 min), and rinsed in water. All cutting boards, elements, and holding vessels were sanitized in the same way before use.

Fruits were hand-peeled and cut into discs (3 cm in diameter and 0.6 cm in thickness) parallel to the longitudinal axis. Apple discs were immediately dipped in distilled water (4–5 °C) for 1 min to eliminate cellular fluids and dried with tissue paper.

Pulsed Light Equipment and Dosimetry

PL treatments were performed with an RS-3000B Steripulse-XL system (Xenon Corporation, Woburn, MA, USA), which produced polychromatic radiation in the wavelength range of 200–1,100 nm. The system consisted of an RC-747 power/control module, a treatment chamber that houses a Xenon flash lamp (non-toxic, mercury-free), and an air cooling system attached to the lamp housing to avoid lamp overheating during operation. The system generated high-intensity PL at a pulse rate of three pulses per second and a pulse width of 360 μs. According to the specifications supplied by the manufacturer, each pulse

delivered 1.27 J/cm^2 for an input of 3,800 V at 1.9 cm from the quartz window surface of the lamp. The different PL doses were obtained in this work by altering the number of applied pulses and/or the distance to the quartz window of the Xenon lamp.

Fluence measurements were taken by a pyroelectric head model ED500 (Gentec Electro-Optics, Québec, Canada) connected to an oscilloscope model TDS 2014 (Tektronix, Beaverton, USA), with an aperture cover of 20.3 cm^2. Measurements were performed in triplicate.

PL Treatment

Apple discs were exposed to a wide range of fluencies in order to select a dose which was microbiologically effective, but which did not modify quality to a great extent. For treatments, samples were put on an adjustable stainless steel shelf in the PL unit and exposed to irradiation for 2, 10, 20, 60, and 100 s at 5 or 10 cm distances from the quartz window. The corresponding fluencies applied were 4.4, 22.1, 44.2, 132.7, and 221.1 J/cm^2 when samples were located at 5 cm, and 2.4, 11.9, 23.9, 71.6, and 119.4 J/cm^2 in those exposed at 10 cm. Irradiated apples were compared with untreated fruits (controls). Control and irradiated samples were packed in closed plastic boxes permeable to air and stored in the darkness at 5 °C (±1 °C) for a week.

Temperature Measurement

The temperature evolution of apple discs during irradiation was monitored using a T-type thermocouple placed just 1–2 mm under the surface of the fruit and connected to a data logger Digi-Sense model 69202–30 (Barnant Company Division, Barrington, USA). Measurements were made in triplicate in apple samples located at different vertical distances from the lamp (5, 10, and 15 cm) and at different positions on the shelf.

Color Measurement

Color of irradiated surface of cut apples was measured with a handheld tristimulus reflectance spectrocolorimeter (Minolta Co. Model CM-508-d, Japan) by using a 1.4-cm measuring aperture and a white background. Values were obtained for C illuminant and 2° observer. Before the test, the instrument was calibrated with a standard white provided by the manufacturer.

The CIE color coordinates (X, Y, Z) and the L^*, a^*, b^* components of the CIELAB space were recorded, where L^* indicates lightness or luminance, a^* indicates chromaticity on a green (−) to red (+) axis, and b^* chromaticity on a blue (−) to yellow (+) axis. These numerical values were converted into "browning index" (BI), using the following equations:

$$\mathrm{BI} = [100\,(\times - 0.31)] / 0.172 \tag{1}$$

where:

$$x = X / (X + Y + Z) \tag{2}$$

Color was evaluated at 0, 3, and 7 days of storage in control and in irradiated samples. Ten independent samples were used for each condition, and five readings were taken at different positions on the irradiated surface. To minimize biological variability between fruits, values were expressed as differences with respect to the corresponding average value for the fresh fruit just before undergoing irradiation.

Microbiological Studies

Listeria innocua ATCC 33090, *Escherichia coli* ATCC 11229 and *Saccharomyces cerevisiae* KE 162 single inocula were tested. Bacteria strains were subcultured, purified weekly in Trypticase Soy Broth supplemented with 0.1% *w/w* Yeast Extract (TSBYE, Britania S.A., Argentina) and Trypticase Soy plus Yeast Extract Agar (TSAYE, Britania S.A., Argentina) at 37 °C and stored at 4 °C. The initial inoculum was prepared by transferring a loopful of a stock culture maintained on agar slants to 20 mL of TSBYE contained in 50-ml Erlenmeyer flasks. Microorganisms were incubated at 37 °C (±1 °C) until stationary phase was reached (≈24 h). A similar procedure was repeated for yeast culture. The initial inoculum was prepared by transferring a loopful of a stock culture maintained on Sabouraud Dextrose Agar (SDA, Biokard Diagnostic, France) slants to flasks with 20 mL of Sabouraud Dextrose Broth (SDB, Biokard Diagnostic, France). Organism was grown at 27 °C (±1 °C) until it reached the stationary phase (≈36 h).

Before inoculation, apple discs were prepared as described in "Sample Preparation" section inside a Class II Security Cabinet (Nuaire, Plymouth, MA, USA) to prevent post-contamination. The inocula (0.1 mL) were widespread with an alcohol-flamed glass spreader onto the surface of apple discs to mimic mid-/post-processing contamination at initial levels of approximately 1×10^6–3×10^7 CFU/cm^2. The inoculated apple discs were immediately irradiated at different vertical distances from the lamp (5 and 10 cm) and at different exposure times (2–100 s). Inoculated and no irradiated apple discs were used as controls.

For enumeration, the inoculated apple discs were put into stomacher bags (Whirl-Pak, Nasco, USA) containing 20 mL of sterile peptone water and were pummeled in a laboratory blender (AES Laboratories, France) at high speed for 3 min. Tenfold dilutions of homogenated samples

were made in 0.1% *w/v* peptone water, and 0.1-ml sample suspension was surface plated using Tryptone Soy Agar (TSA, Biokard Diagnostic, France) (bacteria) or SDA (yeast). Two plates were used for each dilution. Experiments were made by triplicate. Plates were incubated for 48 h at 37 °C (±1 °C), in the case of bacteria, or 27 °C (±1 °C) for the yeast. Survival curves were generated from experimental data by plotting log N/N_0 (where N is the number of colony-forming unit per square centimeter at a given exposure time and N_0 the initial number of colony-forming unit per square centimeter) versus time of irradiation.

Survival of inoculated microorganisms along storage was evaluated in controls and in samples irradiated at 10 cm from the lamp during 10 and 60 s. Samples were stored in stomacher bags at 4–5 °C in darkness and analyzed at 0, 3, and 7 days.

The effect of PL irradiation on native flora was also evaluated. Apple samples were prepared as described in "Sample Preparation" section. The apple discs were exposed on both sides to PL during 10 or 60 s at 10 cm from the lamp, stored in plastic boxes at 4–5 °C in the darkness and analyzed at 0, 3, and 7 days of storage. Three replicates were examined for each condition and experiments were made in duplicate. The enumeration procedure was similar to that described above except that the culture media used for aerobic microorganisms count was Plate Count Agar (PCA, Britania S.A, Argentina) and for mold and yeast count was Potato Dextrose Agar (PDA, Britania S.A., Argentina). Plates were incubated for 72 h at 37±1 °C (PCA) or 27±1 °C (PDA). Results were expressed as N (where N is the number of colony-forming unit per gram).

Microscopic Observations

For light microscopy (LM) of fresh and PL irradiated apples (60 s exposure, 10 cm distance from the lamp), ≅3 mm^3 cubes (including the irradiated cut surface in the case of treated samples) were fixed in glutaraldehyde solution (3 g/100 g) and then in 0.1 M potassium phosphate buffer (pH=7.4) overnight at room temperature. Cubes were then rinsed three times with distilled water, post-fixed in OsO_4 solution (1.5 g/100 g) at room temperature and dehydrated in a graded acetone series prior to be embedded in low-viscosity Spurr resin. Sections (1–2 μm thick) of the Spurr-embedded tissue corresponding to the irradiated surfaces were cut on a Sorvall MT2-B Ultracut microtome and stained with toluidine blue (1 g/100 g) and basic fuchsin (1 g/100 g) solutions. Samples were then examined in a Zeiss Axioskop 2 microscope (Carl Zeiss AG, Jena, Germany). All reagents were from Merck Química Argentina S.A. (Argentina).

Statistical Analysis

All statistical analyses were carried out using Infostat v. 2009 sofware (Universidad Nacional de Córdoba, Argentina). Results were expressed as mean ± standard deviation of the mean (mean ± SD). Two-way analysis of variance with repeated measures was done to establish the presence or absence of significant differences in color measurements according to the factors "treatment" and "time". Significance level was set at $p<0.05$. In case of significant interactions between factors, single effects were examined (i.e., effects of one factor holding the other fixed), and multiple comparisons were performed using the Tukey's test (Zar 1999).

Results and Discusion

Fluence and Temperature Measurements

As shown in Table 1, fluence decreased with the distance to the lamp. Furthermore, on the shelf, fluence was higher beneath the central point of the lamp and was reduced about a half on the extremes. Concomitant with fluence increase, the temperature of apple surface gradually increased with the number of flashes and at closer distances to the lamp. After 100 s of irradiation, the temperature of apple slices centered individually on the adjustable shelf, located at 5, 10, and 15 cm, was about 86, 60, and 44 °C, respectively (Fig. 1). The temperature increase also varied according to the relative position on the shelf. This variation was more pronounced when the shelf was located closer to the lamp (Fig. 1).

The heat build-up in PL treatments is mainly caused by the infrared portion of the spectrum. Sample heating could limit the processing time and the place where sample is located relative to the lamp. Ozer and Demirci (2006) found a temperature increase of 100, 86, and 76 °C on the skin of

Table 1 Fluence of PL per pulse (J/cm^2) at different distances from the lamp and at different positions on the shelf

Results were expressed as mean ± standard deviation

Vertical distance beneath the lamp (cm)	Central point	Extreme 1	Extreme 2
5	0.737±0.003	0.420±0.003	0.419±0.001
10	0.398±0.001	0.192±0.008	0.226±0.001
15	0.256±0.001	0.144±0.002	0.166±0.001

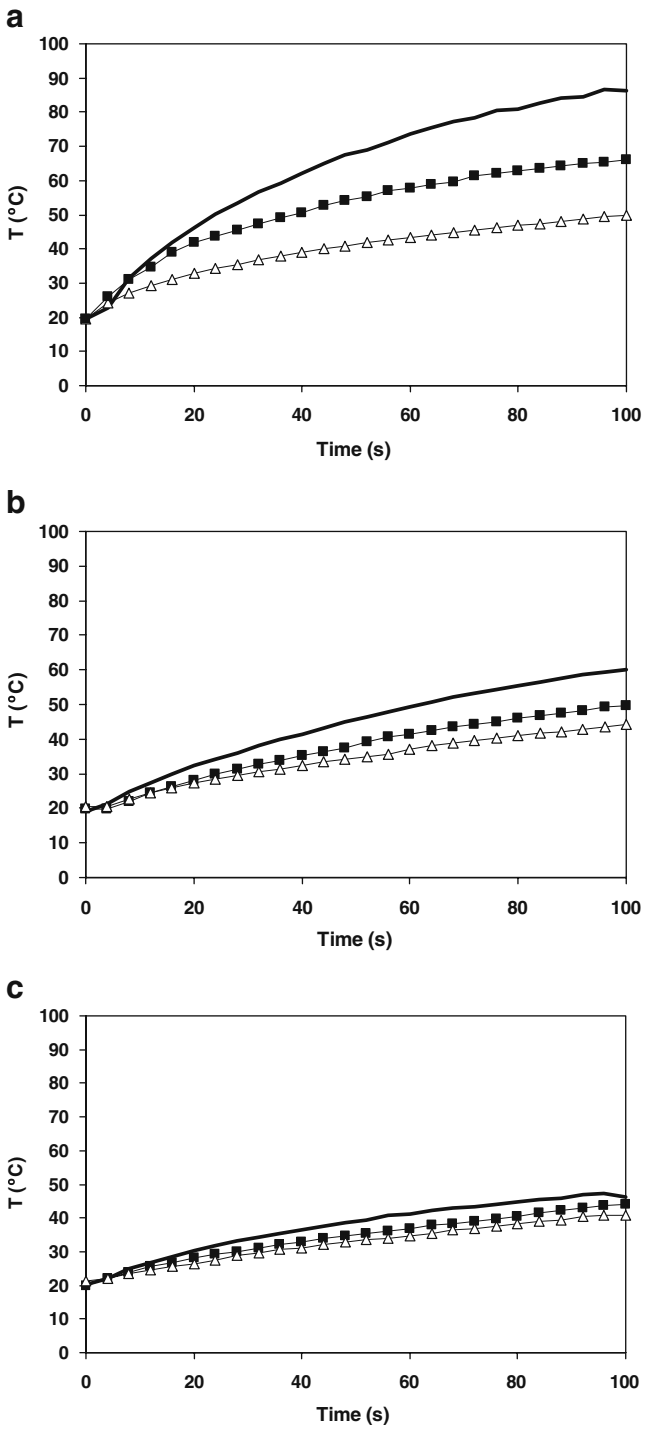

Fig. 1 Temperature increase in cut apple discs irradiated with PL at different distances beneath the lamp and at different positions on the shelf. Apple irradiated at 5 cm (**a**), 10 cm (**b**), and 15 cm (**c**) from the lamp. (*solid line*) shelf central point, (*black squares* and *white triangles*) shelf extreme points

raw salmon fillets treated with pulsed light during 60 s and placed at 3, 5, and 8 cm distances from the lamp, respectively. Jun et al. (2003), when studying the inactivation of *A. niger* spores on corn meal, found that some experimental factor settings resulted in a sample temperature of 120 °C, leading to a change in food properties and hence to quality loss. Gómez-López et al. (2005b) irradiated different minimally processed vegetables at 12.8 cm from the lamp and observed that treatments longer than 45 s per side were not useful to treat samples without provoking serious damage on quality due to the extensively overheating.

Considering a potential adoption of this technology on an industrial scale, the position and the orientation of the sample with respect to the lamp would be one of the primary concerns that had to be addressed to assure a uniform efficiency for microorganism inactivation. Gómez-López et al. (2005a) arrived at the same conclusions when studying the influence of the relative position between the sample and the lamp. These authors demonstrated that when a group of samples was placed at a short vertical distance from the lamp, those located directly below the lamp were decontaminated while the others underwent almost no decontamination. When vertical distance was increased, the decontamination was less intense in those samples located directly below the lamp, but the rest of the samples were also sanitized. Woodling and Moraru (2007) found that fluence decayed exponentially with distance from the lamp and a minor modification of the light path in PL treatments could have a negative impact on the treatment intensity and effectiveness. According to previous results, variations in PL dose were minimized in this work by placing the samples within a uniform area of the radiation field (directly below the lamp and around the central point).

Effect of PL on Color

The colorimetric average L^*, a^*, and b^* values of apple discs exposed to PL at 5 or 10 cm from the lamp during different time lengths are shown in Fig. 2. Values were expressed as differences with the parameters of the fresh fruit just before the irradiation treatment. A significant interaction between treatment and time factors was found for all color parameters ($p<0.0001$, F ranging between 4.5 and 25.5). Fresh apple discs had a creamy-white color, represented by L^* value≈70, a^*≈6.3, b^*≈22. At both sample locations, apple irradiated during 20, 60, and 100 s showed a further decrease in luminosity (greater ΔL^*) and a further increased in a^* value than control samples throughout the different storage periods of time. This behavior was more pronounced in apples exposed to PL at the lowest distance from the lamp assayed. Although just after exposure to PL (day 0) the variations in these parameters were higher as irradiation time increased, no significant differences were overall observed between treatments at the end of storage, except for samples exposed for 2 s, which behaved as the control ($p>0.05$).

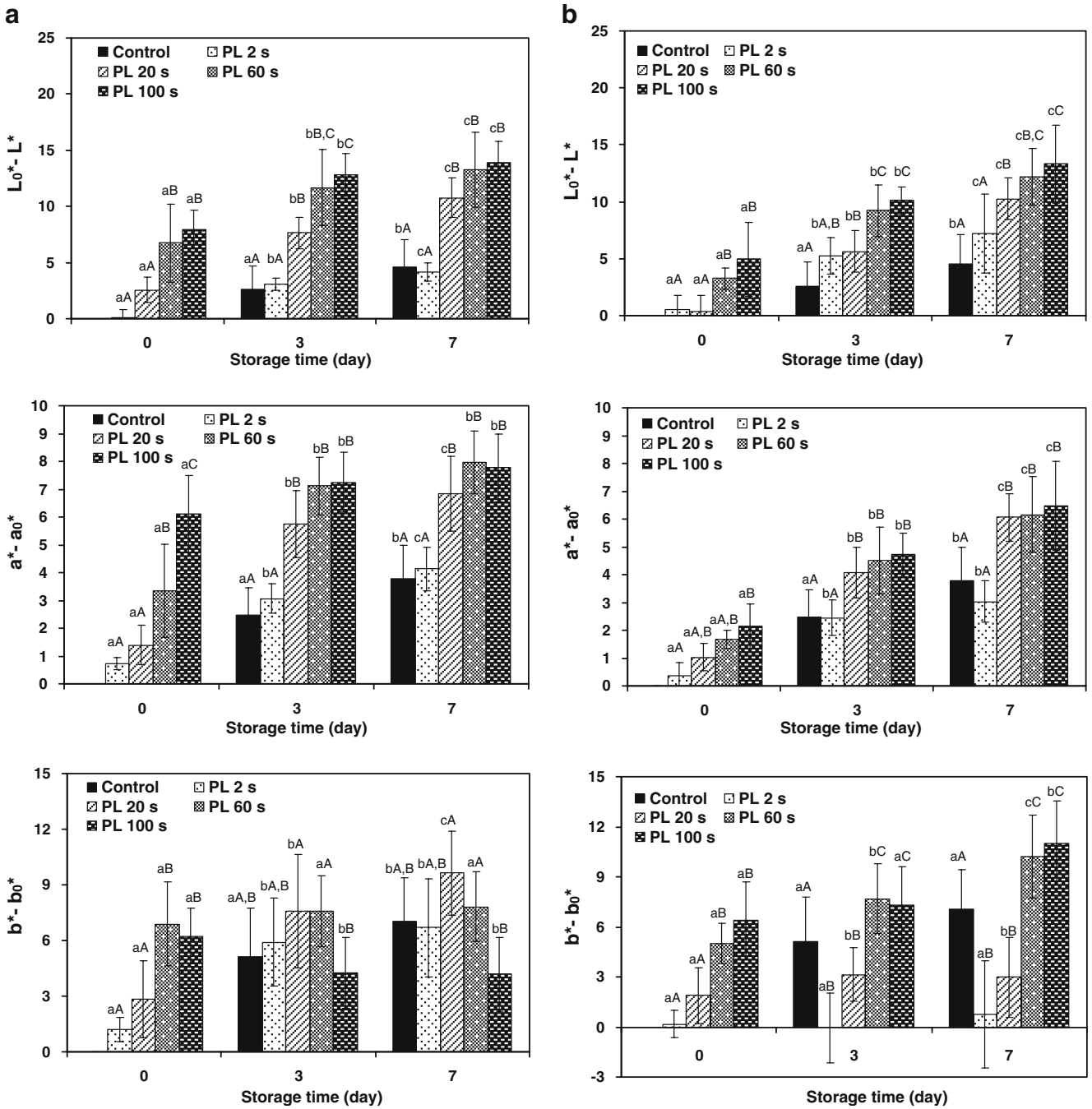

Fig. 2 Effect of PL fluence on lightness (L_0*–L*), a* value (a*–a_0*) and b* value (b*–b_0*) differences of cut apple discs during storage at 5 °C. Apple irradiated at 5 cm (**a**) and 10 cm (**b**) from the lamp. Single effects were analyzed by Tukey's test. For each treatment, means followed by same *lowercase letter* were not significantly different throughout storage time at $p<0.05$. At the same storage day, means of different treatments followed by same *uppercase letter* were not significantly different at $p<0.05$

The b* parameter initially increased with irradiation time. But during storage, the changes in b* did not follow a clear trend. Variation in apples irradiated at 5 cm was similar to that observed in the control. On the other hand, apple exposed to LP at 10 cm during 60 and 100 s showed a further increased in b* value when compared with the control throughout storage, while b* variations of samples irradiated 20 s were less than in the control. For the apple samples irradiated 2 s, b* values did not change significantly until 7 days of storage.

Simultaneous changes in L* and a* values have been associated with browning development on apple surface (Monsalve-González et al. 1993; Goupy et al. 1995; Sapers and Douglas 1987; Gómez et al. 2010). Samples exposed to

PL turned darker (lower L^* values) and less green (higher a^* values) than the control, and this effect was more pronounced as PL dose and/or storage time increased, probably resulting from browning reactions accelerated by temperature increase during irradiation.

The evolution of BI function (expressed as difference with the BI value for fresh apple discs) during storage in apple samples irradiated at different PL doses can be observed in Table 2. At day 0, BI of irradiated apples increased with irradiation time. After 60 and 100 s PL exposure, browning was higher when samples were located nearer the lamp, while for smaller irradiation periods, differences in ΔBI values between samples located at 5 and 10 cm from the lamp were insignificant ($p>0.05$). At days 3 and 7, ΔBI values of apple irradiated during 20, 60, and 100 s at 5 cm from the lamp were similar and higher than those of samples irradiated 2 s and the control. At 10 cm from the lamp, browning was in general less pronounced in all cases than at 5 cm, excepting after long irradiation periods at the end of storage, where ΔBI did not differ significantly ($p>0.05$) with respect to samples irradiated during the same time but at shorter distance from the lamp. On the other hand, apple exposed to PL for 2 s at 10 cm (2.4 J/cm^2) showed lower ΔBI than non-irradiated ones, this indicating an inhibition of browning due to application of PL at low doses. Dunn et al. (1989) observed the same behavior in potato slices subjected to PL for short periods of time and found that browning decrease was associated with polyphenol oxidase decrease. Oms-Oliu et al. (2010) reported also the PPO inactivation in fresh-cut mushrooms just after exposure to PL at 28 J/cm^2.

Table 2 Effect of PL fluence on browning index difference ($BI-BI_0$) of cut apple discs during storage at 5 °C

Storage time (day)	Irradiation time (s)	d=5 cm	d=10 cm
0	0	0a	0a
	2	2.4 (0.9)aA	1.0 (0.8)a,bA
	20	6.2 (2.2)bA	3.8 (2.5)bA
	60	16 (4)cA	9.5 (1.8)cB
	100	21(5)dA	13.2 (3.2)dB
3	0	10.4 (3.7)a	10.4 (3.7)a
	2	12.1 (3.6)aA	4.4 (3.4)bB
	20	20.2 (3.3)bA	13.6 (5.4)a,cB
	60	24.1 (4.1)bA	18.1 (5.9)c,dB
	100	20.3 (3.2)bA	20.1 (3.2)dA
7	0	14.1 (5.8)a	14.1 (5.8)a
	2	15.8 (4.2)aA	7.2 (3.6)bB
	20	26.4 (3.3)bA	18.1 (4.6)aB
	60	27.1 (3.9)bA	24.8 (7.7)cA
	100	24.6 (5.4)bA	28.7 (6.3)cA

Results were expressed as mean ± standard deviation. For each storage time and irradiation distance, means at different irradiation times followed by same lowercase letter were not significantly different at $p<0.05$. For each storage time and at the same irradiation time, means at different irradiation distances followed by same uppercase letter were not significantly different at $p<0.05$

Effect of PL on Microstructure

Optical microscopy studies were performed to evaluate structure changes produced at cellular level by PL. The photomicrographs in Fig. 3 correspond to parenchyma apple tissue localized at the surface of fresh cut apple and PL-treated cut apple at day 0 and at the end of storage (day 7). In fresh tissue, cells and intercellular spaces were loosely arranged in a net-like pattern that was inhomogeneous and anisotropic. Intercellular spaces exhibited various shapes and sizes. Cells, more or less regular in shape, appeared moderately turgid with intact and well-stained cell walls. In few cells, membranes appeared broken probably due to a cutting effect. Also, some cells showed incipient plasmolysis, while in others, a parietal cytoplasm was observed (Fig. 3a–b). Exposure to 60 s PL caused extensive breakage of membranes (plasmalemma and tonoplast), whose rests appeared joined to cell walls. Cell walls appeared much less stained, indicating a PL-induced degradation of biopolymers (Fig. 3c–d).

Non-irradiated tissue stored 1 week in refrigeration showed folding of cell walls but cementing sites between cells (middle lamella) and cell walls appeared with a darker staining than at day 0 (Fig. 3e–f). In contrast, after 7 days of storage, irradiated tissue was visualized with a general disruption. Cells appeared collapsed, with broken membranes, and rupture and folding of cell walls, due to part to the loss of turgor and in part to alteration of wall components. Furthermore, cell walls appeared substantially less stained, especially in the area of adhesion between cells. This would clearly indicate an effect of PL at the level of pectins present in the cell wall (Fig. 3g–h).

The rupture of membranes also was previously observed in cut apple discs irradiated during 20 min with continuous UV-C light at 27 °C (250 J/cm^2) (Gómez et al. 2010). But walls of irradiated tissue appeared densely stained. Folding of walls observed after 1 week storage puts forward evidence of the important impact on walls caused by the UV-C-induced breaking of membranes and the consequent loss of turgor. Therefore, although the structural changes induced by PL can have similarities with those caused by continuous UV-C at membrane level, some differences exist regarding wall impact.

According to microscopic observations, the color changes observed in PL-irradiated apple slices could be at least partially ascribed to the breakage of cellular

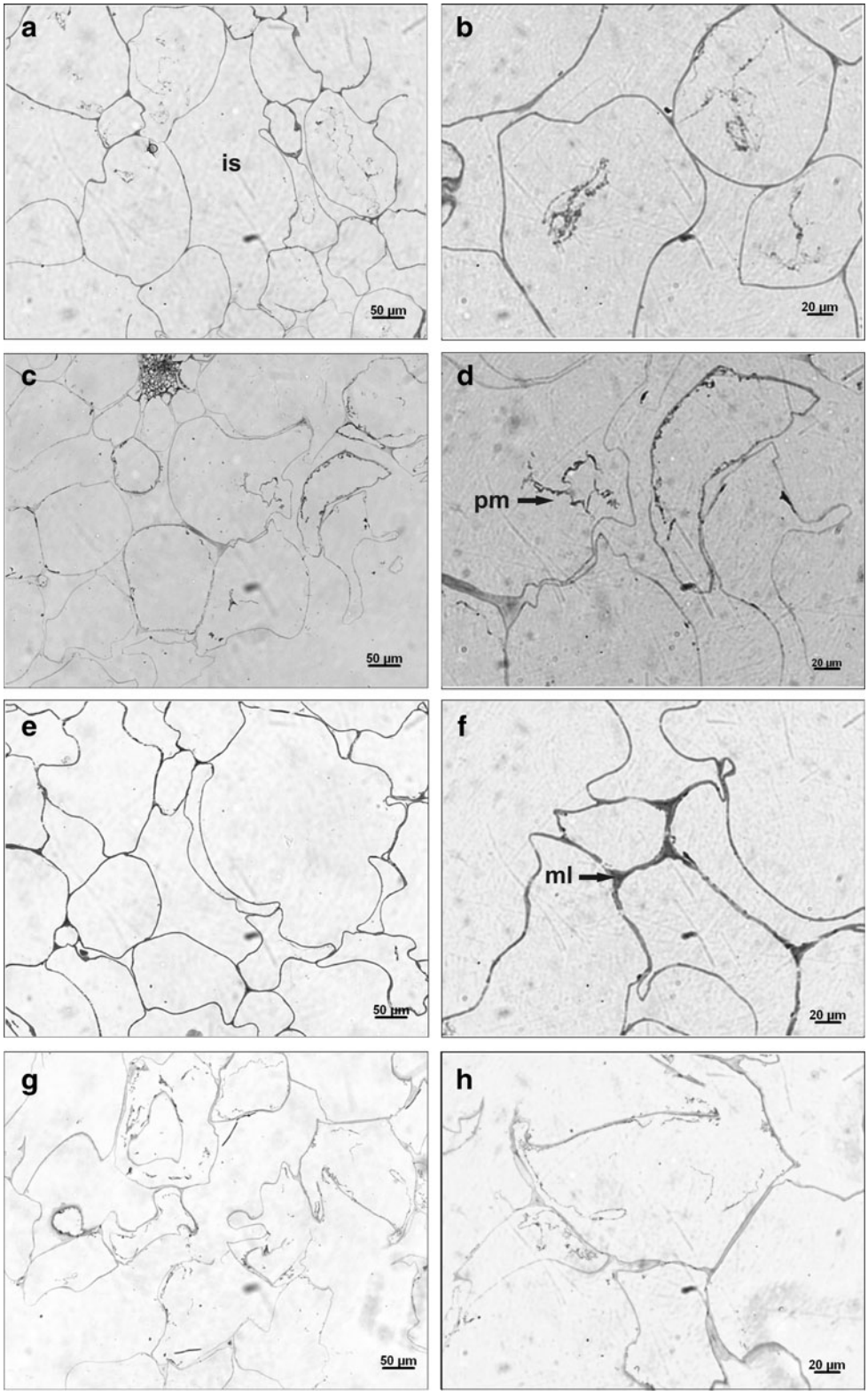

Fig. 3 Light microscopy images from PL-irradiated surface of apple tissue at 0 and 7 day storage. **a**–**b** Control (day 0); **c**–**d** apple irradiated 60 s at 10 cm (day 0); **e**–**f** control (day 7); **g**–**h** apple irradiated 60 s at 10 cm (day 7). *is* intercellular space, *pm* plamalemma, *ml* middle lamella

membranes, which would cause a loss of functional cell compartmentalization. This would increase enzyme-substrate contact with the consequent increase in tissue browning. While low doses of PL provoked an inhibition of browning, exposure to prolonged irradiation times resulted in greater tissue damage which would favor enzymatic browning reactions. However, the increase in temperature in apples exposed to longer

irradiation times could also generate non-enzymatic browning reactions.

Effect of PL on Microbial Response

Figure 4 shows the semilogarithmic survival curves of *L. innocua*, *E. coli*, and *S. cerevisiae* in apple discs irradiated with PL at 5 and 10 cm distance from the quartz window. The curves did not exhibit a linear trend, but in general, the shape was approximately sigmoid. In general, reductions of inoculated microorganisms were approximately 0.5 log cycles between up to six pulses, but then, the inactivation curves tended to level off. The inactivation patterns for *E. coli* and *L. innocua* exhibited a plateau after 2 s up to approximately 60 s PL application, not showing in this period of time great changes between both distances to the lamp. This plateau could be partially due to an injury phase and/or the need of a threshold level of energy for total destruction, as observed by Gómez-López et al. (2007). After 100-s exposure, greater population decrease was observed for *E. coli* in apples irradiated at 5 cm (2.25 log cycles) and for *L. innocua* irradiated both at 5 and 10 cm (1.4 and 1.7 log cycles, respectively). *S. cerevisiae* inactivation curve exhibited also a plateau until 40 s of irradiation without a significant effect of the distance to the lamp. However, after 60 s of exposure, the inactivation was higher at the closer distance to the lamp. The yeast reduction achieved in apple discs exposed 100 s at 10 cm was 1.6 log cycles, while in samples irradiated at 5 cm, yeast colonies were not detected.

The different microbial responses to PL treatments could not only be due to differences in microorganism susceptibility to the UV region of the spectrum and/or different doses of radiation depending on the distance to the lamp, but also to the heating effect when high-PL doses were applied and the different thermal resistance between the organism assayed. Moreover, the inoculated microorganisms could show different sensitivity to the combined action of PL and high temperature. These results would confirm that PL inactivation should be regarded not only as a multitarget technology but as a multifactor process whose mechanisms of action depend on the dose and the microorganism. It is to be noticed that shielding of microorganisms by rough apple surface and microorganism internalization in apple tissue pores greatly influence inactivation patterns (Gómez-López et al. 2007). These situations could also explain the flattening of inactivation curves since light is absorbed at the surface.

Nonlinear inactivation curves have been also reported in the literature for PL treatments. Uesugi et al. (2007) who studied the inactivation kinetics of *L. innocua* in clear suspensions and on stainless steel surfaces (fluence up to 13 J/cm^2) obtained non-linear semilogarithmic survival curves with pronounced tailing. The flattening of the curves occurred at similar doses to this work. Maximum inactivation levels of nearly 7 log cycles were obtained when

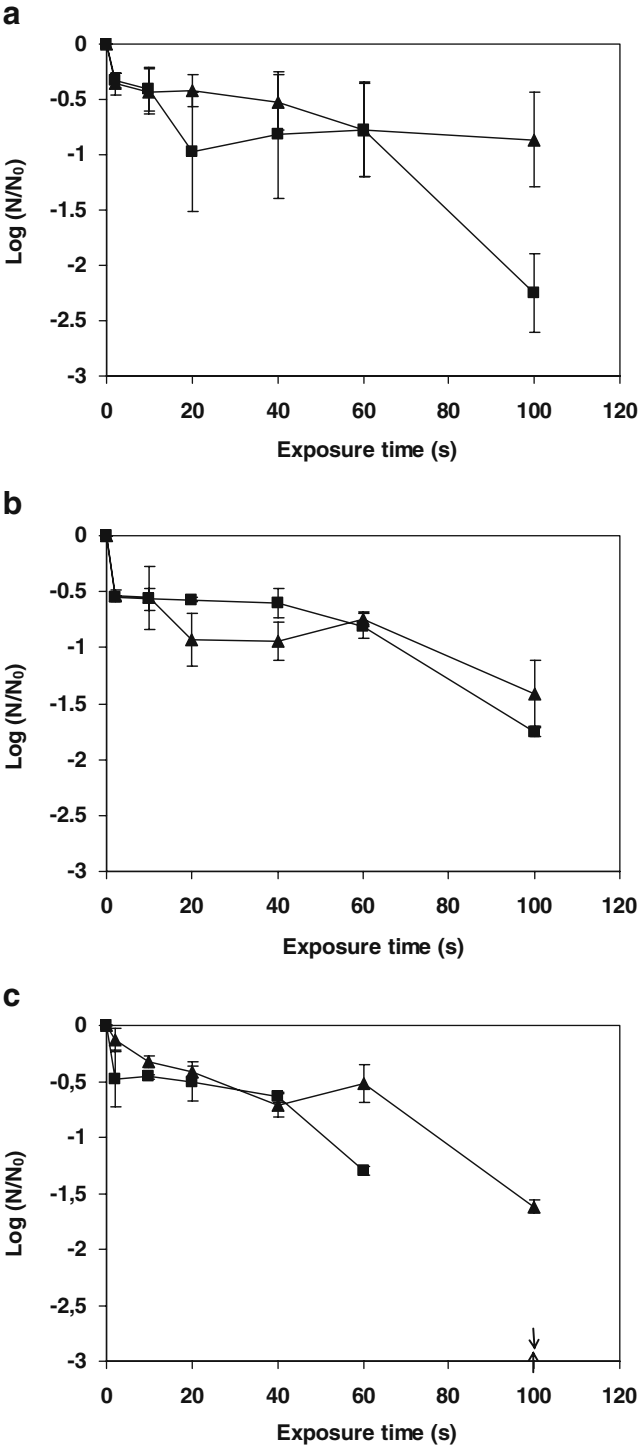

Fig. 4 Effect of PL treatment on semilogarithmic survival curves of microorganisms inoculated in cut apple discs. *E. coli* ATCC 11229 (**a**), *L. innocua* ATCC 33090 (**b**), and *S. cerevisiae* KE 162 (**c**). (*black squares*) *d*=5 cm, (*black triangles*) *d*=10 cm. *E. coli* No.: 2×10^7 CFU/cm^2; *L. innocua* No 4×10^6 CFU/cm^2. *S. cerevisiae* No. 4×10^5 CFU/cm^2. ↕ Not detected counts. *d* distance from the lamp

treating the clear cell suspension with more than 9 J/cm^2. For the PL treatment performed on stainless steel surface, the maximum reduction was lower (3–4 log cycles) and was established at a lower fluence. Sauer and Moraru (2009) found nonlinear kinetics for the inactivation of *E. coli* ATCC 25922 and *E. coli* O157:H7 inoculated in apple cider and juice and inactivation curves also exhibited pronounced tailing. For apple juice, the maximum inactivation levels obtained was about 2.5–2.7 log with a fluence of 13 J/cm^2. In cider, the reductions were about 2.3–3.2 log cycles. Turbulent treatments resulted in higher inactivation (5.8–7.1 log cycles), facilitating the achievement of the required 5-log reduction of *E. coli* O157:H7 in apple juice and cider. It must be noticed that in these studies, the fluence range used was lower compared with the one used in this work, and therefore, the inactivation mechanism would be given only by the photochemical effect and would not participate the photothermal mechanism. Bialka et al. (2008) studied the inactivation of *E. coli* O157:H7 on raspberries and strawberries resulting at PL fluencies between 5 and 72 J/cm^2. They found that the shape of survival curves were different for each berry, with strawberry exhibiting a tailing effect. Inactivation levels at the highest fluence assayed were about 2.5 logs in strawberries and 4.5 logs in raspberries.

Although the application of prolonged irradiation times allowed obtaining in some cases a higher microbial reduction, especially at closer distance from the lamp, severe changes in color and a substantial dehydration of apple disc surface are limiting factors for such long treatments. Accordingly, the survival of inoculated microorganisms during refrigerated storage was analyzed in samples irradiated at 10 cm during only 10 s (fluence 11.9 J/cm^2), where the quality was minimally affected by PL irradiation, and, for the purpose of comparison, during a greater time (60 s, fluence 71.6 J/cm^2) (Table 3). *E. coli* population did not significantly grow in untreated and treated apple discs until 7 days storage, as expected due to the low temperature and pH. On the other hand, *L. innocua* population decreased during storage, both in control and irradiated samples. After 7 days storage, reduction achieved in control and apple discs irradiated 10 s was about 1.4 log cycles, while for samples irradiated 60 s was 2.5 log cycles. On contrary, *S. cerevisiae* grew in control discs (0.3 log cycles) and in PL irradiated apple discs during storage. In irradiated samples, PL application during 10 and 60 s decreased counts by 0.6 and 0.8 log cycles, respectively, but the population increased about 0.4 log cycles at 3 days storage and then remained approximately constant until 7 days. In spite of the microbial growth observed during

Table 3 Effect of PL treatment on the survival of inoculated microorganisms and native flora in cut apple discs throughout storage at 5 °C in the dark

	Microorganism	Storage time (day)	Control	10 s PL[a]	60 s PL[a]
Inoculated microorganisms			log (N/N_0)		
	E. coli ATCC 11229	0	0	−0.34±0.06	−0.56±0.05
		3	−0.09±0.05	−0.3±0.2	−0.5±0.3
		7	−0.1±0.2	−0.26±0.03	−0.35±0.04
	L. innocua ATCC 33090	0	0	−0.30±0.05	−1.0±0.2
		3	−0.6±0.1	−1.0±0.3	−1.8±0.4
		7	−1.4±0.3	−1.5±0.2	−2.5±0.1
	S. cerevisiae KE 162	0	0	−0.6±0.1	−0.8±0.2
		3	−0.1±0.1	−0.12±0.05	−0.3±0.3
		7	0.28±0.04	−0.31±0.04	−0.3±0.2
Native flora			N (CFU/g)		
	Aerobic mesophilic	0	5±4	ND	ND
		3	6±2	2±1	2±1
		7	198±35	4±2	3.5±0.5
	Molds and yeasts	0	61±11	ND	ND
		3	99±11	40±6	7±2
		7	236±75	40±27	7±2

Results were expressed as mean ± standard deviation. *E. coli* No. 2×10^7 CFU/cm^2; *L. innocua* No. 3×10^6 CFU/cm^2; *S. cerevisiae* No. 1×10^6 CFU/cm^2

ND not detected

[a] Apples exposed to irradiation at 10 cm from the lamp

storage in irradiated apples, counts were lower than those in control discs.

The response of native flora (mesophilic aerobes and yeasts and molds) after the different PL treatments and during refrigerated storage can be observed in Table 3. Mesophilic aerobes population remained without significant variation in flashed apples. By contrast, the increase in aerobic count on untreated discs was about 2 logs after 7 days at 5 °C. On the other hand, the proliferation of yeasts and molds was about 2.5 logs in control samples at the end of storage. Counts in apples irradiated during 10 and 60 s were reduced in 0.8 and 1.6 log, respectively. Oms-Oliu et al. (2010) found that the irradiation of mushrooms slices at fluencies between 4.8 and 28 J/cm^2 allowed extension of the microbiological shelf life by 2–3 days in comparison to untreated samples, but the high PL fluencies dramatically affected texture and promoted enzymatic browning. In fresh cut vegetables such as spinach, celery, green paprika, soybean sprouts, radicchio, carrot, iceberg lettuce, and white cabbage, PL application for 45 and 180 s per side provided log reduction in mesophilic aerobic counts between 0.21 and 1.67, and between 0.56 and 2.04, respectively. But when samples were processed for shelf-life studies, PL treatment did not increase the shelf life of the vegetables in spite of the reduction in the initial microbial load excepting in iceberg lettuce where one extra storage day at 7 °C was achieved (Gómez-López et al. 2005b). The results here obtained suggested that the shelf life of cut apples from the microbial point of view would be prolonged even applying a few flashes of PL.

Conclusions

The higher the PL dose, the greater the effect on microbiological stability. Cut apple surface exposed to high PL fluencies turned darker (lower L^* values) and less green (higher a^* value) than the control, and this effect was more pronounced as PL dose and/or storage time increased. Color changes could be at least partially ascribed to the breakage of cellular membranes, which would cause a loss of functional cell compartmentalization and an increase in enzyme-substrate contact with the consequent increase in tissue browning. But increases in temperature at high doses could also cause non-enzymatic browning.

Although the application of high PL fluencies allowed obtaining greater microbial reductions, they also promoted browning of apple. Application of PL at a dose of 11.9 J/cm^2 could extend the shelf life of cut apple with minimal modification in color.

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