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Storage quality of strawberry fruit treated by pulsed light: Fungal decay, water loss and mechanical properties

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ABSTRACT

The effect of different pulsed light (PL) doses (2.4–47.8 J/cm^2) on water loss, fungal spoilage, mechanical properties and structure of strawberries stored for up to 8 days at 6 °C was studied. Incidence of postharvest molds on strawberry fruits was reduced by over 16–42% with PL application. There were no significant differences in maximal rupture force (F_R), mechanical work (W) and deformability modulus (E_d) values between treated and untreated fruits immediately after treatments. After 8 days storage at 6 °C, untreated strawberries showed a pronounced softening (≈48% reduction in F_R), but stored strawberries exposed for 10 s and 40 s to PL presented slight or not significant changes in the mechanical parameters regarding day 0, while F_R and W values of 20 s-PL treated samples were increased by 35% and 88% compared to those at 0 day storage. Micro and ultrastructure changes evaluated by LM and TEM images demonstrated ITW cell wall strengthening and a major integrity of walls of hypodermis cells induced by PL stress, while cell wall disassembly and reduction of cell-to-cell contact were detected in stored untreated fruit. There were no significant differences in weight loss among untreated and PL treated fruits after storage, excepting at the highest PL dose. PL technique would be able to simultaneously provide disinfection and delete softening of the tissues along cold storage. Present results make this non-thermal, residue-free alternative promising for extending shelf-life of traditional and organic strawberry production.

Industrial relevance: The present results demonstrated that pulsed light (PL) treatment is a promising alternative for extending the shelf-life of strawberries. A decrease in fungal incidence and a depletion of softening, important factors which limit the strawberry postharvest storage life, were achieved by the application of PL.

1. Introduction

Consumption of fresh fruits has dramatically increased during the past few decades and far exceeded the increases observed for processed fruit products (Barth, Hankinson, Zhuang, and Breidt, 2009). This rise in produce consumption was driven, at least in part, by increased awareness in healthy habits. The "soft fruit" group, which includes strawberries, raspberries, blueberries, cranberries and gooseberries, invariably ranks high among fresh fruits due to their powerful antioxidant content and its putative role in the prevention of several chronic and degenerative diseases associated with oxidative damage like cancer and heart disease (Battino et al., 2009). The storage life of soft fruits is greatly shortened by both physiological and pathological deterioration (Barkai-Golan, 2001). Strawberry, the most important world crop in this group, is a popular and attractive fruit due to its high visual appeal and desirable flavor. Strawberry belongs to the family Rosaceae and is considered a false fruit since the edible structure originates from the expansion of the receptacle as a pseudocarp (Aharoni and O'Connell, 2002; Vicente and Sozzi, 2007). Physical, sensory and nutritional qualities of strawberry fruits are associated with traits like size, firmness, color, taste and aroma, vitamin C and phenolic contents (Mazur et al., 2014). Dynamic changes in chemical composition, and tissue structure during ripening, senescence, and processing cause variations in sensory, chemical and physical properties. Although strawberry is characterized by a high metabolic rate, decay development is the primary cause of loss. Postharvest diseases are the result of latent infections that occur in the field during the growing season and infections from wounding during harvest and handling operations and contribute to major economic losses to growers, processors, marketers and consumers (Michailides, Morgan, and Luo, 2010). The major postharvest pathogen of strawberry is *Botrytis cinerea*, the causal agent of gray mold. It survives on organic debris in the field; during the flowering and fruiting season fungal spores are common in the atmosphere and are deposited on flowers. The disease is manifested only during the postharvest phase, when the fruit ripens, during transit and marketing (Ceredi et al., 2009).

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Other fungi responsible for postharvest rot include species of *Mucor*; *Rhizopus*, the causal organisms of "leak" disease; *Colletotrichum*, which cause anthracnose, and *Phytophora*, which initiate leather rot (Barkai-Golan, 2001).

Postharvest fungicidal applications are not practical for ripe strawberries because of their sensitivity to wetting. The minimal growth temperature of *Botrytis* is about −2 °C and cold storage can only retard decay development. Extension of strawberry postharvest life has been an ongoing challenge and some studied alternative antifungal agents to reduce fruit losses, alone or in combination, include heat, UV-C irradiation, pulsed light, ozone, chlorine dioxide, ultrasound, natural antimicrobials and edible film coatings (Barkai-Golan, 2001; Vicente and Sozzi, 2007; Aday and Caner, 2014; Marquenie, Michiels, Van Impe, Schrevens & Nicolaï, 2003; Gómez-López, Devlieghere, Bonduelle, and Debevere, 2005; Lagunas-Solar, Piña, MacDonald, & Bolkan 2009).

Pulsed light (PL) involves the use of intense and short-duration (1 μs–0.1 s) pulses of broad spectrum light of wavelength ranging from UV to near-infrared (200–1100 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) (Gómez-López, Ragaert, Debevere, and Devlieghere, 2007). It has, comparatively to continuous UV-C light, higher penetration depth and emission power (Krishnamurthy, Demirci, and Irudayaraj, 2007). Its use has been approved by the FDA (1996) for the decontamination of food and food surfaces. PL is one of the most promising non-thermal surface decontamination technologies for food produce due to the significant microbial reduction in very short intervals (tens of seconds) compatible with the logistics of the fresh fruit industry, the limited energy cost, the low environmental impact, the lack of residual compounds and its great flexibility (Lagunas-Solar, Piña, MacDonald, and Bolkan, 2006; Oms-Oliu, Martín-Belloso, and Soliva-Fortuny, 2010). Its efficacy has been mainly attributed to microbial DNA damages by thymine dimmer formation (photochemical effect) and/or to localized overheating of microbial cells (photothermal effect) and/or to structural damage caused by the pulsing effect (photophysical effect) ((Wekhof, 2000; Krishnamurthy et al., 2007).

The objective of this work was to investigate the effect of different PL doses on water loss, fungal spoilage, mechanical properties and structure of strawberries stored for up to 8 days at 6 °C. How differences in tissue structure were expressed by penetrometric parameters and water loss was also studied.

2. Materials and methods

2.1. Plant material

Strawberries (*Fragaria* × *ananassa* Duch., cv. Camarosa; pH 3.5 ± 0.3; 7.4 ± 0.5 °Brix) having 100% surface red color were purchased at a local orchard and immediately transferred to the laboratory. Fruits were selected for uniformity of ripeness and size, and absence of physical injuries or microbial infection. Then, they were randomly distributed into polyethylene boxes, stored at 5–7 °C and processed within a day.

2.2. Pulsed light equipment and dosimetry

PL treatments were performed with a RS-3000B Steripulse-XL system (Xenon Corporation, Woburn, MA, U.S.A.), which produced polychromatic radiation in the wavelength range of 200 to 1100 nm. The system consisted of a RC-747 power/control module, a treatment chamber that houses a xenon flash lamp and an air cooling system attached to the lamp housing to avoid lamp overheating during operation. The system generated high intensity pulsed light at a pulse rate of 3 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 3800 V at 1.9 cm from the quartz window surface of the lamp.

Different fluences were obtained by altering the number of applied pulses at a fixed distance from the 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.

2.3. Pulsed light treatment

To perform the PL treatments up to four strawberries were treated at the same time. Fruits were put on a sterile glass tray and placed on a stainless steel shelf in the PL unit at 10 cm distance from the quartz window of the lamp. Variations in radiation dose absorption were minimized by placing the samples within a uniform area of the radiation field (beneath the lamp and around the central point). Samples were exposed to irradiation for 2, 10, 20 and 40 s, corresponding to fluences of 2.4, 11.9, 23.9 and 47.8 J/cm^2, respectively. Fruits were irradiated on one side and then they were turned upside down on another sterile tray and treated during the same period of time on the opposite side.

Temperature measuring of strawberry during PL treatment was monitored by using T-type thermocouples whose tips were placed immediately beneath the surface and in the center of the fruit. The thermocouples were connected to a data logger Digi-Sense model 69202-30 (Barnant Company Division, Barrington, USA). Temperature measurements were done in triplicate.

PL treated strawberries were compared with untreated fruits (control). Control and irradiated samples were packed in closed plastic boxes permeable to air (26 cm × 19 cm × 6 cm) and stored at (6 ± 1) °C for 8 days. Each box contained about 10–11 fruits. Samples were analyzed immediately after treatment (0 day) and at selected days of storage.

2.4. Decay incidence

Postharvest strawberry disease was assessed by incidence, by visually recording the presence or absence of fungal development, regardless of the severity of the infection. Results were expressed as percentage of infected fruit (Aday and Caner, 2014; Michailides et al., 2010). The observations were made in 32 fruits for each condition. The whole experiment was repeated three times.

2.5. Mechanical properties

Puncture test was performed with an Instron Testing Machine model 3345 (Canton, Massachusetts, USA) with a flat-end cylindrical probe (4.8 mm in diameter), 50 N-load cell, and a crosshead speed of 30 mm/min. Each specimen was penetrated on the equatorial side. From the force–displacement and stress–deformation curves, three mechanical parameters were computed: the maximal rupture force (F_R, expressed in N) that represents the force required to puncture the fruit epidermis, the mechanical work (W, expressed in J) that corresponds to the energy needed to break the epidermis and was estimated by the area under the curve up to the epidermis rupture point, and the deformability modulus (E_d, expressed in mPa) calculated from the initial linear portion of the stress–deformation curve. Puncture measurements were done on 22 strawberries for each condition. The whole experiment was repeated twice.

2.6. Weight loss

Weight loss along storage of treated and untreated strawberries was recorded in a balance (Precisa 180 A, Switzerland) with a precision of ±0.0001 g. Ten fruits were used for each condition. Results were

expressed as percentage of weight loss with respect to fresh fruit without treatment or storage, according to Eq. (1):

$$PP(\%) = 100\,x\,(p_0 - p_t)/p_0 \quad (1)$$

where PP: percentage of weight loss, p_0: initial weight of fresh strawberry sample and p_t: weight of strawberry sample at time t.

2.7. Microscopic observation

For light microscopy (LM) observations, sections (≅3 mm^3) of fresh and treated strawberries, including the PL exposed surface, were fixed in glutaraldehyde solution (3 g/100 g) and then in 0.1 M potassium phosphate buffer (pH = 7.4) during 48 h at room temperature. Sections were then rinsed three times with distilled water, postfixed 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 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). For transmission electron microscopy (TEM), samples embedded in Spurr resin were cut in ultrathin sections (1 μm thick) using a glass knife with a ultracut microtome, collected on copper grids and double stained with uranyl acetate and Reynolds lead-citrate. Sections were examined using a JEOL JEM-1200 EX II (Japan) transmission electron microscope at an accelerating voltage of 80 kV. All reagents were from Merck Química Argentina S.A. (Argentina).

2.8. Statistical analysis

Statistical analyses were carried out using the Infostat v. 2010 software (Universidad Nacional de Córdoba, Argentina). Mechanical data was analyzed by multivariate analysis of variance (MANOVA). Previously to conduct the analysis, multivariate outliers were detected by Mahalanobis distance and removed from data set. Post-hoc multiple comparisons among multivariate means were performed by Hotelling tests based on Bonferroni correction. Two-way analysis of variance (ANOVA) was performed on weight loss results according to the factors "treatment" and "storage time". Because of the existence of significant interactions between factors, single effects were examined (i.e. effects of one factor holding the other fixed). Multiple comparisons were performed using the Tukey test. In all the analyses significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Temperature evolution during PL exposure

The initial temperature of the fruits was (12.6 ± 0.6) °C. During PL treatment, temperature increased with time, being the increase higher in the surface than in the center of the fruit. After 4, 10, 20 and 40 s of PL exposure, the temperature was (13.8 ± 2.0) °C, (20.7 ± 2.0) °C, (29.3 ± 2.3) °C and (40.2 ± 2.9) °C near the surface, and (13.0 ± 0.8) °C, (14.2 ± 0.4) °C, (15.1 ± 0.5) °C and (16.3 ± 0.2) °C in the center, respectively. A similar increase of temperature was found by Luksiene, Buchovec, and Viskelis (2013), who reported that the temperature on the surface of the strawberry fruit after PL exposure never exceeded 42 °C (UV light density 5.4 J/cm^2; 10 cm from the lamp, temperature of untreated fruit: 22 °C).

3.2. Decay incidence

The effectiveness of PL treatment to inhibit fungal development on strawberries was visually inspected. Overall there was a marked increase in the number of infected fruits during storage but delays were observed on the onset of infection in treated fruits. Strawberries treated with PL for 2 to 40 s (fluences between 2.4 and 47.8 J/cm^2) showed a diminution in fungal incidence compared to untreated fruits (control) (Fig. 1). This decrease was more pronounced as PL fluence increased. After 8 days of storage, a significant reduction ($p < 0.005$) of about 16% in the percentage of infected fruit of 2 s PL exposed fruits compared to the percentage of infected fruits showed by stored control was observed. For higher doses (10, 20 and 40 s) the reduction achieved was significantly greater ($p < 0.005$) (between 30% to 42%), the differences among these doses being not statistically significant.

These results are in partial agreement with the literature. Marquenieet al. (2003) studied the use of different doses of PL (0–250 s; 25 cm distance from the lamp) to inactivate conidia of the fungi *B. cinerea* and *Monilia fructigena* suspended in 10 mM phosphate buffer. An inactivation of 1 log unit and 3–4 log units was observed after 40 s or 100 s PL treatments respectively. However, when these authors evaluated the effect of PL (40–250 s) on conidia of *B. cinerea* inoculated on strawberries, there was no significant difference between any of the treated groups and the untreated fruits (Marquenie et al., 2003). In opposition to these findings, Luksiene et al. (2013) indicated that the decontamination of strawberries by PL (UV light dose of 3.9 J/cm^2) was significant compared to control. Mesophilic bacteria and yeasts and molds naturally distributed on the surface of strawberries were inactivated by 2.2 logs and 1 log respectively, while inoculated *Bacillus cereus* and *Listeria monocytogenes* were inactivated by 1.5 and 1.1 log, respectively. The strong dependence of the efficacy of the PL treatment on critical process parameters (voltage, electric current intensity, dose received by the sample, uniformity of the treatment, pulse repetition rate) could explain, at least in part, the contradictory literature results.

The trial results showed the possibility of containing fungal decay by PL as an alternative treatment to synthetic fungicides. Because fungi may reside in crevices or irregularities present on fruit surface or may penetrate under the strawberry epidermis, a great PL shielding effect could be responsible of the only partial disinfection. Lagunas-Solar et al. (2006) remarked the need to introduce techniques to create multidirectional direct or reflected UV beams combined with random movements of the fruits to provide uniform PL exposure of all fruit surfaces.

3.3. Water loss

The weight loss (associated with the loss of water) for control and PL-treated strawberries along refrigerated storage is presented in Fig. 2. Significant interaction between the factors "treatment" and "storage time" was observed (ANOVA $F_{8, 135} = 3{,}93$; $p < 0.0001$). Immediately after processing, PL irradiated samples showed a slight weight

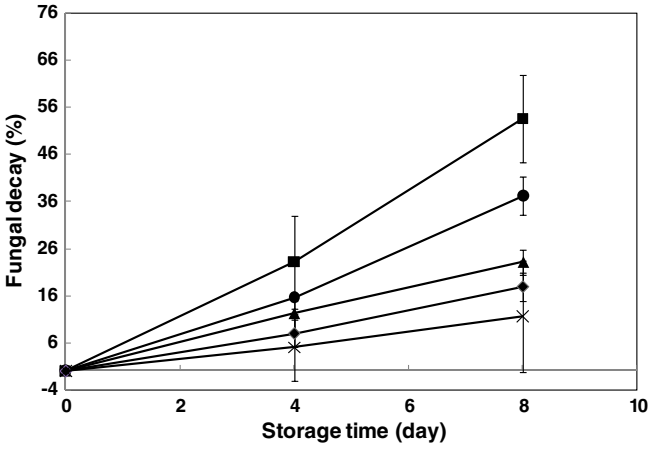

Fig. 1. Fungal decay incidence in untreated and PL-treated strawberry fruits stored at 6 °C. (■) control; (●) 2 s-PL; (▲) 10 s-PL; (♦) 20 s-PL; (X) 40 s-PL.

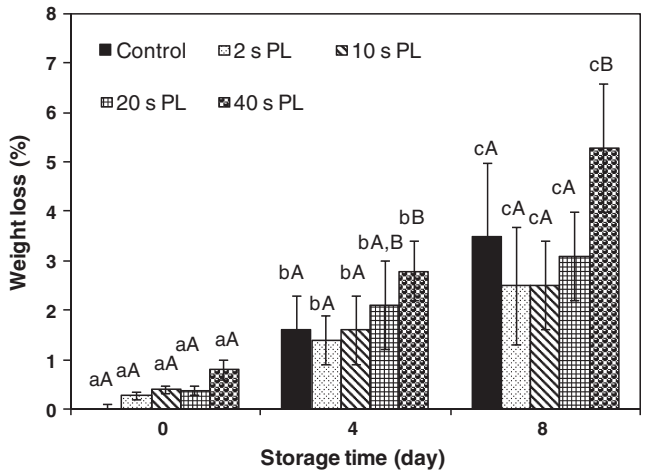

Fig. 2. Weight loss (%) for non-treated and PL-treated strawberry fruits throughout storage at 6 °C. For each treatment, means followed by same lowercase letter were not significantly different throughout storage time at $p < 0.05$. At the same storage day, means of different treatments followed by same uppercase letter were not significantly different at $p < 0.05$.

loss (<1%), although it was not statistically significant. Both in untreated and treated fruits a significant decrease in weight was observed throughout storage. The reduction was significantly higher in fruits exposed to PL during 40 s (5.3 ± 1.3%) than in control fruit (3.5 ± 1.5%) and in fruits irradiated for shorter times (2.5 ± 1.2%–3.1 ± 0.9%).

3.4. Mechanical properties

Typical force–displacement curves determined on 0 and 8 day for control and 10 s, 20 s and 40 s PL-treated strawberries are presented in Fig. 3. The fruit exhibited an approximately linear increase of the stress with strain up to the failure point. The first peak in the curves corresponds to the force required to puncture the epidermis. The breakdown occurred at low strain, which is the typical behavior of many fresh fruits and vegetables. Subsequent peaks showed the resistance force to penetration in the inner region of the fruits. It can be observed that the epidermis of the strawberry fruit (treated or untreated) contributed approximately 57–63% of the firmness of the fruit before the rupture force, while the role of the hypodermal and parenchymal tissues was minor but not negligible. Control and PL-exposed samples exhibited similar mechanical behavior immediately after irradiation (Fig. 3 A). At 8 days of storage, force–displacement curves of control fruits showed lower forces than those of treated strawberries, not only in the epidermis (where F_R decreased about twofold) but also throughout the whole fruit, while forces exhibited by stored PL exposed fruits were similar or greater than at day 0 (Fig. 3 B).

Mean values of mechanical parameters (rupture force, F_R; mechanical work, W; deformability modulus, E_d) obtained from the first peak of force–displacement and stress–deformation curves are shown in Table 1. Overall, the mechanical parameters were found to be dependent on PL dose and storage time. MANOVA results indicate highly significant differences among multivariate means (MANOVA $F = 10.22$, $p < 0.0001$). Statistical analysis did not reveal significant differences in the mechanical parameters between treated and untreated fruits immediately after treatments. However, after 8 days storage at 6 °C, control strawberries showed a significant decrease in F_R, W and E_d values, reflecting the softening of untreated fruit during storage. On the contrary, stored strawberries exposed during 10 s and 40 s to PL presented slight or not significant changes in the mechanical parameters regarding day 0, while 20 s-PL treated samples were observed with higher F_R and W values than those at 0 day storage.

The obtained results are in accordance with prior reports about the impact of PL on firmness of strawberry fruits just after irradiation process. According to Luksiene et al. (2013), PL treatment does not affect fruit firmness as no changes were observed in comparison with control, but these authors evaluated this parameter only after PL exposure. Other authors reported that fruit firmness of strawberries (cv. Elsanta) decreased during 10 days storage period, but they found no significant differences between PL treated and control fruits (Marquenie, Michiels, et al., 2003).

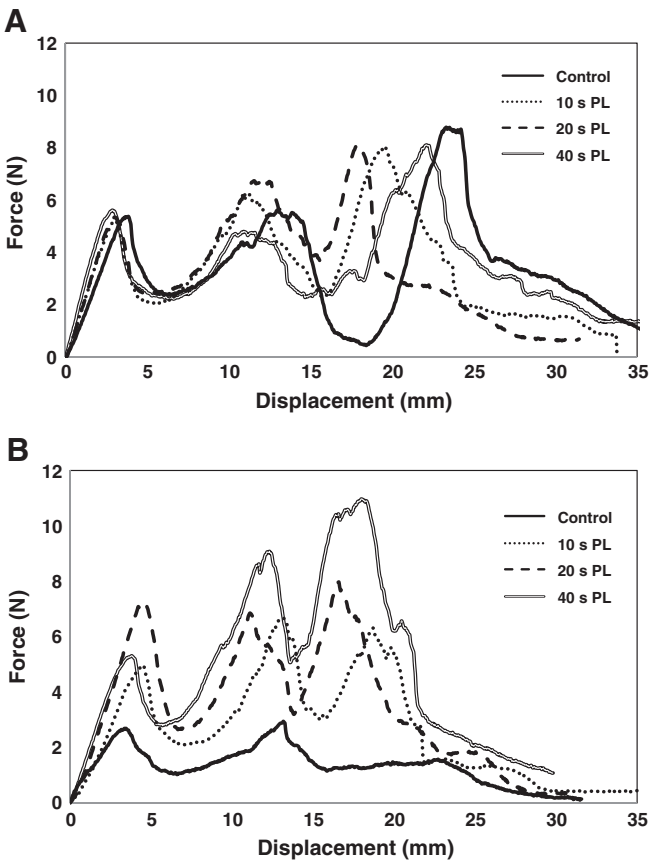

Fig. 3. Typical penetrometric force–displacement curves for non-treated and PL-treated strawberry fruits stored at 6 °C. A) Day 0, B) Day 8.

3.5. Microscopic features

Structure knowledge is critical to understanding how pulsed light process alters fruit properties since there is a causal connection between structure and functionality (Aguilera, Stanley, and Baker, 2000; Jackman and Stanley, 1995). Figs. 4–5 show ultra and microstructural aspects of strawberry fruits as affected by PL treatment and posterior storage.

Table 1
Mean value ± SD (standard deviation) of mechanical parameters obtained from puncture test for non-treated and treated strawberries at 0 and 8 day storage at 6 °C.

Storage time (day)	PL treatment (s)	F_R (N)	W (mJ)	E_d (mPa) ($\times 10^{-2}$)	
0	0	5.4 ± 1.9	9.8 ± 4.8	1.1 ± 0.2	A
	10	5.1 ± 1.7	8.8 ± 4.8	1.1 ± 0.2	A
	20	5.1 ± 1.8	8.7 ± 4.2	1.1 ± 0.2	A
	40	5.7 ± 1.2	10.5 ± 3.6	1.1 ± 0.2	AB
8	0	2.8 ± 1.3	8.1 ± 4.6	0.4 ± 0.2	D
	10	4.9 ± 1.4	12.2 ± 3.9	0.7 ± 0.2	C
	20	6.9 ± 2.1	16.4 ± 6.2	0.9 ± 0.4	E
	40	5.2 ± 1.4	10.7 ± 3.8	0.9 ± 0.2	CB

Post-hoc multiple comparisons using Hotelling tests based on Bonferroni correction $\alpha = 0.05$. Different letters indicate significant differences.

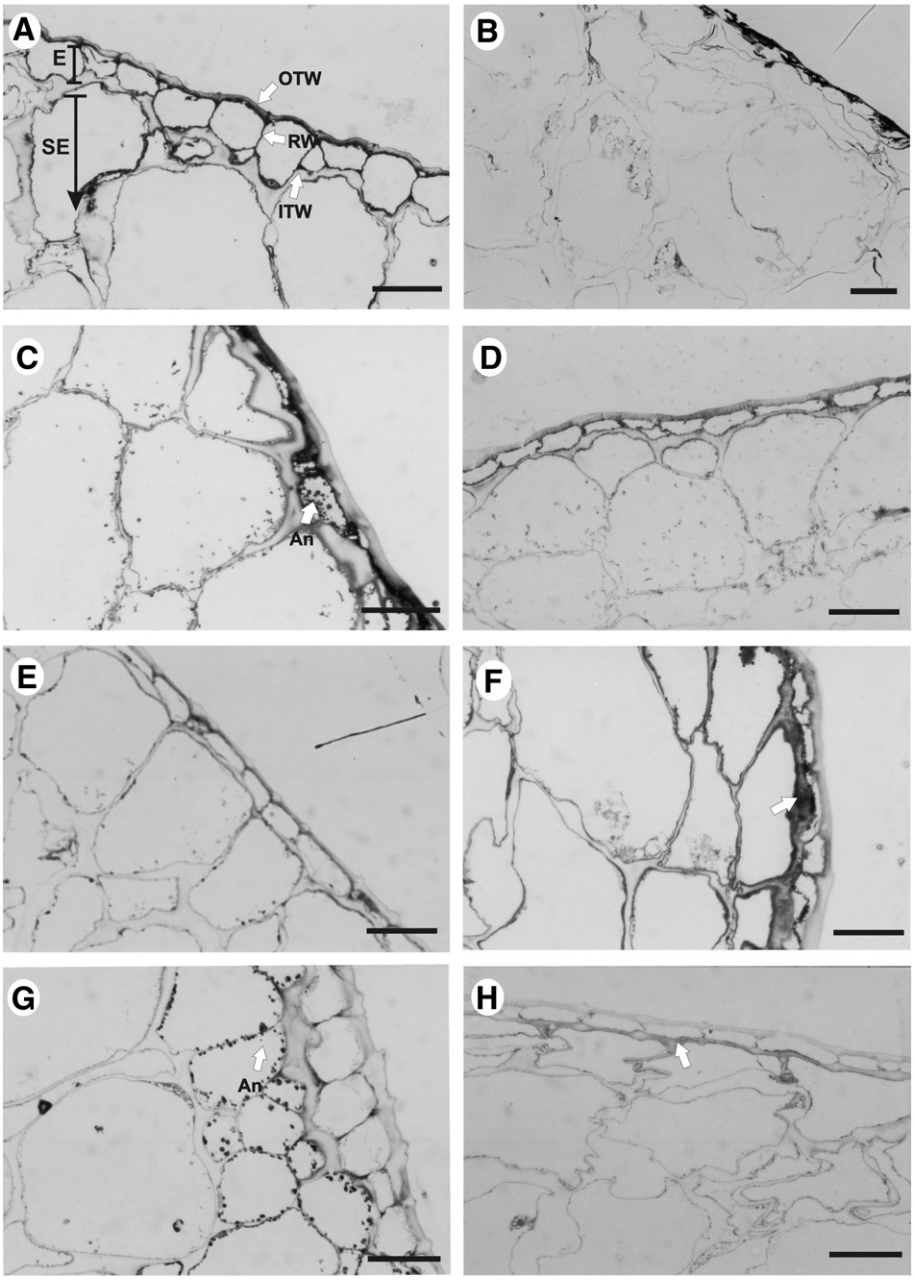

Fig. 4. Light microscopy (LM) micrographs showing epidermal (E) and subepidermal cells (SE) of PL-treated strawberries stored at 6 °C. A, B: control; C, D: 10 s-PL; E, F: 20 s-PL; G, H: 40 s-PL. A, C, E, G: day 0; B, D, F, H: day 8. OTW = outer tangential wall; ITW = inner tangential wall; RW = radial epidermal cell wall; An = anthocyanin contents. Scale: 50 μm.

In LM observations of raw tissues, epidermal cells, rectangular or irregular in shape, appeared organized in a continuous (without air spaces between cells) monolayer of strongly attached cells with an asymmetrical cell wall deposition. Most of them were observed turgid, with parietal cytoplasm and with scarce disrupted membranes. Outer tangential epidermal walls (OTW) were visualized undulated. Inner tangential epidermal walls (ITW) appeared thicker and less densely stained than OTW and radial epidermal walls (RW) (Fig. 4 A). MET analysis from the cross section of the epidermis showed four discernable layers from outer to inner: 1. epicuticular wax layer, 2. cuticular membrane, 3. pectic layer, and 4. non-cutinized cellulose layer (Fig. 5 A, B). Epicuticular waxes were visualized as a thin and discontinuous layer, although it is not very likely that waxes survive TEM preparation procedures without change in structure or dislocation (Schreiber and Schönherr, 2009). Cuticular membrane was electron dense, with reticulate pattern and slight irregular contour. Pectin layer, around the epidermal cell wall–cuticular membrane interface, was electronically denser than the other layers and protrude into the cuticular membrane at an acute angle forming a dense reticulum (Fig. 5 B). Cellulose layer looked less electronically dense, excepting in the innermost region, which presented higher electronic density, with the cellulose microfibrils arranged in a longitudinal pattern. Radial walls appeared dark and straight (Fig. 5 A). Subepidermic cells presented well stained walls with a notorious middle lamella cementing neighboring cells in some regions (Fig. 4 A). Tissues exposed to 10 s-PL showed contracted epidermal cells with OTW and ITW slightly thicker than control samples and with large amount of anthocyanins in vacuoles (Fig. 4 C). In TEM, the cuticle was observed with minor electronic density and radial epidermal walls showed tightly packed and darkly stained fibrillar material and a conspicuous middle lamella in between (micrographs not shown). Subepidermic cells showed well stained walls but most of them appeared with disrupted membranes

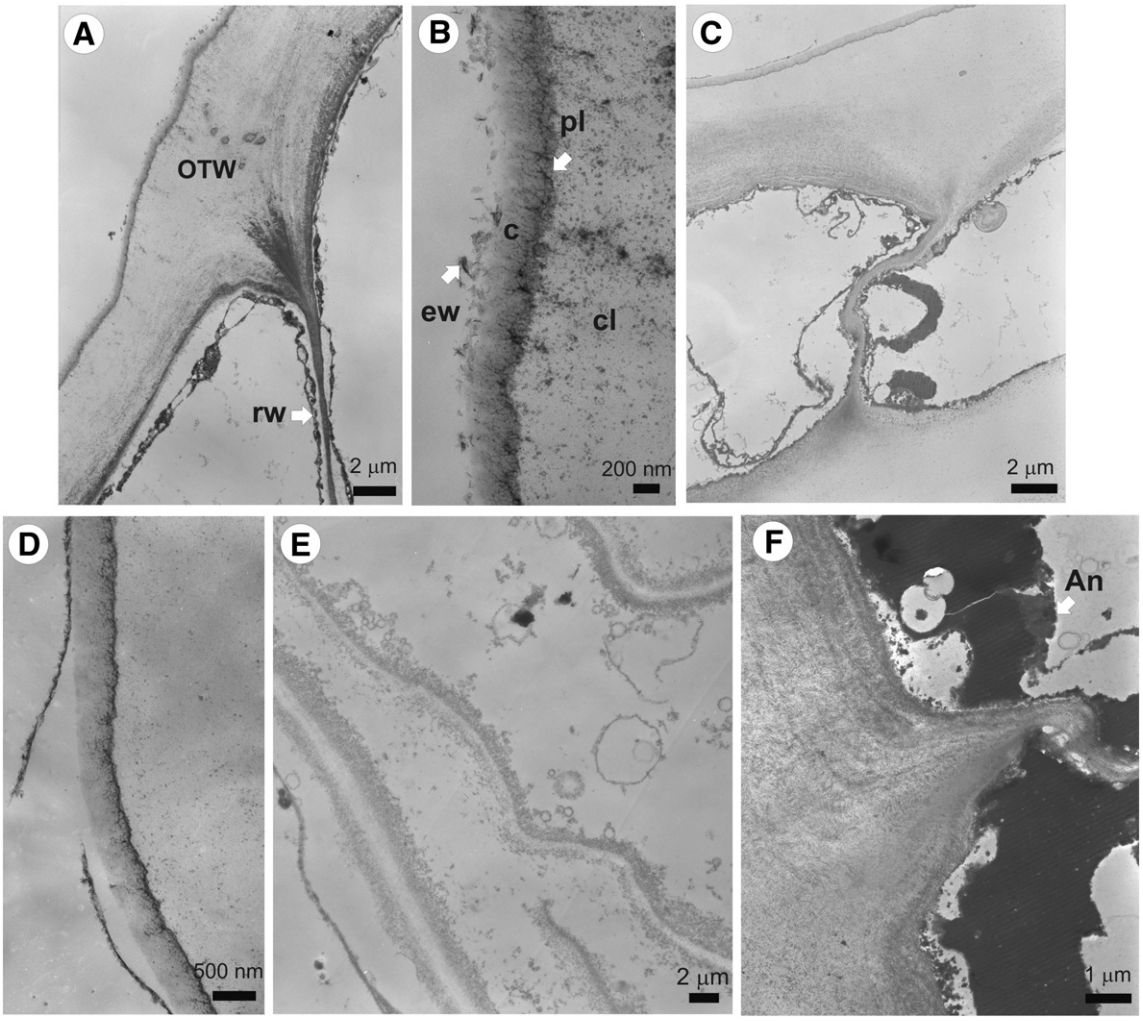

Fig. 5. Transmission electron microscopy (TEM) micrographs from 20 s-PL treated strawberries at 0 and 8 days of storage. A, B, E: control; C, D, F: 20 s-PL. OTW = outer tangential cell wall; rw = radial epidermal cell wall; ew = epicuticular wall layer; c = cuticle; pl = pectic layer; cl = cellulose layer; An = anthocyanin contents.

(Fig. 4 C). Strawberries exposed to 20 s and 40 s-PL exhibited cells clearly affected by plasmolysis with the plasma membrane detached from the wall (Fig. 4 E, G). Unlike control samples, epicuticular waxes were observed as a continuous and well defined layer, in some regions separated from the cuticle, and pectin layer looked electronically dense and sharply delimited (Fig. 5 D). Most radial walls appeared folded and cellulose layer did not present notorious changes compared to non-treated tissues (Fig. 5 C D). Cold storage had a dramatic effect on untreated fruit structure. Control tissues were visualized markedly degraded. Epidermal cells were collapsed and flattened. Subepidermic cells showed broken and collapsed membranes, with formation of vesicles. Cell walls were hardly visualized and showed pronounced folding and disruptions in some areas (Fig. 4 B). TEM images indicated noticeably deteriorated cell walls, with loss of electron density and disruptions (Fig. 5 E). In contrast, stored PL-treated strawberries were visualized structurally better maintained than untreated fruits. Tissues exposed to 10 s-PL showed epidermal cells with slightly folded walls. OTW and ITW appeared stained (Fig. 4 D). Subepidermic cells arrangement was maintained after cold storage but walls showed a moderate staining. Some cells showed broken plasmalema and tonoplast (Fig. 4 D). Structure integrity of tissue exposed 20 s to PL appeared better preserved with respect to the 10 s-PL irradiated tissue one. Epidermal cells were flattened and irregular in shape, with well stained OTW (Fig. 4 F). Cuticle, pectic and cellulose layers of the epidermis were visualized with marked electronic density in TEM observations (micrographs not shown). Radial walls showed pronounced folding, indicating a greater loss of turgor (Fig. 5 F). Curiously, the ITW appeared clearly reinforced, very well defined and thick, with a darker staining than in control tissue at 0 day (Figs. 4 F and 5 F). Anthocyanins were observed in epidermal cells forming large clusters of indefinite shape (Fig. 5 F). Cell walls of subepidermic cells were well stained. Some cells appeared with disrupted membranes (Fig. 4 F). Epidermal cells of 40 s-PL treated tissues showed an appearance similar to that of 10 s-PL exposed fruits (Fig. 4 H), except that ITW were markedly more stained as in tissues irradiated during 20 s (Fig. 4 H). Epicuticular waxes, cuticle, pectic and cellulose layer were observed in TEM with good electronic density (micrographs not shown). LM images of subepidermic cells irradiated for 40 s revealed greater intercellular spaces, a pronounced decrease in cell-to-cell contact and walls much more folded than tissues irradiated for 10 s and 20 s (Fig. 4 H).

3.6. Changes in fruit structure and their role in mechanical properties and water loss

Preserving the structure is a major objective of the postharvest processing of fruits, as changes in structure lead to detrimental changes in texture, flavor, appearance and mechanical and nutritional properties (Aguilera and Stanley, 1999). The relationship between some materials' properties, in particular transport and rheological ones, and microscopic features of plant tissues is well known. Strawberry fruits are not

isotropic materials and their structure varies in the different tissues (the epidermis, the hypodermis, the cortical cells, the vascular bundles and the pith). As seen in Fig. 3, the force–displacement curve at low deformations is determined not only by the mechanical properties of the epidermis, but also by the other tissues. In general, at the cellular and tissue levels, the three major structural factors that contribute to mechanical behavior of plant-based foods are turgor pressure within individual cells (i.e. the force exerted on the cell membrane by intracellular fluid), the cell wall rigidity, and the cell–cell adhesion, determined by the integrity of the middle lamella and the plasmodesmata (Alzamora, Castro, Nieto, Vidales, and Salvatori, 2000; Alzamora, Viollaz, Martínez, Nieto, and Salvatori, 2008; Jackman and Stanley, 1995; Waldron, Smith, Parr, Ng, and Parker, 1997). In the epidermis, cuticle composition and architecture also affect the mechanical behavior of the epidermal cell walls (Domínguez, Cuartero, & Heredia, 2011; Lara, Belge, & Goulao, 2014).

The cuticle is a thin extracellular polymeric membrane, with a layered structure, that covers aerial parts of higher plants such as leaves, fruits, flowers and seeds, protecting themselves from the environment (Domínguez et al., 2011). Plant cuticles are characterized by a pronounced heterogeneity in both chemical compositions as well in fine structure. Cuticle is mainly composed of cutin, a lipophilic polyester polymer, and, in minor proportion, cutan, an aliphatic compound assembled mainly by ether bonds. Amorphous waxes and a minor fraction of phenolic are also embedded in the cutin matrix. The outer side of the cuticle is covered by epicuticular waxes (amorphous and crystalline). On the inner side of the cuticle, cutin is mixed with pectin and glucan polysaccharides from the outer epidermal cell wall, which can be regarded as a cutinized cell wall (Domínguez et al., 2011; Esau, 1977). This cutinized layer is attached to the epidermal cell walls (composed of peptic substances, celluloses and hemicelluloses) by the peptic layer.

In fruits characterized by thick and well developed cuticle, this lipid membrane is a mostly viscoelastic and stress-hardening material that stiffens the more elastic epidermal cell walls, adding mechanical support to the epidermal tissue (Domínguez et al., 2011). Thus, the peel (epidermal cells plus cuticle) is a more rigid structure than the inner tissues. On the contrary, in the case of strawberry fruit, characterized by a weaker cuticle, the mechanical role of subepidermal cells is also significant, as evidenced in Fig. 3.

The close dependency of mechanical properties on fruit structure could be recorded. In the different tissues of stored untreated fruits, cell wall disassembly, reduction of cell-to-cell contact and plasmolysis or rupture of membranes with loss of turgor would be the main processes leading to tissue softening (Figs. 4B, 5E). Some of these observations are in agreement with previous results reported by Rosliet al. (2004), who found that strawberries show a continuous decrease of cell wall content during ripening. They found that softening of Camarosa and Pajaro cultivars could be closely related to pectin solubilization and depolymerization, particularly at the end of ripening.

On the contrary, microscopic observations of stored irradiated tissues demonstrated cell wall strengthening by PL stress, which would be correlated to the corresponding force–displacement curves at the end of storage. Epidermis in treated fruits appeared better maintained than in control one (Fig. 4 B, D, F, H). In spite of turgor loss, the reinforcement of the middle lamella of radial cells and a very dark stained ITW could be observed mainly in 20 s-PL exposed fruits. Walls of subepidermic cells also appeared more stained than in stored untreated fruit. Consequently the epidermis strength of PL irradiated samples was higher than that of the untreated fruit. The W value, i.e. the energy required to break the epidermis, indicated that the epidermis was more prone to rupture in the untreated than in PL irradiated fruits. Turgor pressure leads to rigidity of the cells and tissues, and together with the cell wall, provides mechanical support to maintain the shape of cells and tissues. No turgor could be maintained after storage and consequently, stored treated and untreated tissues showed lower deformability modulus than at day 0, but reinforcement in cell wall structure would account by the observed greater stiffness (>Ed) of stored irradiated tissues.

Cell wall reinforcement is a well-known defense mechanism of plants. To withstand plants attacks, a series of temporally and spatially regulated events are expressed and defense genes are activated, leading to phytoalexin accumulation, pathogenesis related protein synthesis and cell wall strengthening (Brisson, Tenhaken, and Lamb, 1994; Lesniewska, Adrian, Klinguer, and Pugin, 2004; Somssich and Hahlbrock, 1998). These defense mechanisms are inducible by biotic or abiotic (such as metallic salts or UV light) elicitors. There is scarce information about the hormetic effects of PL irradiation. According to our knowledge, this is the first report on the softening delay caused by PL treatment. However, cell wall modifications induced by UV-C are well documented. For instance, Pomboet al. (2009) reported that UV-C radiation delayed strawberry fruit softening due to the decrease of the transcription of a set of genes encoding enzymes and proteins involved in wall degradation, during the first hours after treatment. Lesniewska et al. (2004) characterized wall changes of suspended grapevine cells in response to UV-C stress by atomic force microscopy. After 3 h, the elicited cells displayed sprouted expansions around the cell wall that corresponded to pectin chains resulting in a Young's modulus greater than that of untreated cells.

The cuticle functions as a permanent biological barrier and is a determinant factor of some important traits related to postharvest quality, such as water loss (Lara et al., 2014). Schreiber and Schönherr (2009) proposed that two parallel paths for water exist in the cuticle membrane: water can move either in aqueous pores of the polar polymers or diffuse in cutin, which fills the space between the polar polymers. Additionally, the epicuticular wax layer at the outer surface of cuticle would reduce water permeability of cutin and cover a fraction of aqueous pores, which reduces the amount of water that moves in aqueous pores. Structure images of cuticle and epicuticular waxes would not account for differences in cuticular transpiration among 40 s PL treated samples and the rest of the fruits (treated or untreated). Perhaps the exposure to the greatest PL dose would provoke an important injury to strawberry tissue than generated during exposure for shorter times, which would be translated in an increase in respiration and transpiration rates.

4. Conclusions

Incidence of postharvest molds on strawberry fruits was reduced by over 16–42% with PL application in doses ranging 2.4–47.8 J/cm^2. There were no significant differences in FR, W and Ed values between treated and untreated fruits immediately after treatments. After 8 days storage at 6 °C, untreated strawberries showed a pronounced softening (≈48% and 64% reduction in FR and W respectively), but stored strawberries exposed for 10 s and 40 s to PL presented slight or not significant changes in the mechanical parameters regarding day 0, while FR and W values of 20 s-PL treated tissues were increased by 35% and 88% compared to those values at 0 day storage. The stiffness (E_d) of stored 20 s- 40 s and 10 s PL irradiated tissues was 125% and 75% greater than that stored untreated fruit. Micro and ultrastructure changes evaluated by LM and TEM images demonstrated ITW strengthening and a major integrity of walls of hypodermis cells by PL stress, while cell wall disassembly and reduction of cell-to-cell contact were detected in stored untreated fruit. These structure features could be correlated to the corresponding force–displacement curves at the end of storage and explained the greater F_R, W and E_d values of PL treated fruits. PL technique would be able to simultaneously provide disinfection and delete softening of the tissues without increasing water loss (excepting at the highest PL dose) along cold storage. Present results make this non-thermal, residue-free alternative promising for extending shelf-life of traditional and organic strawberry production.

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