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Impact of combined ascorbic acid/$CaCl_2$, hydrogen peroxide and ultraviolet light treatments on structure, rheological properties and texture of fresh-cut pear (William var.)

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ABSTRACT

This work aimed to evaluate and correlate rheological properties (dynamic oscillatory, creep/recovery and double compression tests), texture (sensory evaluation) and structure (optical and transmission electron microscopy observations) of fresh-cut pear as affected by ascorbic acid/$CaCl_2$ dipping, hydrogen peroxide and short-wave ultraviolet light radiation (UV-C). All pear samples showed a solid behavior ($G' > G''$), but both dynamic moduli decreased in response to the treatments. For treated tissues, the instantaneous elastic (J_0) and the retarded (J_1, J_2) compliances increased, while the steady-state viscosity (η_N) and all mechanical parameters decreased. PLS regression models revealed that texture could be well explained by rheological properties. Deformability modulus (E_d) was positively correlated to sensory fracturability and hardness and negatively correlated to juiciness. J_0, J_1 and J_2 were negatively related to sensory hardness. Compression and creep parameters evidenced changes in structure (mainly rupture of membranes, degradation of middle lamella and cell walls) of surface tissues.

1. Introduction

Development of new technologies to minimize microbial risk while preserving the fresh-like characteristics of fruits is crucial to satisfy the increasing demand for ready-to-eat products (Soliva-Fortuny and Martín-Belloso, 2003). A good procedure to reduce microbial risk involved in the consumption of fresh-cut fruits and/or extend their shelf life includes the reduction or elimination of microbial load by using emerging decontamination techniques, like high hydrostatic pressure, pulsed electric field, pulsed light, ultrasound and short-wave ultraviolet light (Gómez et al., 2011a).

UV-C is a radiation in the range of 200–280 nm which cross DNA pyrimidine bases of cytosine and thymine, impairing formation of hydrogen bonds with the purine base pair on the complimentary strand of DNA and thus reproduction of microorganisms (Bintsis et al., 2000; Shama, 2006). It has also been proved to cause significant damage in the cytoplasmic membrane integrity and in the cellular enzyme activity (Schenk et al., 2011). Hydrogen peroxide (H_2O_2) is a strong oxidizing proposed as an alternative to decontaminate fruits and vegetables due to its low toxicity and safe decomposition products. It is effective against a wide spectrum of bacteria, yeast, molds, viruses and spore-forming organisms (Cords et al., 2005). H_2O_2 has been shown to damage bacterial proteins, DNA and cellular membranes of microbial cells and to remove protein from the coat of the bacterial spores (Juven and Pierson, 1996). H_2O_2 is classified as GRAS to be used in food products as a bleaching, oxidizing and reducing agent and antimicrobial agent (Sapers and Miller, 1998). Combining (UV-C) irradiation and H_2O_2 has been applied successfully in industrial sterilization or disinfection processes and in food preservation. Both processes in combination have been reported to show a synergistic action. Waites et al. (1988) suggested that the mechanism for UV-C–H_2O_2 synergy is mainly related to enhanced production of hydroxyl radicals from H_2O_2 due to irradiation.

During minimal processing, mechanical injury results in cellular delocalization of enzymes and their substrates, leading to biochemical deteriorations such as enzymatic browning, off-flavors and texture breakdown, as well as increased respiration rate and ethylene synthesis. Enzymatic browning, caused mainly by the action of polyphenol axidase (PPO), is a major effector limiting the shelf-life of minimally processed fruits (Lamikanra, 2002). Ascorbic acid as a reducing agent has for long been applied in combination with organic acids or calcium salts to prevent enzymatic browning

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and maintain firmness of fruits (Lamikanra, 2002; Wang et al., 2007). Recently, Gomez et al. (2010) and Schenk (2010) found that the application of an antibrowning pretreatment containing 1% (w/v) ascorbic acid – 0.1% (w/v) calcium chloride helped in maintaining the original color of apple and pear slices after UV-C light exposure respectively.

Effects of UV-C light, alone or combined with other treatments, on quality attributes (texture, flavor, color) and microbial response is quite diverse depending on the type of produce and the dosages applied (Shama, 2006; Charles and Arul, 2007). Gómez et al. (2010) examined the effect of UV-C irradiation at different doses on surface color of apple slices stored in refrigeration for 7 days. They also explored the use of some pretreatments (hot water blanching, dipping in a solution containing ascorbic acid and calcium chloride) to minimize apple browning caused by UV-C light. Color parameters were found to be dependent on UV-C dose, storage time and type of pretreatment. Both pretreatments contributed to maintain the original color of apple slices after UV-C light exposure. Gómez et al. (2011b) studied the effect of UV-C (11.2 kJ/m^2) combined with an antibrowning pretreatment on texture, rheological properties (viscoelastic and mechanical properties) and structure of apple slices during refrigerated storage. Overall, both dynamic moduli decreased, and instantaneous compliance, decay compliances and fluidity significantly increased after treatments and storage at 5 °C. However, texture attributes (hardness, fracturability, juiciness and crispness) of fresh apple and apple dipped into the antibrowning solution and then UV-C irradiated did not show significant differences after 5 days refrigerated storage. Recently, Schenk et al. (2012) demonstrated that immersion of pear slices in 3% v/v H_2O_2 solution (300 s) in combination with UV-C irradiation (3.7 kJ/m^2, 450 s) achieved significant reductions in *Escherichia coli*, *Listeria inocua* and *Zygosaccharomyces bailii* populations (2.4–3.6 log red.). They also examined surface color and texture of pear slices during refrigerated storage (8 days). During storage, processed samples with the combined treatment (H_2O_2/UV-C) turned darker than control samples and this effect was more pronounced in pear discs treated with UV-C treatment alone. The combined treatment kept optimal microbial stability and exhibited a greater value of the CIE L* parameter (lightness) than the UV-C treatment alone. Texture profile analysis conducted using a trained panel showed that H_2O_2/UV-C processed pear discs were perceived with significantly less hardness and fracturability but as juicy as untreated discs.

It is well known that mechanical properties of biologic tissues depend on contributions from the different levels of structure: the molecular level (i.e., the chemicals and the interactions between the constituting polymers), the cellular level (i.e., the architecture of the tissue cells and their interactions) and the organ level (i.e., the arrangement of cells into tissues) (Ilker and Szczesniak, 1990; Jackman and Stanley, 1995; Alzamora et al., 2008). At the cellular level, the three major structural aspects that contribute to textural properties of plant-based foods are turgor (the force exerted on the cell membrane by intracellular fluid), cell wall rigidity, and cell–cell adhesion, determined by the integrity of the middle lamella and the plasmodesmata (Ilker and Szczesniak, 1990; Alzamora et al., 2008). In recent years, several studies have been carried out in fruits and vegetables in an effort to understand the relationships between structure, texture and rheological changes induced by processing (Jack et al., 1995; Alzamora et al., 2008; Gómez et al., 2010, 2011b; Garcia Loredo et al., 2011).

The objectives of the present investigation were: (a) to analyze the rheological properties (derived from dynamic oscillatory, creep/recovery and double compression tests), texture attributes, and structure (by light and transmission electron microscopy observations) of fresh cut pear as affected by ascorbic acid/$CaCl_2$ dipping, hydrogen peroxide and ultraviolet light radiation; (b) to examine the correlations between rheological parameters and texture, using partial least square linear regression; and (c) to explore how differences in pear tissue structure were expressed by viscoelastic, mechanical and sensory parameters. This combined treatment (antibrowning solution/H_2O_2/UV-C) was aimed to reduce the potential contamination of "ready-to-eat" cut pear to be consumed within 1–2 days of refrigerated storage (catering industry, restaurants, schools) (Ahvenainen, 2000).

2. Materials and methods

2.1. Preparation of samples

Ripe pears (*Pyrus communis*, William cv; $a_w = 0.98 \pm 0.03$; 12.7 ± 1.0 °Brix; pH 4.0 ± 0.3) were purchased at a local market and maintained at 4–5 °C until use. Before being processed, whole fruit was washed in water, dipped into sodium hypochlorite solution (100 ppm free chlorine, 3 min) and rinsed in water. All cutting boards, tools and holding vessels were sanitized in the same way before use. Pears were hand peeled and slices of parenchymatous tissue were cut parallel to the axis through the calyx and the stem. The slices were cut out vertically with a cork borer to obtain 0.03 m in diameter and 0.01 m in thickness discs. All slices were taken from the middle part between the center and the surface of the fruit, where there were few vascular bundles. Pears discs were dipped in distilled water (4–5 °C) for 1 min to eliminate cellular fluids, slices were dried in tissue paper and immediately subjected to the different treatments to avoid the loss of moisture.

Ten measurements of the thickness were made at different points with a Teclock dial micrometer model SM-124 (±0.0001 m, Japan). Only slices with a standard deviation of the required thickness lower than 0.005 m were used.

The same lot of fruit was used in all the experiments to minimize the inherent variation due to age and/or cellular structure of the biological tissue, and the influence of agronomic practices and time of harvest in the field.

2.2. UV-C equipment and dosimetry

The UV-C irradiation device consisted of one bank of two reflectors with unfiltered germicidal emitting lamps (maximal emission at 253.7 nm, TUV-15 W G 13 T8 55 V, Philips, Holland) located 0.1 m above the produce tray. The UV-C lamps and the treatment area were enclosed in a wooden box covered with aluminum foil with a cover protection for the operators. A ventilation device was installed in a corner of the box to avoid temperature increase due to UV-C radiation. The average air temperature during the treatments was 27 ± 1 °C. Prior to use, the UV-C lamps were allowed to stabilize by turning them on at least 15 min.

The UV-C intensity emitted from the lamps was determined by using the iodure/iodate chemical actinometer (Rahn, 1997). All reactive employed in UV-C dosimetry were analytical grade from Merck Química Argentina S.A. (Argentina). The test was made by quadruplicate and the mean value was reported. Variations in radiation dose absorption were minimized by placing the samples within a uniform area of the radiation field (between the lamps and equidistant with respect to lamp extremes).

Based on previous study by Schenk et al. (2008), demonstrating the effectiveness of UV-C dose of 3.7 kJ/m^2 to inactivate spoilage microorganisms, this dose was applied to pear discs. Treatment application lasted 450 s.

2.3. Treatments

Pear discs were dipped into an antibrowning solution (DIP) containing 1% (w/v) ascorbic acid (food grade, Química Oeste S.A.,

Argentina) plus 0.1% (w/v) calcium chloride (food grade, Saporiti S.A., Argentina), pH 3.5, for 180 s at 4 °C (Schenk et al., 2011; Ponting et al., 1972). Then they were immersed into 15 ml sterile citric acid-Na_2HPO_4 buffer solution (pH 3.0) containing 3.0% v/v H_2O_2 (P), during 300 s. The reaction with H_2O_2 was stopped by neutralization. The neutralizing solution was obtained from 4% w/v $Na_2S_2O_3{\cdot}5H_2O$ diluted in 0.25 M KH_2PO_4 buffer solution adjusted at pH 7.0 with 1 N NaOH (Raffellini et al., 2008). All reagents were purchased from Anedra S.A. (Bs. As., Argentina).

Pear discs treated with the antibrowning and the H_2O_2 solutions (DIP + P) were exposed to irradiation during 450 s (fluence: 3.7 kJ/m^2) (DIP + P + UV-C) on one side. Single effect of DIP and combined effect of DIP + UV-C were also studied. Treated samples were compared with untreated pear discs (C) held at a temperature similar to the one corresponding to the treated fruit (27 °C ± 1 °C).

2.4. Evaluation of viscoelastic properties

Viscoelastic properties were characterized at 25 °C in a Paar Physica MCR 300 rheometer (Anton Paar GmbH, Graz, Austria) using a 0.030 m diameter parallel plate geometry. The slabs were placed between the lower plate of the rheometer and the measuring plate with rough surface (model PP/30), using only as much compression as necessary to provide maximum contact area and minimum slip (≅1 N). Temperature was controlled by an external liquid bath thermostat model Viscotherm VT2 (Anton Paar GmbH, Graz, Austria). Dynamic oscillatory tests were performed in the controlled strain mode. To ensure that all measurements were carried out within the linear viscoelastic region (LVR), a strain sweep was carried out at an angular frequency of 10 s^{-1}. Thereafter, storage (G') and loss (G'') moduli and loss tangent (tan δ) were measured in the frequency range 0.1–100 s^{-1} using a strain amplitude value of 0.005% (within the limits of linearity previously established). Storage moduli values were fitted using a linear regression of log (G') vs. log (ω):

$$\log(G') = n\log(\omega) + k \tag{1}$$

where n is the slope of the regression and k is G' value at 1 s^{-1} of angular frequency.

Creep-recovery tests were conducted by applying a constant shear stress of 35 Pa during 100 s. A previous stress sweep varying the applied stress from 10 to 50 Pa showed that, in the selected condition, deformation was proportional to the stress applied. After removal of stress, sample recovery was registered for an additional period of 200 s. Each pear sample was subjected to three consecutive creep-recovery assays. The first two trials were conducted in order to remove any surface irregularity in the specimen (Mittal and Mohsenin, 1987). Compliance data from creep experiments were fitted by a mechanical model consisting of a spring connected in series with two Kelvin–Voigt elements (each Kelvin–Voigt element has a spring and a dashpot in parallel) and a dashpot element described by the following equation (Sherman, 1970):

$$J(t,\tau) = (J_0) + \sum_{i=1}^{2}(J_i)(1 - e^{-t/\lambda_i}) + t/\eta_N \tag{2}$$

where $J(t,\tau)$ is the creep compliance ($J = \gamma(t)/\tau$ with $\gamma(t)$ being the strain at the time t and τ the applied constant stress). J_0 is the instantaneous compliance at $t = 0$; J_i are the retarded compliances; λ_i ($J_i = \eta_i \times J_i$) are the retardation times and η_i are the coefficients of viscosity associated with the Kelvin–Voigt elements; η_N is the coefficient of viscosity associated with Newtonian flow and its inverse the steady-state fluidity of the material.

Data were obtained using a minimum of 10 and 15 replicates for dynamic oscillatory and creep-recovery tests respectively.

2.5. Instrumental texture evaluation

An Instron Universal Testing Machine model 3345 (Canton, Massachusetts, USA), with a 5000 N compression load cell interfaced with a series data acquisition software (Bluehill 2, v. 2.17, Instron, USA), was used to conduct the Texture Profile Analysis (TPA) (Bourne, 1978). Two cycles of compression were applied to 70% of deformation. Tests were performed with a crosshead speed of 0.001 m/s and a 0.035 m diameter cylindrical probe. The following parameters were obtained from the force–time curves, according to the definitions of Bourne (1978): fracturability (F), hardness (H) during the first compression cycle, hardness (H_2) during the second compression cycle, area (A_1) under the curve during the first compression, area (A_2) under the curve during the second compression, cohesiveness (Coh), adhesiveness to palate (Adh), springiness (S), gumminess (G), and chewiness (Chew).

The deformability modulus (E_d) was calculated using equations 3–5 (Calzada and Peleg, 1978):

$$E_d = \sigma_R/\varepsilon_R \tag{3}$$

$$\sigma_R = F(t)[(H_0 - \Delta H)/A_0H_0] \tag{4}$$

$$\varepsilon_R = \ln[H_0/(H_0 - \Delta H)] \tag{5}$$

where $F(t)$: compression force at time t; H_0: height of the sample before compression; ΔH: difference of the height of the sample before compression and during compression; A_0: cross-sectional area of the cylinder before compression. For each condition, 20 replicate samples were measured and the correspondent mean value was reported.

2.6. Sensory descriptive analysis

Nine panelists (three males, six females), all between the ages of 21–38, composed the sensory panel. For the selection of panelists, twelve people were recruited from staff from Buenos Aires University based on their interest, availability, previous experience in sensory evaluation and familiarity in texture terminology. Prescreening procedure was done in which the panelists were evaluated for normal sensory acuity through basic taste test, sequential triangle test (Meilgaard et al., 2006) and an intensity ranking test using the hardness scale (Civille and Szczesniak, 1973). The panelists who passed the prescreening tests were trained with the texture profile method following the procedures described by Civille and Szczesniak (1973) during 35–40 h (2 h per week) for them to be able to recognize and measure the most relevant texture attributes (hardness (*SH*), fracturability (*SF*) and juiciness) using the standard rating scales (Hough et al., 1994; Szczesniak and Ilker, 1988).

On each session, three samples were presented to the panelists in white plastic cups coded with random three-digit numbers. Two well-known food references and an evaluation form were also provided with the sample. This form included instructions and the line scale corresponding to the texture attribute with the positions of the references indicated on it. The panelists scored intensities on the line scale (0–17, depending on the texture scale). A glass of water and unsalted crackers were used for rinsing their mouth and cleaning their teeth between sample evaluations. All sessions were carried out in individual booths under white light. The descriptive analysis of the samples was replicated two times in the same session.

2.7. Microscopic observations

For light microscopy (LM), cubes of fresh and treated apples (≅1 mm^3) 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 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 (D'Ambrogio de Argüeso, 1986). 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).

For transmission electron microscopy observations (TEM), samples immersed in Spurr resin were cut in ultrathin sections (1 μm thick) using a glass knife with a Sorvall MT 2-B 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.

2.8. Statistical analysis

Instrumental and sensory data were expressed as mean ± standard deviation of the mean (mean ± SD). The significance among storage modulus curves was analyzed using the methodology reported by Costell and Durán (1978).

Two-way analysis of variance (ANOVA) was done to establish the presence or absence of significant differences among texture parameter values according to the factors "treatment" and "assessor". Significance level was set at $\alpha < 0.05$. When significant differences were found the Turkey's test was performed.

Multivariate analysis of Variance (MANOVA) was used detect differences among samples in rheological data (viscoelastic properties and instrumental texture profile). Significance level was set at $\alpha < 0.05$ when significant differences were found the Hotelling corrected for Bonferroni test was performed. Principal analysis component (PCA) of mean ratings for each attribute was used to illustrate the relationship among variables and samples. These statistical analyses were carried out using Infostat v2009 software (Córdoba, Argentina).

Linear Partial Least Squares Regression Analysis (PLS) was used to analyze the relationships between sensory (Y-block) and rheological properties (X-block) matrices. Both sensory and instrumental variables were standardized previously to the PLS analysis. The GenStat statistical language (GenStat discovery edition 3, Oxford, UK) was used for these analyses.

3. Results and discussion

3.1. Dynamic moduli

Linear viscoelastic limits ranged between 0.001% and 0.008% for fresh fruit and between 0.001% and 0.02% for treated samples (data are not shown). Accordingly, a strain amplitude value equal to 0.005% was selected for frequency sweep test for all assayed samples to ensure linearity. The average mechanical spectra are presented in Fig. 1. All pear samples had predominant solid behavior, with G' exceeding G'' over the entire frequency range. UV-C treatments combined with antibrowning pretreatment (DIP + UV-C and DIP + P + UV-C) decreased G' and G'' moduli in pear tissues. G' showed a linear dependence with the angular frequency. The lineal regression slopes were slightly greater for treated samples (n values were 0.050 ± 0.004; 0.06 ± 0.01; 0.07 ± 0.01 and 0.06 ± 0.01 for C, DIP, DIP + UV-C, and DIP + P + UV-C samples, respectively). Statistical analysis of storage modulus curves showed significant differences among control and treated samples; and among treated samples. The dependence of G'' with frequency was more complex.

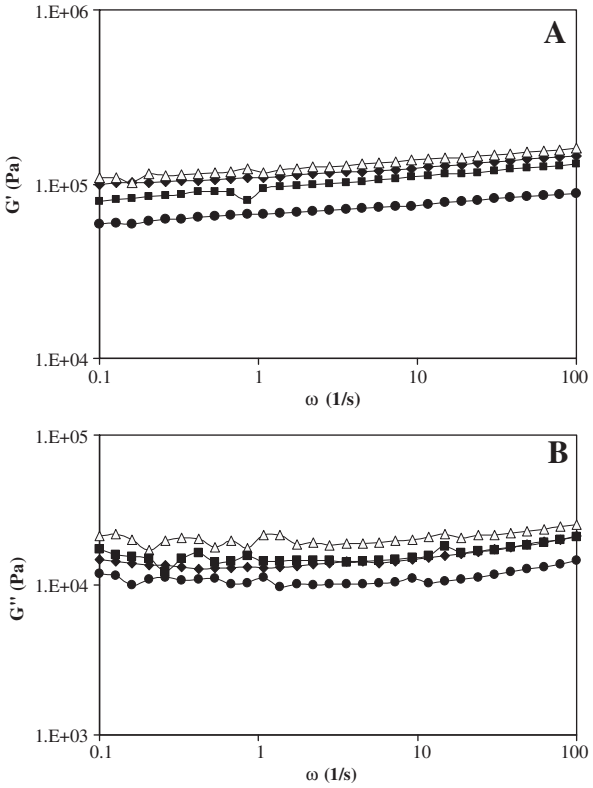

Fig. 1. Mechanical spectrum for fresh and treated pear discs. (A) Storage modulus G'; (B) loss modulus G''. (♦) Fresh, (Δ) dipped into the antibrowning solution (DIP), (■) dipped into the antibrowning solution and UV-C irradiated (DIP + UV-C), (●) dipped into the antibrowning solution, treated with hydrogen peroxide and UV-C irradiated (DIP + P + UV-C).

The values of tan δ for the different treatments ($G''/G' = 0.15–0.23$) were slightly higher than those related to control samples ($G''/G' = 0.12–0.15$), indicating a small decrease of the solid component compared to the viscous component.

Different dynamic mechanical spectra of raw and treated tissues revealed a change in tissue microstructure. Decreasing levels of elastic modulus and increasing values of the slope n might be correlated to loss of rigidity.

Dynamic spectra followed patterns in agreement with those previously reported for osmotically dehydrated apple (Garcia Loredo et al., 2011), melon (Martínez et al., 2005) and pear (Wu and Guo, 2010).

3.2. Creep-recovery behavior

Average creep/recovery curves for fresh and treated samples are presented in Fig. 2. Treated pear tissues showed greater deformations than control (raw tissue). Creep response of all samples was well characterized (correlation coefficient >0.999) by the mathematical model represented by Eq. (2) and the corresponding parameters are supplied in Table 1. According to interpretation of Sherman (1970), J_0 would be related to those bonds of structural units that are elastically stretched when the stress is applied, and show instantaneous and complete recovery when the stress is removed. J_i parameters would be related to bonds that break and reform at different rates, weaker bonds breaking at smaller values of time than the stronger ones. As these bonds completely recovered after a certain time, they show retarded elastic recovery. The linear region of Newtonian compliance t/η_N would be related to those bonds that are ruptured during the shear creep step. The time required for them to reform is longer than the creep-recovery

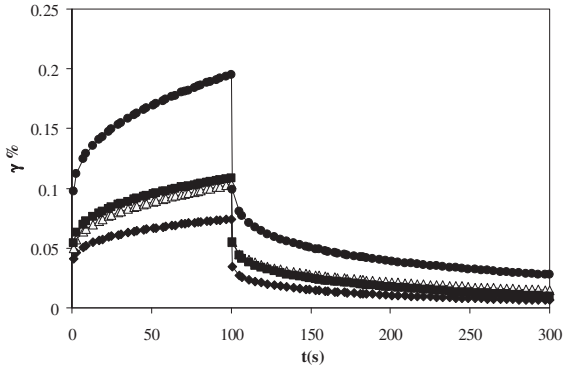

Fig. 2. Average experimental creep/recovery curves (deformation (γ%) vs time (t)) for fresh and treated pear discs. (♦) Fresh, (Δ) dipped into the antibrowning solution (DIP), (■) dipped into the antibrowning solution and UV-C irradiated (DIP + UV-C), (●) dipped into the antibrowning solution, treated with hydrogen peroxide and UV-C irradiated (DIP + P + UV-C).

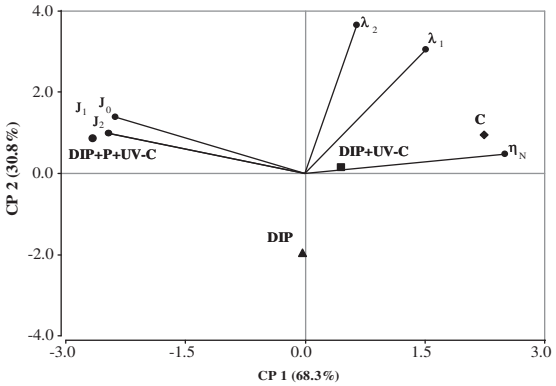

Fig. 3. Principal component analysis bi-plot of creep curve parameters used to differentiate fresh and treated pear tissues by different parameters. (♦) Fresh, (▲) dipped into the antibrowning solution (DIP), (■) dipped into the antibrowning solution and UV-C irradiated (DIP + UV-C), (●) dipped into the antibrowning solution, treated with hydrogen peroxide and UV-C irradiated (DIP + P + UV-C).

period; so the released units will flow and part of the structure will not be recovered. The relatively large variability associated with creep parameters (standard deviations) observed in Table 1 are common when evaluating viscoelastic properties of plant tissues and can be attributed to many factors, such as inhomogeneity of tissues, age, sample location within the fruit, differences in intercellular space interconnectivity and cell size, etc. (Mittal and Mohsenin, 1987; Pitt, 1992). Creep parameters showed significant differences ($p < 0.0001$) among fresh and treated pears and also among treated pears.

Compliances (instantaneous elastic (J_0), retarded (J_1 and J_2) and steady-state viscous ($1/\eta_N$)) of treated tissues increased in value respect to control, revealing a decrease in their respective modules ($E_i = 1/J_i$), while retardation times (λ_1 and λ_2) slightly decreased. Use of DIP and/or hydrogen peroxide combined with UV-C provoked loss of elasticity and rigidity, and an increase in fluidity of pear tissue. On the contrary, Gómez et al. (2011b) did not find significant differences between creep parameters of fresh apple tissue and that pretreated with DIP and then UV-C irradiated (DIP + UV-C).

PCA showed the spatial relationships of the six creep parameters for each pear sample (Fig. 3). The first two principal components explained 68.3% and 30.8% of the variance, respectively. The first component contrasted positively J_0, J_1 and J_2 compliances and negatively η_N parameter. The second axis was positively defined by λ_1 and λ_2. Fresh pear, placed to the right of the graph, showed high values of η_N and λ_1. DIP + P + UV-C pear sample, placed to the left of the graph, were mainly characterized by an increase in J_0, J_1, and J_2 and a decrease in η_N. On the other hand, DIP and DIP + UV-C pear samples, located in the middle of the graph, exhibited compliances values greater than those corresponding to control but lower than those corresponding to hydrogen peroxide treated sample (DIP + P + UV-C).

The average increase in the overall compliance at the end of the creep phase of treated samples as compared to fresh pear tissue was 147% (DIP), 138% (DIP + UV-C) and 260% (DIP + P + UV-C). For all pear samples, the relative contribution of each type of compliance was in the range 40–51% for the instantaneous elastic; 20–30% for the slow-rate viscoelastic; 13–15% for the fast-rate - viscoelastic and 15–22% for the steady-state viscous compliance (Table 1). All tissues exhibited plastic strain that remained unrecovered at the end of the creep/recovery test. Plasticity values (the ratio of unrecoverable or permanent deformation, t/η_N, to the total deformation at the end of the creep phase, J (100 s,τ)) of DIP + P + UV-C pears were slightly greater than those of the other samples.

3.3. Texture Profile Analysis

Typical compression-time curves for fresh and treated tissues are displayed in Fig. 4. Compression curves of all pear samples exhibited the typical shape of hard materials, with abrupt rupture peaks. UV-C combined with the antibrowning solution and/or hydrogen peroxide treatments (DIP + UV-C, P + UV-C; DIP + P + UV-C) provoked a decrease in the maximum peak. Table 2 shows the means and the standard deviations corresponding to the mechanical parameters of evaluated samples. Significant differences ($p < 0.0001$) were observed among fresh and treated pear samples. There were no significant differences between DIP + UV-C and DIP + P + UV-C samples. All mechanical parameters (fracturability, hardness, hardness 2, area 1, area 2, springiness, cohesiveness, gumminess, chewiness and deformability modulus) decreased for all treated tissues regarding control sample.

A two-dimensional representation of PCA is presented in Fig. 5. The first two principal components explained 89.5% and 10.2% of the variance, respectively. The first contrasted positively the following parameters: fracturability, hardness, hardness 2, area 1, area 2, springiness, chewiness, modulus of deformability and gumminess. The second axis was positively defined by cohesiveness.

Table 1
Means of viscoelastic parameters derived by fitting Eq. (2) to compliance curves from creep phase for fresh (C), DIP, DIP + UV-C and DIP + P + UV-C pears.

Treatment	J_0 (1/Pa) ($\times 10^5$)	J_1 (1/Pa) ($\times 10^5$)	J_2 (1/Pa) ($\times 10^5$)	λ_1 (s)	λ_2 (s)	η_N (Pa.s) ($\times 10^{-7}$)
C	1.1 ± 0.2 50.54**	0.46 ± 0.15 20.46**	0.29 ± 0.06 13**	26.3 ± 6.1	2.71 ± 0.56	2.77 ± 0.59 a 15.97**
DIP	1.3 ± 0.4 40.01**	0.59 ± 0.18 29.46**	0.39 ± 0.13 12.2**	22.2 ± 5.4	2.28 ± 0.67	1.68 ± 0.97 b 18.27**
DIP + UV-C	1.5 ± 0.5 47.65**	0.67 ± 0.26 22.23**	0.44 ± 0.18 14.6**	24.9 ± 5.7	2.51 ± 0.48	2.27 ± 1.84 c 15.5**
DIP + P + UV-C	2.4 ± 0.5 45.46**	1.1 ± 0.27 19.03**	0.7 ± 0.2 13.4**	23.8 ± 2.7	2.57 ± 0.27	0.85 ± 0.19 d 22.04**

Post-hoc multiple comparisons using Hotelling tests based on Bonferroni correction $\alpha = 0.05$.
Different letters indicate significant differences at $p \leqslant 0.05$ between treatments.
** % contribution to the overall compliance at the end of the creep phase.

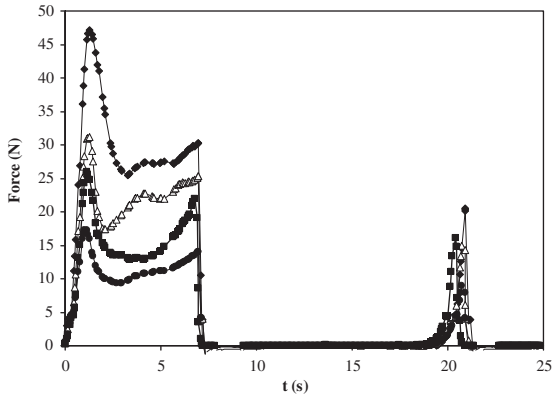

Fig. 4. Typical force/time curves for fresh and treated pear discs. (♦) Fresh, (Δ) dipped into the antibrowning solution (DIP), (■) dipped into the antibrowning solution and UV-C irradiated (DIP + UV-C), (●) dipped into the antibrowning solution, treated with hydrogen peroxide and UV-C irradiated (DIP + P + UV-C).

The location of treated samples in the graph showed a decrease in all mechanical parameters because of the treatments.

3.4. Sensory texture attributes

Means and their standard deviations for to the evaluated attributes in fresh and treated pear samples are presented in Table 3. Although the panel discerned differences in sensory hardness, fracturability and juiciness among all pear samples assayed, these differences were not statistically significant ($F_{3,15} = 2.35$, $p = 0.1$; $F_{3,15} = 0.48$, $p = 0.78$; and $F_{3,18} = 2.83$, $p = 0.053$, respectively). As far as hardness and juiciness were concerned, the evaluations of panel were consistent and homogeneous ($F_{5,15} = 2.58$, $F_{5,15} = 2.02$, respectively). In regard to fracturability attribute, ''assessor'' factor was significant ($F_{6,18} = 63.1$, $p < 0.05$), suggesting that panel performance was not consistent in their evaluation. However, all panelists scored the treated pears as less fracturable than control samples. Thybo and Martens (1998) and Garcia Loredo et al. (2011) also reported that trained assessors had difficulty detecting differences by texture profile method in oral fracturability of cooked potatoes and osmotically dehydrated apples, respectively.

Similar results on the sensory impact of UV-C radiation on fruits were also observed by other authors. Manzocco et al. (2011b) did not detected significant differences in hardness and juiciness between fresh and UV-C irradiated watermelon. Gómez et al. (2011b) studied the effect of UV-C irradiation combined with an antibrowning pretreatment in texture (hardness, fracturability, juiciness and crispness) of cut apple refrigerated 5 days and found no significant differences with respect to raw tissue in all evaluated attributes.

Sensory (*y*-variables) and instrumental measurements (*x*-variables) were correlated by PLS regression analysis. About 64.1% of the variability of sensory attributes could be explained by TPA mechanical parameters using a PLS regression model with 6 PLS-factors. Approximately 15% of the variability was explained by

Table 3
Mean values and standard deviation of texture parameters for fresh and treated pear tissues evaluated by the panelists.

Treatment	*SH*	*SF*	Juiciness
C	4.83 ± 0.72[a]	4.41 ± 2.01[a]	4.92 ± 1.01[a]
DIP	4.55 ± 1.12[a]	3.89 ± 1.75[a]	4.99 ± 0.74[a]
DIP + UV-C	4.31 ± 0.65[a]	3.91 ± 2.19[a]	5.33 ± 1.4[a]
DIP + P + UV-C	4.18 ± 0.65[a]	3.7 ± 2.28[a]	5.23 ± 0.61[a]

Means in same column with the same letter are not significantly different ($p < 0.05$).

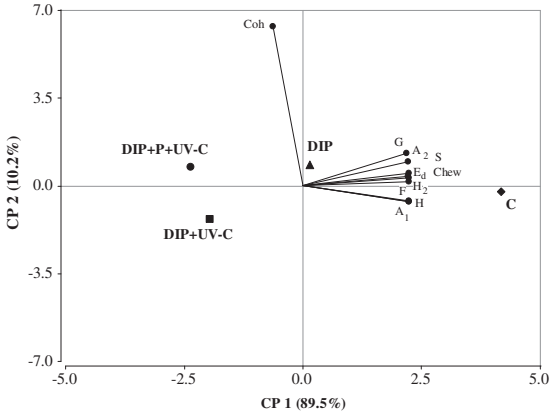

Fig. 5. Principal component analysis bi-plot of mechanical parameters used d to differentiate fresh and treated pear tissues by different texture attributes. (♦) Fresh, (▲) dipped into the antibrowning solution (DIP), (■) dipped into the antibrowning solution and UV-C irradiated (DIP + UV-C), (●) dipped into the antibrowning solution, treated with hydrogen peroxide and UV-C irradiated (DIP + P + UV-C).

PLS-factor 1, 17% by PLS-factor 2, 8.9% by PLS-factor 3, 6.9% by PLS-factor 4, 4.5% by PLS-factor 5 and 11.4% by PLS-factor 6. An inclusion of more than six PLS-factors did not improve the prediction (data not shown), and therefore, the remaining PLS-factors were omitted from the analysis. According to this model, sensory hardness was the best explained attribute (approximately 21.8% of the variance explained), while juiciness and fracturability were scarcely explained (approximately 5.5% and 7.5% of the variance explained, respectively). The position of hardness, fracturability, area 2, gumminess and chewiness on the right side of the plot along PLS-factor 1 (Fig. 6) indicated that these parameters were positively related to sensory fracturability and negatively correlated to juiciness. Along the PLS-factor 2, sensory hardness was positively correlated to modulus of deformability (Fig. 6).

Seventy-nine percent of the variance in sensory attributes was explained by creep parameters through a PLS model with five PLS factors (20.7, 17.5, 6.5, 8 and 26.2%, respectively). Juiciness was the best explained sensory property (approximately 13.1%), while sensory hardness and fracturability were less explained (approximately 6.7% and 1.9% of the variance explained, respectively). Fig. 7 shows that juiciness was positively correlated to the steady-state viscosity (η_N) along PLS-factor 1. Sensory hardness was negatively correlated to instantaneous and retarded compliances

Table 2
Mean values and standard deviation of TPA parameters and deformability modulus for fresh (C), DIP, DIP + UV-C and DIP + P + UV-C pears.

Treatment	*F*(N)	*H* (N)	H_2 (N)	A_1 (J)	A_2 (J)	*S* (-)	Coh (-)	*G* (N)	Chew (N)	E_d (N/mm^2)
C	39.5 ± 8.1	39.5 ± 8.1	15.2 ± 3.9	0.14 ± 0.03	0.011 ± 0.002	0.22 ± 0.04	0.04 ± 0.01	1.5 ± 0.5	0.35 ± 0.18	0.55 a ± 0.12
DIP	24.1 ± 8.4	24.1 ± 8.4	11.6 ± 3.6	0.09 ± 0.03	0.004 ± 0.001	0.18 ± 0.03	0.04 ± 0.01	1.04 ± 0.39	0.2 ± 0.1	0.35 b ± 0.15
DIP + UV-C	19.2 ± 5.9	19.2 ± 5.10	9.2 ± 3.2	0.08 ± 0.02	0.003 ± 0.001	0.16 ± 0.03	0.04 ± 0.02	0.71 ± 0.36	0.12 ± 0.08	0.18 c ± 0.07
DIP + P + UV-C	16.29 ± 3.97	16.29 ± 3.98	8.65 ± 1.73	0.07 ± 0.01	0.0031 ± 0.0005	0.17 ± 0.03	0.04 ± 0.03	0.72 ± 0.15	0.12 ± 0.04	0.15 c ± 0.03

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

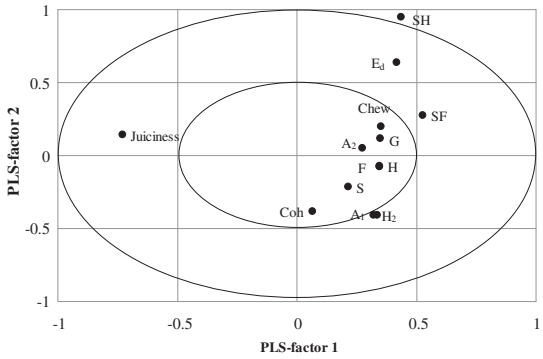

Fig. 6. Plot of X-loadings and Y-loadings for mechanical parameters of fresh and treated pear tissues.

(J_0, J_1 and J_2) and positively related to the first retarded time (λ_1) along PLS-factor 2.

Large-strain testing refers to deforming a sample to the point of permanent structure changes, and its information had been traditionally used to correlate with sensory evaluation since in the mouth irreversible deformations take place (Tabilo-Munizaga and Barbosa-Cánovas, 2005). Instead, small-strain testing in the linear viscoelastic region involves probing the structure of the sample in a non-destructive manner and is widely used to obtain information about internal structure of the system. However, the most noteworthy observation from present results is that a good degree of correlation was found between creep parameters and juiciness and sensory hardness. This fact is in agreement with our previous results (Gómez et al., 2012), in which a high degree of correlation between crispness and juiciness with creep and dynamic parameters in cut apple were assessed.

3.5. Structure features

Typical LM and TEM images of pear tissue localized at the pear disc surface are illustrated in Figs. 8 and 9, respectively. LM microphotographs of fresh tissue showed rounded parenchyma cells with small intercellular spaces formed by the conjunction of three or four cells. Turgor pressure forced the plasmalemma tightly against the cell wall. The large amount of cell volume was occupied by the central vacuole, and the protoplasm, bounded by the plasmalemma and the tonoplast, was present as a thin layer lining the cell surface (Figs. 8A and B; 9A and B). In TEM, fresh tissue exhibited electronic dense cell walls (Fig. 9A and B). In many micrographs, greater electronic intensity was visualized toward the margin and in the central zone corresponding to the middle lamella (Fig. 9B). Tonoplast and plasmalemma appeared intact.

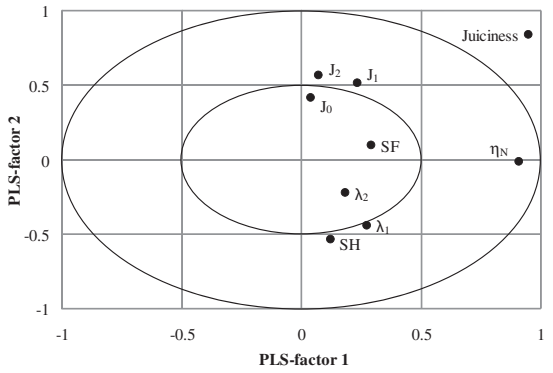

Fig. 7. Plot of X-loadings and Y-loadings for creep parameters of fresh and treated pear tissues.

Cell of pear tissues dipped into the ascorbic acid/calcium chloride solution became slightly less rounded in shape (Fig. 8C and D). Fibrillar organization was modified (Fig. 9D). In some areas, the middle lamella appeared clearly reinforced (Fig. 9C). DIP + UV-C treatment provoked rupture of cell membranes (plasmalemma and tonoplast) and consequently, loss of turgor and severe folding of cell walls (Fig. 8E and F; Fig. 9E and F). Cell walls appeared with very low electron density (Fig. 9E and F). In some cells, the middle lamella could be visualized (Fig. 9F). The UV-C induced breakage of cell membranes was also reported by Gómez et al. (2011b) and Manzocco et al. (2011a) in parenchyma of cut apple tissue. In addition, Charles et al. (2008) studied the effect of a hormic UV-C dose (3.7 kJ/m^2) on structure of tomato fruit during storage. They reported that in the epicarp cells, after 6 h of treatment, the cytoplasm retracted from the cell wall, and a rupture of plasma membrane was observed in some places. Wall structure appeared modified with a more wavy aspect.

As seen in LM, cell walls of DIP + P + UV-C sample showed low staining density. Cells looked slightly more rounded in shape than those of DIP + UV-C pear tissues (Fig. 8G and H). Overall, cell arrangement clearly showed cells which were apart from each other, with reduced intercellular contacts. This fact would indicate an effect of hydrogen peroxide on pectins of the middle lamella, altering the connection between adjacent cells. In TEM micrographs, walls with very much reduced electron density, alteration of fibrillar pattern, and degradation of the middle lamella, presence of vesicles in the cell lumen and slightly plasmolyzed and fragmented cytoplasm were observed (Fig. 9G and H).

From microscopic studies it is evident that DIP + UV-C and DIP + P + UV-C treatments provoked alterations in the network of biopolymers of cell walls (cellulose, hemicelluloses and pectins) joined with chemical bonds of variable strength, these modifications being much greater in tissues undergone hydrogen peroxide treatment.

3.6. Relationship among structure, rheological properties and texture perception

Changes in pear tissue structure after treatments would mainly occur in surface of irradiated samples due to the low penetration of UV-C irradiation. In spite of this modification in structure was only at surface level, assayed rheological tests (instrumental texture profile analysis, oscillatory and creep-recovery tests) were sensitive distinguishing differences of small magnitude in structure among fresh and UV-C irradiated pear samples (with antibrowning pretreatment and with or without hydrogen peroxide treatment). On the other hand, no significant changes (although discernable) in texture attributes (hardness, fracturability and juiciness) were detected among fresh and treated tissues.

Rheological parameters, and so texture perception, have been demonstrated to be associated with some structural components of the fruit tissue, reflecting changes that occur at cellular level (Jackman and Stanley, 1995; Garcia Loredo et al., 2011; Alzamora et al., 2008). The elastic response of plant tissues has been attributed to: 1) cellulose, the main component of the cell wall, which provides individual cells with rigidity and resistance to rupture (John and Dey, 1986; Pitt, 1992); 2) the occluded air in the porous matrix; and 3) the turgor pressure (Alzamora et al., 2008). These structure elements would mainly influence the values of the storage modulus (G') of dynamic spectra, and the modulus of deformability (E_d) determined in TPA test. On the other hand, Jackman and Stanley (1995) proposed an interpretation of a 6 element creep mechanical model to analyze the multiple softening mechanisms in tomato pericarp tissue during ripening. This interpretation has been successfully used to explain osmotically dehydrated melon and apple creep behavior and apple affected by UV-C and pulsed

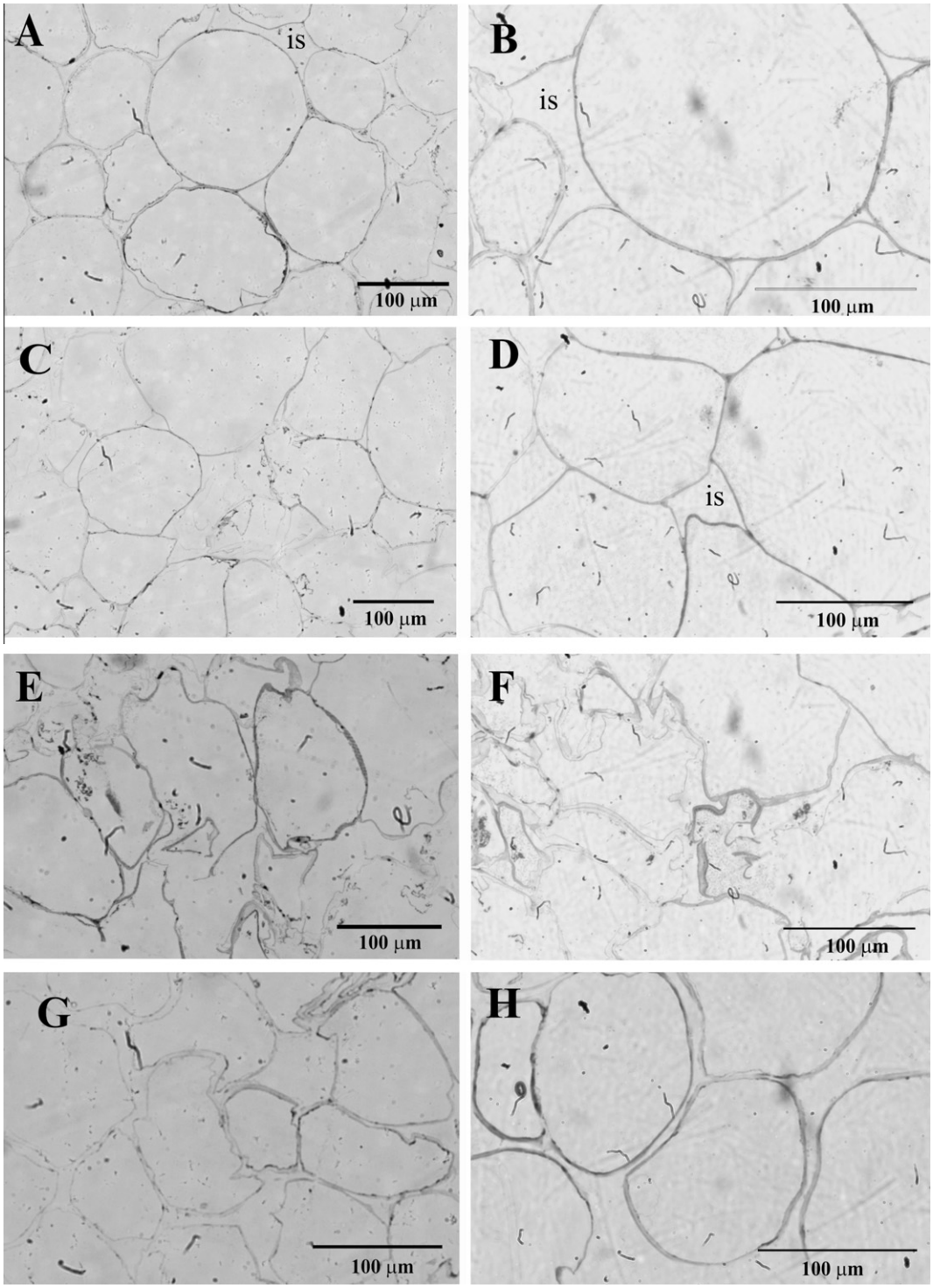

Fig. 8. LM images from fresh and treated pear surface tissues. (A and B): Raw (control), (C and D): dipped into the antibrowning solution, (E and F): dipped into the antibrowning solution and UV-C irradiated, (G and H): dipped into the antibrowning solution, treated with hydrogen peroxide and UV-C irradiated. is: intercellular space.

light (Garcia Loredo et al., 2011; Martínez et al., 2005; Gómez et al., 2011b; Gómez et al., 2012). Instantaneous elastic compliance J_0 would be related to the combination of turgor and primary cell wall strength as dictated by cellulose. Viscoelastic compliances J_1 and J_2 could be attributed to time – dependent changes in pectins and hemicelluloses, respectively. Steady state viscosity could be related to cell wall fluidity arising from exosmosis and/or solubilization and degradation of polymers and less water binding capacity due to treatments.

In general, UV-C radiation (with or without hydrogen peroxide treatment) provoked a decrease in G' and G'' moduli and an increase in J_0 regarding fresh pear tissue. These changes, associated with loss of turgor by disruption of cellular membranes and alteration of fibrillar cellulose pattern, were more pronounced in tissues treated with hydrogen peroxide accordingly with the greater severity of the changes in these structure elements.

Folding and collapse of cell walls, modification of fibrillar pattern and degradation of middle lamella (with loss of the adhesion

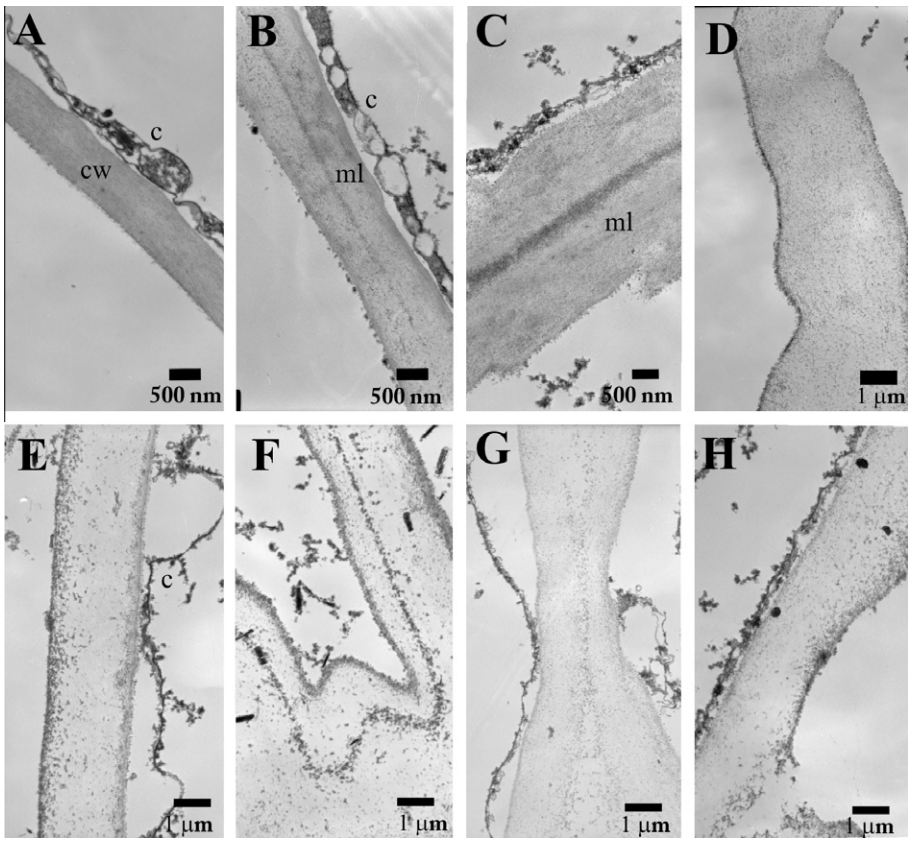

Fig. 9. TEM micrographs from fresh and treated pear surface tissues. (A and B): Raw (control), (C and D): dipped into the antibrowning solution, (E and F): dipped into the antibrowning solution and UV-C irradiated, (G and H): dipped into the antibrowning solution, treated with hydrogen peroxide and UV-C irradiated. cw: cell wall; c: cytoplasm; ml: middle lamella.

cell-to-cell in hydrogen peroxide treated tissue), would expressed in an increase in J_1 and J_2. Again, this increase was more abrupt in the case of tissue treated with hydrogen peroxide, accordingly with the greater damage in wall structure observed in MET (Fig. 9G and H).

Regarding η_N response, although this creep parameter is usually affected by great standard errors due to biological variability (Alzamora et al., 2008), it can be observed that there was a decrease in η_N values of irradiated samples (with DIP and with or without P) indicating an increase in fluidity of the cell wall matrix. This could be attributed to the greater amount of apoplastic water due to membrane breakage and to solubilization and degradation of pectins and other wall biopolymers.

Rupture of membranes with consequent loss of turgor, degradation of cell walls and cell–cell adhesion observed in MO and TEM micrographs (Figs. 8 and 9) of treated pear tissues would be related to the mechanical parameters decrease.

4. Conclusions

Changes in surface tissue structure and in TPA, dynamic and creep behavior were in general evidenced due to DIP and UV-C treatments (DIP + UV-C; DIP + P + UV-C). Sensory differences detected in hardness, fracturability and juiciness were not significant, though discernable. PLS analysis founded clear correlations between rheological properties (creep and mechanical parameters) and sensory attributes. Sensory hardness was negatively related to instantaneous (J_0) and retarded (J_1, J_2) elastic compliances and positively correlated to the modulus of deformability.

Rheological behavior could be partially ascribed to observed changes in structure at the surface level, i.e. loss of turgor ($<E_d$ and G'; $>J_0$) and alteration and degradation of cell wall and middle lamella (>juiciness, $<E_d$ and $>J_1$, J_2 and $1/\eta_N$).

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