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ORIGINAL PAPER

Potential of UV-C Light for Preservation of Cut Apples Fortified with Calcium: Assessment of Optical and Rheological Properties and Native Flora Dynamics

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Abstract The use of UV-C light (fluence 11.2 kJ/m^2) to preserve calcium fortified cut apples was investigated. Impregnation treatments with calcium salts at atmospheric pressure (AI) (with and without previous blanching (B)) were performed to incorporate calcium into the apple matrix. An antibrowning dipping (AD) (ascorbic acid/calcium chloride solution) was also applied to non-blanched fortified apples. The impact of treatments on surface color, viscoelastic properties, microstructure, and native flora during refrigerated storage was analyzed. UV-C light was useful not only reducing microbial load of calcium-enriched apples (between 1.3 log cycles to non-detectable levels) but decreasing microorganisms' growth (between 0.7 to 2.6 log cycles as compared with apples without UV-C exposure) during 7-day storage at 5 °C. AI caused marked browning, and not significant additional color changes due to UV-C exposure were observed. Blanching not only facilitated calcium incorporation (four times higher than in non-blanched tissues) inside the fruit but also helped in reducing browning. AD was also effective to minimize color changes but diminished the UV-C germicidal effect. AD + AI and B + AI treatments negatively affected viscoelastic properties, being the changes more pronounced in heated tissues. Both storage and loss moduli were reduced due to processing and during storage of samples, indicating that fortified apple tissues became less elastic and less viscous. However, exposure of AD + AI and B + AI apples to UV-C did not modify creep response neither at 0 day nor at day 7 and also had a negligible effect on dynamic spectra. Modifications in rheological properties and color were partially ascribed to microstructure features (breakage of cellular membranes with loss of functional cell compartmentalization and loss of turgor; modifications in cell walls). These findings suggest that UV-C irradiation could be used for prolonging shelf life of calcium fortified cut apples with minimal or negligible impact on color and viscoelastic properties.

Keywords Apple · Calcium impregnation · UV-C light · Color · Viscoelastic properties · Native flora

Introduction

Calcium intake is recognized to be essential for bone health as well as for lowering the risk of a host of other disorders such as hypertension, colon cancer, and obesity (Rafferty et al. 2007; Theobald 2005). Despite of awareness of the importance of calcium to health, deficiency of this nutrient has been demonstrated to occur in large segments of populations in developed and developing nations (Ervin et al. 2004; Pacín et al. 1999; Zhai et al. 2006). This suboptimal calcium consumption through different life stages would led to negative calcium balance that could be exacerbated by high dietary intake levels of protein, sodium, fiber, and phosphate, which may decrease the bioavailability of this nutrient (Gueguen and Pointillart 2000; Knox et al. 1991).

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Food fortification with nutrients is a good strategy for dealing with widespread nutrient deficiencies, as it has the best chance of reaching the population segments most at risk, as contrasted with the attempts at changing individuals' food choices or relaying on voluntary supplement taking (Rafferty et al. 2007). Previous studies have demonstrated the feasibility of fruit and vegetable tissues to incorporate minerals by vacuum and atmospheric impregnation techniques (Alzamora et al. 2005). In particular, calcium fortification has been assessed in different matrices such as apple (Anino et al. 2006; Joshi et al. 2010; Park et al. 2005; Salvatori et al. 2007), mushroom (Gras et al. 2003; Ortiz et al. 2003), melon (Tapia et al. 2003), mango (Ostos et al. 2012), and carrot and eggplant (Gras et al. 2003). During impregnation processes conducted at atmospheric pressure, plant cellular structure acts as a semi-permeable membrane, and the component is transferred from the concentrated solution to the cell by a process mainly considered as diffusion driven. When impregnation treatments are combined with previous blanching, profound structure alterations may occur (swelling of cell walls, disruption of membranes), which affect mass transport phenomena, increasing calcium uptake inside the tissues (Ortiz et al. 2003; Salvatori et al. 2007).

Most of the reported literature examined the impact of calcium impregnation treatments immediately after processing, but there are scarce studies in which preservation methods and shelf life of the fortified fruit and vegetable products were evaluated. To extend the shelf life of these products, in addition to storage in refrigeration, the application of other antimicrobial stressor(s) that ensure microbiological safety during storage without detriment of quality attributes would be necessary. Shortwave ultraviolet light (UV-C) radiation is an alternative method to reduce the number of microorganisms on the surface of fresh and cut fruits and vegetables (Allende and Artes 2003; Bintsis et al. 2000; Fonseca and Rushing 2006; Rodoni et al. 2012; Schenk et al. 2008; Shama 2006). The maximum lethal effect of UV-C light is in the range of 250–260 nm. The most severe cell damage occurs at the nucleic acid level, crossing DNA pyrimidine bases of cytosine and thymine to form cross-links and impairing formation of hydrogen bonds with a purine base pair on the complementary strand of DNA. Cellular death occurs after the threshold of cross-linked DNA molecules is exceeded (Guerrero-Beltrán and Barbosa-Cánovas 2004; Shama 2006). UV-C light also been proved to cause damage in the cytoplasmic membrane integrity and in the cellular enzyme activity (Schenk et al. 2011). The inactivation level depends on the specie, density of microorganisms, food characteristics, and UV-C dose (Guerrero-Beltrán and Barbosa-Cánovas 2004). Its use has been approved by the Food and Drug Administration for decontamination of foods and food surfaces (USDA-FDA 2002).

The application of high UV-C doses can result in a negative impact on food quality characteristics. In fresh-cut fruits and vegetables, the main quality depletion observed due to prolonged UV-C light exposure was a marked increase in surface browning (Erkan et al. 2001; Gómez et al. 2010; Manzocco et al. 2011).

In previous studies, we evaluated the potential of UV-C light to preserve fresh-cut apples (Gómez et al. 2010). We concluded that (1) the use of antibrowning agents prior to UV-C irradiation can minimize the undesirable changes in color; (2) survival patterns of inoculated microorganisms and native flora were influenced by the UV-C dose, the type of microorganism, and the apple pretreatment; (3) structure and optical and rheological changes occurred mainly during storage and not immediately after treatments; (4) changes on rheological and color characteristics depended on pretreatments/treatments and storage and were correlated with structure features. These results remarked the role of the (pre)treatments and of storage on structure, microbiological aspects, quality changes of the processed fruit. On the other side, we also analyze the ability of apple matrix for incorporation of calcium by two different impregnation techniques (in vacuum or at atmospheric pressure) (Anino et al. 2006). Operative impregnation conditions that allow the incorporation of great calcium quantities in apple tissue minimizing the impact on structure, optical, and rheological properties were established. However, the preservation and the corresponding shelf life of these "functional" apples and the effect of storage on their structure and quality were not investigated.

The aim of this study was to assess the potential of UV-C light irradiation to preserve calcium fortified cut apples. The impact of treatments on surface color, linear viscoelastic properties (as derived from dynamic oscillatory and creep/recovery tests), microstructure, and native flora dynamics was analyzed after processing and during refrigerated storage.

Material and Methods

Sample Preparation

Raw apples (*Malus pumila*, Granny Smith var.; $a_w \cong 0.98$; 11.3–12.2°Brix, pH 3.3–3.5) were purchased at a local market and maintained at 4–5 °C during 1 day 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, tools, and holding vessels were sanitized in the same way before use.

Apples were hand peeled. Slices of parenchymatic tissue (3 mm in diameter and 6 mm in thickness) were cut with a cork borer. The fruit slices were dipped in distilled water (4–5 °C) for 1 min to eliminate cellular fluids and immediately subjected to the different treatments.

Preparation of Fortified Cut Apples

The incorporation of calcium on apple matrix was performed through atmospheric impregnation (AI), with or without previous blanching (B). The experimental conditions were selected according to the technologies previously designed in our laboratory for calcium fortification of cut apples (Anino et al. 2006; Salvatori et al. 2007). Aqueous solutions containing 12.6 % (*w*/*w*) glucose, 1.1 % (*w*/*w*) Ca^{2+} lactate, and 4.4 % (*w*/*w*) Ca^{2+} gluconate were used as impregnation medium. The pH was adjusted to 3.5 (similar to the natural apple tissue pH) with citric acid. All reagents were of food grade and from Saporiti S.A., Argentina. The impregnation medium was isotonic regarding to the content of apple native soluble solids to avoid water transfer mechanisms. The impregnation was conducted during 4.5 h under forced convection by using a mechanical stirrer at 400 rpm.

For blanching prior to calcium impregnation, samples were exposed to saturated vapor (2 min) and then immediately cooled in water (4±1 °C, 5 min).

For apples impregnated with calcium without previous blanching, an antibrowning treatment was applied before and after calcium impregnation to minimize browning caused by treatments. Apples were immersed into an antibrowning solution (antibrowning dipping, AD) 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 5 min at 4 °C (Ponting et al. 1972).

UV-C Light Treatments

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 G13 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 mean 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).

Fortified apple discs were exposed to UV-C light for 20 min (fluence 11.2 kJ/m^2) on each side. The selected UV-C radiation treatment was suitable to achieve microorganism's inactivation on fresh-cut apple slices, according to previous studies (Gómez et al. 2010). After irradiation, UV-C-treated and non-UV-C-treated samples were packed in closed polyethylene boxes permeable to air and stored in the dark at 5 °C (±1 °C).

Fresh-cut apples, apple samples impregnated with calcium at atmospheric pressure, with or without blanching (B + AI and AI apples, respectively) and/or apple samples with an antibrowning dipping before and after calcium incorporation (AD + AI apples) were used as controls according to the different analysis. Experiences were made in duplicate.

Color Measurement

Cut apple surface color was measured with a handheld tristimulus reflectance spectrocolorimeter Model CM-508-d (Minolta Co., 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 C.I.E. 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), defined as brown color purity, using the following equations (Buera et al. 1986):

$$BI = [100(x - 0.31)]/0.172 \quad (1)$$

$$\text{where } x = X/(X + Y + Z) \quad (2)$$

Color was evaluated at 0, 3, and 7 days of refrigerated storage (5±1 °C) after apple exposure to UV-C light. Ten independent samples were used for each condition with five readings taken at different positions on the surface of each sample.

Analysis of Viscoelastic Properties

Viscoelastic properties of apples subjected to the different treatments and exposed to 20 min UV-C light were analyzed at 25 °C in a Paar Physica MCR 300 rheometer (Antor Paar GMBH, Graz, Austria) using a 30-mm diameter parallel plate geometry. Apple samples (30 mm in diameter) were placed on the lower plate of the rheometer. Slip was minimized by placing a measuring plate with rough surface (model PP/30) and using only as much compression as necessary to provide maximum contact area (normal force=1 N).

Temperature was controlled by an external liquid bath thermostat model Viscotherm VT2 (Anton Paar, Graz, Austria). The test conditions used in the analysis of viscoelastic

properties were selected according to previous studies (Martinez 2005; Martinez et al. 2007).

Dynamic oscillatory test were performed in the controlled strain mode. Prior to a frequency sweep, a strain sweep was carried out at an angular frequency (ω) of 1 s^{-1} to determine the linear viscoelastic range (LVR). The LVR was determined with the Paar Physica US 200 software package (Anton Paar GmbhH, Graz, Austria). Thereafter, storage (G') and loss moduli (G'') were measured in the frequency range 0.1–100 s^{-1} using a strain amplitude value of 0.01 % (within the limits of linearity previously established). Storage moduli values were fitted using a linear regression of log (G') vs log (ω):

$$\log(G') = n\log(\omega) + k \tag{3}$$

where n is the slope of regression and k is log G' value at 0.1 s^{-1} of angular frequency.

Creep-recovery tests of apples were conducted by applying a constant shear stress of 35 Pa for 100 s. A previous stress sweep by varying the applied stress from 1 to 100 Pa indicated that in the selected condition, the deformation was proportional to the stress applied. After removal of the stress, sample recovery was registered for a period of time of 200 s. Each apple 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):

$$J(t,\tau) = (J_0) + \sum_{i=1}^{2} (J_i)\left(1-e^{-t/\lambda_i}\right) + {}^{t}/_{\eta_N} \tag{4}$$

where $J(t, \tau)$ is the creep compliance ($=\gamma(t)/\tau$ with $\gamma(t)$ being the strain at the time t and τ the constant stress applied). J_0 is the instantaneous compliance at $t=0$; J_i are the retarded compliances; λ_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.

The Origin v.7.0 software (OriginLab Corporation, Northampton, USA) was use for non-linear regression analyses. The estimation method used to minimize residual sum of squares was Marquardt.

In both tests, data were obtained using 10 replicates for each condition. Measurements were made at 0 and 7 days of storage at 5 °C (±1 °C).

Microbiological Analysis

The effect of UV-C light on native flora of apple slices enriched with calcium was evaluated. Apples subjected to AI with and without blanching/or antibrowning treatment were exposed to UV-C light, stored in plastic boxes at 4–5 °C and analyzed at 0, 3, and 7 days of storage. For enumeration, samples 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 (6 strokes/s) for 3 min. Homogenated samples were serially diluted in 0.1 % (*w*/*v*) peptone water, and 0.1 mL sample suspension was surface plated using plate count agar (PCA, Britania S.A., Argentina) for aerobic microorganisms count and Yeast Glucose Chloramphenicol Agar (YGC, Britania S.A., Argentina) for mold and yeast count. Plates were incubated for 72 h at 37 °C (±1 °C) (aerobic microorganisms) or 27 °C (±1 °C) (molds and yeasts). Three replicates were examined for each condition, and experiments were made in duplicate. Results were expressed as N (where N is the number of CFU/g).

Determination of Calcium Content

Calcium content in apple tissues was measured by atomic absorption spectrophotometry, according to the methodology described by Salvatori et al. (2007). The reference material RM 8435 (whole milk powder) was used to verify the accuracy of the analytical procedures. Triplicates were run with each set of apple samples and reference material. Values within 5 % of the certified value for reference material and <5 % relative standard deviation for apple samples were required for acceptance of the data. Mean values of calcium concentration (expressed as total content of wet impregnated sample) were reported.

Microscopic Observations

For light microscopy (LM) of fresh and treated tissues, cubes of approximately 3 mm^3 (including the surface of samples) were fixed in glutaraldehyde solution (3 g/100 g) and then in 0.1 M potassium phosphate buffer (pH=7.4) overnight at (24 ±1) °C. Cubes were then rinsed three times with distilled water, postfixed in OsO_4 solution (1.5 g/100 g) at (24±1) °C, 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) at 0- and 7-day refrigerated storage. All reagents were from Merck Química S.A. (Argentina).

Statistical Analysis

Statistical analyses were carried out using the Infostat v. 2009 software (Universidad Nacional de Córdoba, Argentina). Results were expressed as mean ± standard deviation of the mean (mean ± SD). Two-way analysis of variance (ANOVA) was performed on microbiological results according to the factors "treatment" and "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 both analysis, a significant level $\alpha=0.05$ was used.

Multivariate analysis of variance (MANOVA) was used to analyze color and rheological data. Hotelling corrected for Bonferroni test was performed in case of finding significant differences. Significance level was set at $p<0.05$. Principal component analysis (PCA) of mean ratings for each attribute was used to illustrate the relationship among variables and samples.

Results and Discussion

Calcium Content

Fresh apples had an average calcium content of (7.4±1.2) μg/g. The amount of calcium incorporated on apple tissue after atmospheric impregnation with and without previous blanching was (2734±207) and (721±104) μg/g, respectively. Therefore, calcium uptake in samples impregnated with previous blanching was approximately four times higher than in apples only impregnated.

Calcium content in 200 g (daily ration) of cut apples subjected to AI would satisfy about 14 % of the calcium adequate intake (1000 mg/day for adults, Institute of Medicine of The National Academies 2010), meanwhile 55 % of the adequate intake would be achieved when apples were blanched before calcium impregnation. Previous preliminary studies on relative absorption of calcium showed that apples impregnated with calcium lactate and gluconate were a good vehicle to provide easily absorbable calcium (Salvatori et al. 2007). Moreover, greater absorption of this nutrient was achieved when apples were previously blanched.

Native Flora

Damage to microorganisms induced by UV-C light and other killing agents can be permanent or sublethal. Sublethal injury cells are capable of repair during storage and may grow, potentially compromising product quality or safety (Lado and Yousef 2002; Leitsner and Gould 2002). Otherwise, microorganisms can be internalized in the fruit, protected in groves, hollows, and other surface irregularities, and/or attached to injured surfaces. These facts impair the accessibility of the decontamination agents to microorganisms, which may survive the treatments. Accordingly, different patterns of growth of microorganisms during storage of UV-C exposed fruits would be expected, as previously reported by Gómez-López et al. (2008), Gómez et al. (2010), and Schenk et al. (2012) in different fruit and vegetables with minimal processing. Microbial populations were observed to grow at the same or at a slower rate than in the untreated product, decrease in number, or keep counts constant without further recovery along storage, but also, decontamination treatments can enhance the growth rate. Therefore, a reduction in microbial load after UV-C exposure not always would lead to a shelf life extension, and it is important to assess the response of native flora not only to UV-C exposure but through the storage time.

Table 1 shows the microbial dynamics during refrigerated storage of treated apple discs. Significant interaction between factors treatment and storage time was found ($F_{10,36}=26.2$ for aerobic mesophilics; $F_{10,36}=22.2$ for molds and yeasts).

Initial loads of native flora in calcium impregnated apples (with and without blanching) were higher than in raw fruits. The exposure of AI samples to UV-C light during 20 min caused a reduction in the microbial population to no detectable levels. In impregnated apples previously blanched, the reduction achieved was about 2.5 log cycles for mesophilic aerobes and 1.2 log cycles for molds and yeasts. In general, all samples showed an increase in microbial population throughout storage. However, irradiated apples presented lower microbial growth than those with the same fortification treatment but non-irradiated. After 7 days of storage, proliferation of mesophilic aerobes was reduced between 1 and 2 log cycles in AI- and B + AI-treated apples exposed to UV-C light compared with samples not subjected to UV-C treatment. On the other hand, the reduction in molds and yeasts was about 2.0–2.5 log cycles. In the case of samples dipped into the antibrowning solution (AD + AI + UV-C-treated apples), the reduction achieved was lower (≈0.5 and 0.6 log cycles for mesophilic aerobes and molds and yeasts, respectively). Therefore, the ascorbic acid/ calcium chloride antibrowning pretreatment would have a protective effect on microorganisms' inactivation induced by UV-C light. This effect was previously reported in fresh-cut apple discs irradiated with UV-C light and pulsed light (Gómez et al. 2010, 2012a, b). It could be attributed to the antioxidant properties of ascorbic acid, which could diminish the germicidal effect of UV-C light on microorganisms, to different radiation absorption and/or to changes on the surface roughness. However, for all fortified apple samples, an extension of shelf life seemed to be possible by using UV-C.

It is necessary to remark that inherent aspects of native flora could influence present results, such as variability in type and level of initial contamination and biological variability in the population (Gómez-López et al. 2008). This fact, as well as

Table 1 Counts of aerobic mesophilic and molds and yeasts (expressed as CFU/g) in cut apple discs subjected to different treatments and stored at 5 °C

Microorganism	Treatment	Storage time (day)		
		0	3	7
Aerobic mesophilic	Control	$(0.6\pm0.2).10^{2aA}$	$(0.9\pm0.4).10^{2aA}$	$(4.2\pm2.1).10^{2bA,C}$
	AI	$(1.9\pm0.6).10^{3aB}$	$(1.6\pm0.7).10^{3aB}$	$(2.5\pm0.8)10^{3aB}$
	AI+20 min UV-C	N.D.	$(2.7\pm0.2).10^{2bC}$	$(2.1\pm0.1).10^{2bC}$
	AD + AI+20 min UV-C	8 ± 7^{aC}	$(1.88\pm0.3).10^{2bCD}$	$(8.9\pm0.8).10^{2cD}$
	B + AI	$(3.6\pm1.2).10^{3aD}$	$(2.1\pm0.5).10^{3aB}$	$(29.2\pm8.4).10^{3bE}$
	B + AI+20 min UV-C	$(0.1\pm0.1).10^{2aC}$	$(1.2\pm0.1).10^{2bA,D}$	$(4.4\pm1.1).10^{2cA}$
Molds and yeasts	Control	$(1.2\pm0.7).10^{2aA}$	$(2.5\pm1.3).10^{2a,bA}$	$(2.9\pm0.4).10^{2bA}$
	AI	$(4.7\pm0.8).10^{2aB}$	$(1.6\pm0.6).10^{3bB}$	$(3.3\pm0.1).10^{3cB}$
	AI+20 min UV-C	N.D.	$(0.3\pm0.2).10^{2bC}$	$(0.31\pm0.07).10^{2bC}$
	AD + AI+20 min UV-C	$(0.15\pm0.04).10^{2aC}$	$(0.22\pm0.09).10^{2aC}$	$(8.9\pm1.1).10^{2bD}$
	B + AI	$(4.4\pm2.7).10^{2aB}$	$(8.4\pm1.2).10^{2bB}$	$(17.7\pm2.2).10^{3cE}$
	B + AI+20 min UV-C	$(0.25\pm0.03).10^{2aC}$	$(0.2\pm0.2).10^{2aC}$	$(0.5\pm0.1).10^{2aC}$

Results were expressed as mean±standard deviation

AI atmospheric impregnation, *AD* antibrowning dipping, *B* blanching, *N.D.* non-detectable

For each type of microorganism and storage time, means followed by same uppercase letter were not significantly different at $p<0.05$. For each type of microorganism and treatment, means followed by same lowercase letter were not significantly different at $p<0.05$

raw fruit, process, and storage variability, would be taken into account in future shelf life studies.

Microscopic Features

Light microscopy observations were performed to evaluate microstructural changes provoked by treatments. The photomicrographs in Figs. 1 and 2 correspond to parenchyma apple tissues localized at the surface of cut apple subjected to different treatments at 0 and 7 days of storage, respectively. Cells in fresh apple tissue appeared turgid, rounded in shape, with parietal cytoplasm and stained walls (Fig. 1a). Tissues treated with AD + AI exhibited many cells affected by plasmolysis and some of them with broken membranes. Cells also appeared more irregular in shape than in the fresh tissue (Fig. 1b). The exposure to UV-C light provoked a severe rupture of membranes (Fig. 1c). Apples treated with B + AI showed a slight contraction of tissue, but cell walls were observed well stained, smoothed, slightly folded, and with few disruptions (Fig. 1d). This would indicate that the great calcium penetration in blanched tissues could improve cell wall structure. This phenomenon was also observed in a previous work on the same fruit matrix and under the same impregnation conditions (González-Fésler et al. 2008). It is well known that calcium ions form cross-links or bridges between free carboxyl groups on adjacent polygalacturonate chains present in middle lamella, contributing to cell-cell adhesion and cohesion (Jackman and Stanley 1995). The subsequent irradiation of blanched tissues caused greater disruption of plasmalemma and tonoplast and tissue contraction (Fig. 1e).

After 1 week of storage, all treated tissues showed folding of cell walls but in a lesser extent than non-treated ones (Fig. 2). The microstructural differences in AD + AI tissues with and without irradiation diminished with storage (Fig. 2c, b, respectively). On the contrary, although impregnated samples previously blanched with and without UV-C treatment showed similar cellular arrangement, cell walls of irradiated tissues (Fig. 2e) looked less stained and with greater disruptions than those non-irradiated (Fig. 2d).

Optical Properties

The variations in the color coordinates and BI function immediately after treatments and throughout refrigerated storage of calcium-enriched apple discs (with and without previous antibrowning dipping), exposed or not to UV-C light, are shown in Fig. 3. Significant differences in the color coordinates among different samples were found (MANOVA $F_{32,540}=7.3$; $p<0.0001$). Apple slices subjected to AI (with and without UV-C irradiation) did not show significant variations in color coordinates with respect to untreated samples just after treatments (day 0). However, changes in color were visualized in treated fruits after 3 days of storage. The variations were reflected in a decrease in luminosity (L*) and an increase in a* and b* coordinates in apples enriched with calcium exposed or not to UV-C light. The modifications observed in color coordinates were associated with the development of

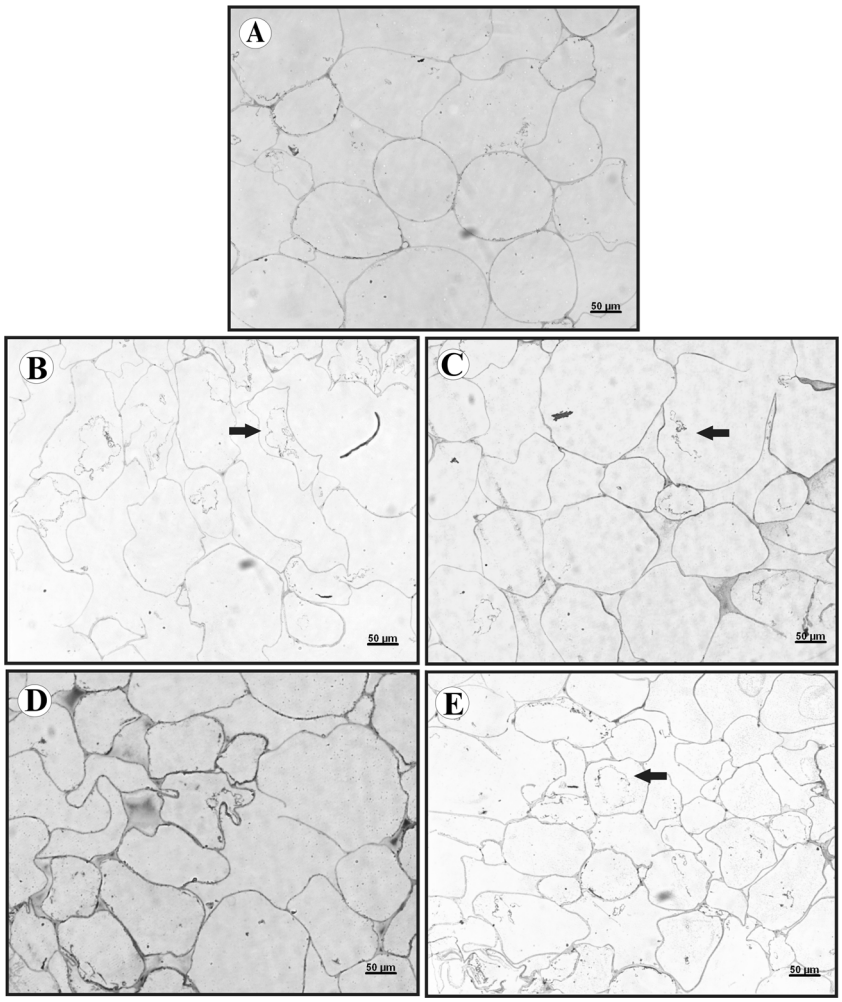

Fig. 1 Light microscopy images from surface of apple tissue subjected to different treatments at 0 day. **a** Raw (control), **b** AD + AI, **c** AD + AI + UV-C 20 min, **d** B + AI and, **e** B + AI + UV-C 20 min. *AD* antibrowning dipping, *AI* atmospheric calcium impregnation, *B* blanching. *Black arrows* indicate disrupted membranes or plamolysis

browning on apple surface. Samples subjected to AI turned browner than control during storage, exhibiting a greater increase in BI values than non-treated fruits. These modifications were slightly more pronounced in samples irradiated 20 min. At 7 days of storage, color changes were slightly accentuated in samples treated only with AI. On the other hand, color coordinates of apples treated with AI and exposed to UV-C light remained, in general, without significant variations ($p>0.05$) and were not significant different ($p>0.05$) to those of non-irradiated calcium fortified samples. Variation in color coordinates throughout storage in calcium impregnated samples (with and without UV-C irradiation) was reduced due to application of the antibrowning treatment. Regarding L* coordinate, AD + AI and AD + AI + UVC-treated samples showed lower values of L* than control but changes in luminosity were lower than in treated samples without AD. In general, changes in a*, b*, and BI values of AD-treated apples (with and without UV-C exposure) were almost negligible. According to these results, the ascorbic acid/calcium chloride solution seemed to be effective to inhibit browning induced by calcium incorporation and UV-C exposure.

The color coordinates and BI function corresponding to impregnated apples with a previous blanching are presented in Fig. 4. Significant differences in the values among different samples were found (MANOVA $F_{16,\ 324}=7.3$; $p<0.0001$). Blanching pretreatment provoked about 11 % decrease in the luminosity of apple samples. Although treated samples showed significant variations in color coordinates and BI function during storage, the modifications, mainly in a*, b*, and BI values, were lower than in AI apples without previous thermal treatment. At the end of storage, no significant changes between B + AI apples exposed or not to UV-C light were observed. Therefore, blanching prior to calcium impregnation and UV-C treatment not only facilitated the mineral incorporation inside the fruit structure but also help in reducing apple surface browning.

Changes in color in treated and/or stored cut apples could be correlated with the structural modifications observed in apple tissue. Browning developed in calcium-enriched samples can be at least partially explained by the breakage of cellular membranes provoked by impregnation treatment. The loss of functional cell compartmentalization would cause

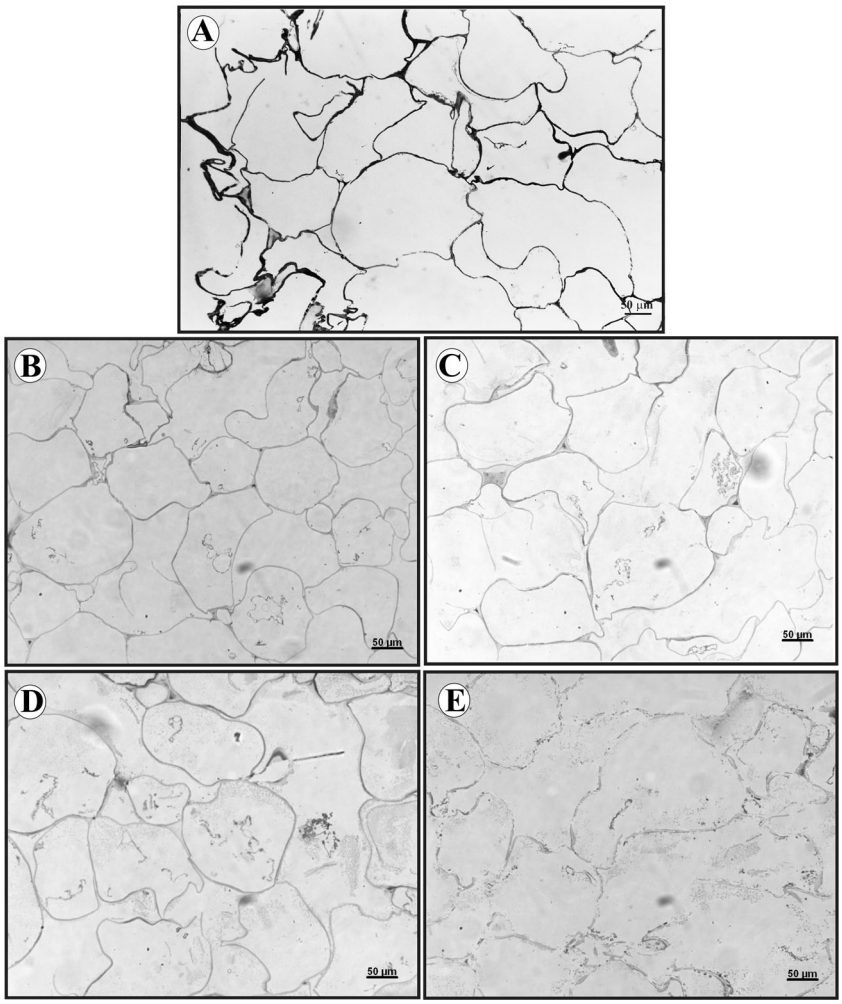

Fig. 2 Light microscopy images from surface of apple tissue subjected to different treatments and stored 7 days at 5 °C. **a** Control, **b** AD + AI, **c** AD + AI+ 20 min UV-C, **d** B + AI, and **e** B + AI+20 min UV-C. *AD* antibrowning dipping, *AI* atmospheric calcium impregnation, *B* blanching

that phenolic substrates come into contact with the enzymes, and thus, browning could be triggered. Exposure of tissues to UV-C light induced an additional damage in membranes that was reflected in a slight increase in browning in irradiated apples at 3 days of storage. In samples previously blanched or dipped into the antibrowning solution, despite membrane rupture, browning could be inhibited or reduced by inactivating the enzymes or by affecting reaction substrates.

Viscoelastic Properties

Dynamic Spectra

Figure 5 shows the average mechanical spectrum of fortified apple slices, blanched or dipped into the antibrowning solution, and exposed to UV-C light, at 0 day (Fig. 5a) and after 7 days of storage (Fig. 5b). All samples assayed showed a dominant solid behavior given by G' values higher than G'' values over the entire frequency range (tan $\delta \approx 0.08$–0.17). The slight linear increase in G' with increasing angular frequency denotes an elastic, cross-linked network. Unlike the G' spectrum, the frequency dependence of G'' consisted of one small negative slope at low frequencies and a positive slope at high frequencies.

Both storage and loss moduli were reduced due to processing and during storage of samples, indicating that apple tissue became less elastic and less viscous. Significant changes in the average values of the slope (n) and ordinate (k) obtained from the linear regression of log G' vs log ω curves among different samples were found (MANOVA $F_{8,\ 180}=6.7$; $p<0.001$) (Table 2). After treatments (day 0), apples immersed into the antibrowning solution and subjected to AI presented lower values of k ($<G'$ values) than untreated fruit and similar n values. Blanched apples showed a more pronounced decline in k and a slight increase in n compared with the control. The diminished in G' values and the slight increase in n values might be correlated to a

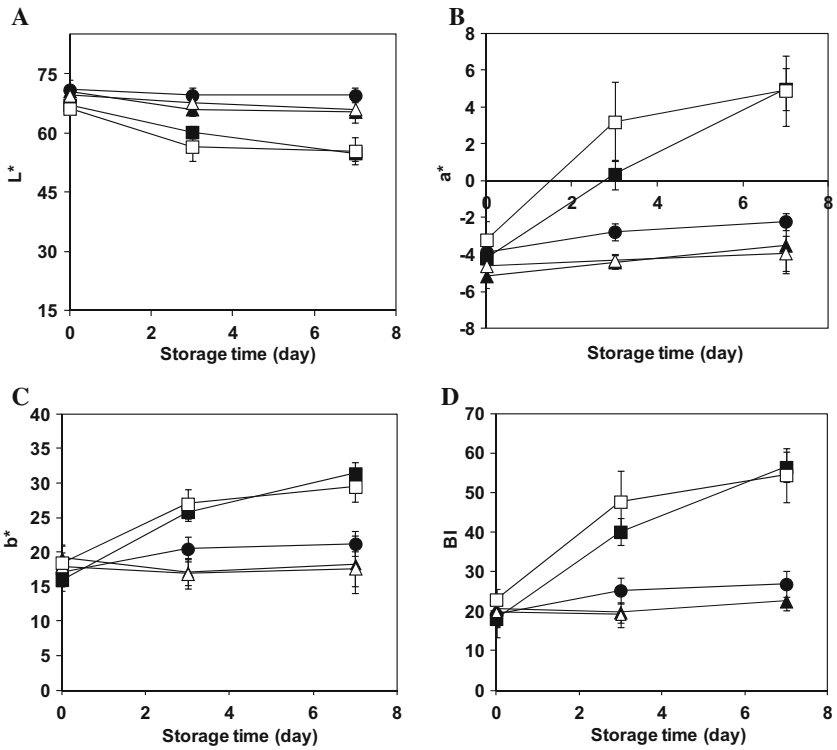

Fig. 3 Effect of antibrowning dipping, atmospheric calcium impregnation, and UV-C light on color coordinates and BI function of cut apple discs stored at 5 °C. **a** L*, **b** a*, **c** b*, and **d** BI. Control (*circles*), AI (*filled squares*), AI+ 20 min UV-C (*empty squares*), AD + AI (*filled triangles*), and AD + AI+20 min UV-C (*empty triangles*). *AD* antibrowning dipping, *AI* atmospheric calcium impregnation

loss of rigidity in the gel network, which was increased when impregnation treatment was combined with previous blanching. Both in B and AD-treated calcium-enriched apples, the subsequent exposure to UV-C light did not provoke significant changes in *n* and *k* parameters. After 7 days of storage, control samples showed a slight decrease in *k* value, but *G′* values remain higher than for treated ones. They also presented a slight decrease in *n* values as compared with the fruit at day 0. This behavior of untreated apple was in agreement with previous findings reported by Gómez et al. (2010), who observed a slight increase in rupture stress in fresh-cut

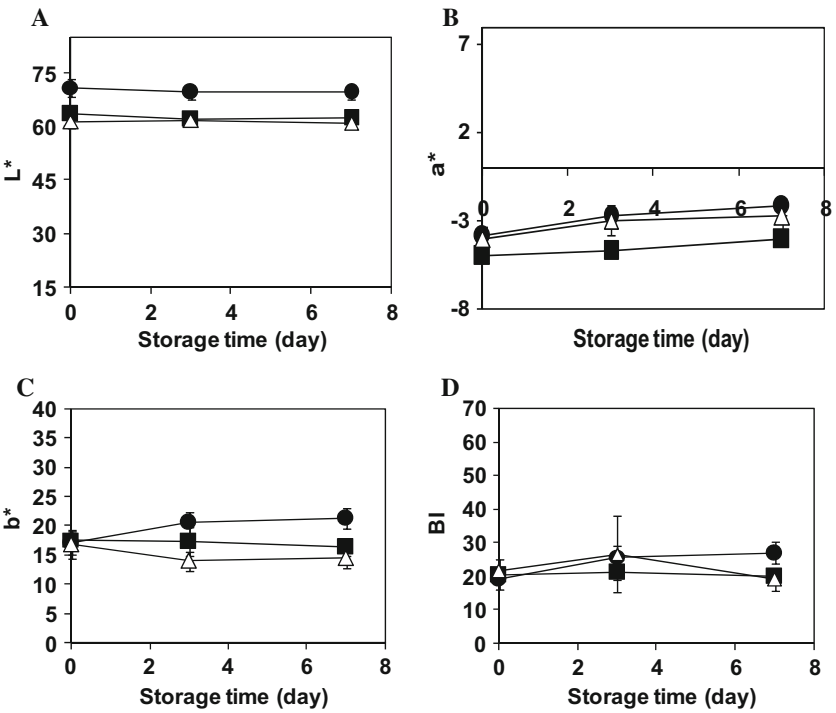

Fig. 4 Effect of blanching, atmospheric calcium impregnation, and UV-C light on color coordinates and BI function of cut apple discs stored at 5 °C. **a** L*, **b** a*, **c** b*, and **d** BI. Control (*circles*), B + AI (*squares*), B + AI+20 min UV-C (*triangles*). *B* blanching, *AI* atmospheric calcium impregnation

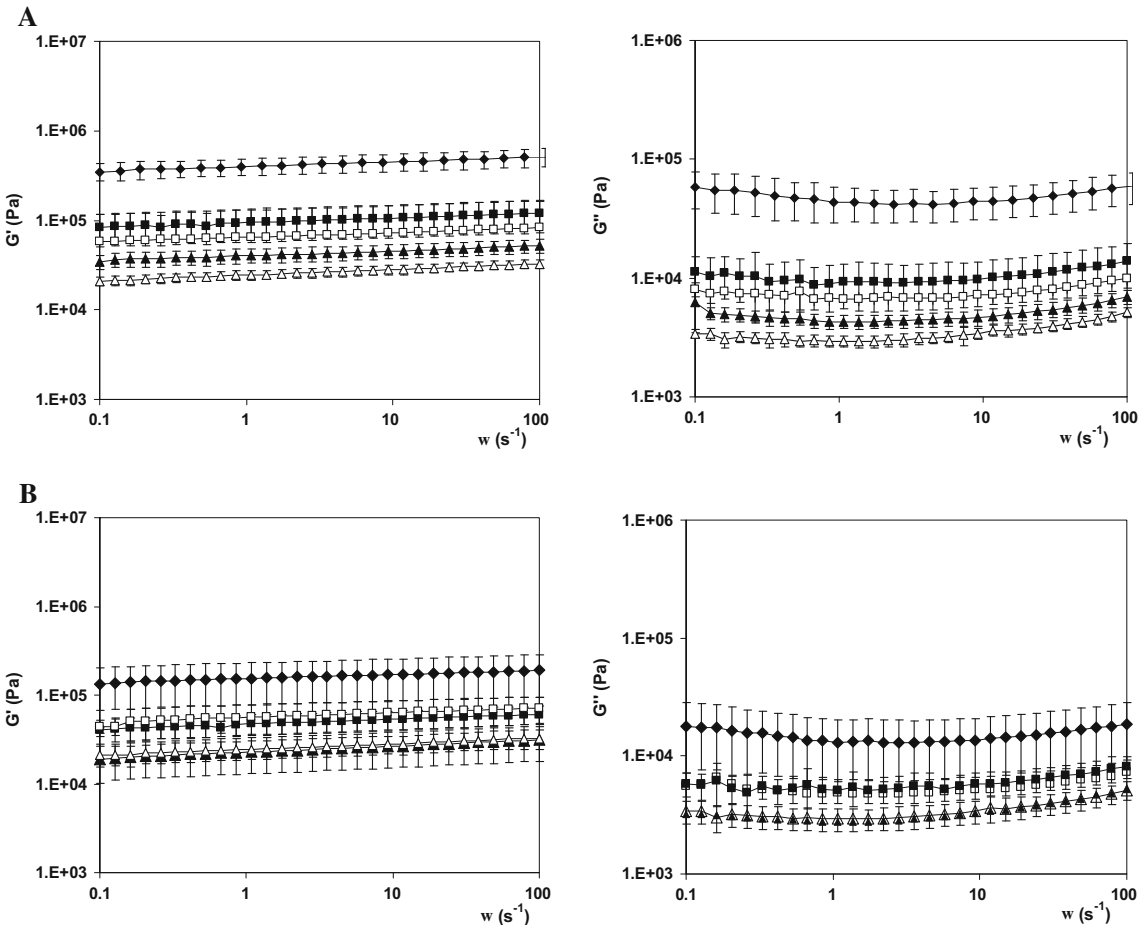

Fig. 5 Variation of storage modulus (G') and loss modulus (G'') with frequency for apple tissue subjected to different treatments and stored at 5 °C. **a** Day 0, **b** day 7. Control (*diamonds*), AD+AI (*filled squares*), AD + AI+20 min UV-C (*empty squares*), B + AI (*filled triangles*), B + AI+ 20 min UV-C (*empty triangles*). *AD* antibrowning dipping, *AI* atmospheric calcium impregnation, *B* blanching. *Bar*: standard deviation

apples stored a week at refrigeration temperatures. The G' pattern across the frequency spectra did not significantly change during storage in apples treated with AD + AI (with and without UV-C irradiation). On the contrary, samples subjected to blanching prior impregnation exhibited a more pronounced decline in k and a slight increase in n, denoting a loss of tissue stiffness also along storage.

Table 2 Parameters derived from linear fitting of log G' vs log ω curves for fresh and treated apples at 0 and 7 days of storage

Storage time (day)	Treatment	n	k (Pa.s^n)	
0	C	0.05±0.01	5.6±0.1	A
	AD + AI	0.051±0.005	4.9±0.2	CE
	AD + AI+20 min UV-C	0.050±0.006	4.81±0.05	E
	B + AI	0.055±0.005	4.7±0.3	DE
	B + AI+20 min UV-C	0.057±0.002	4.50±0.04	D
7	C	0.046±0.002	5.1±0.3	C
	AD + AI	0.056±0.004	4.7±0.1	DE
	AD + AI+20 min UV-C	0.055±0.008	4.7±0.1	DE
	B+AI	0.070±0.004	4.3±0.2	B
	B + AI+20 min UV-C	0.064±0.006	4.4±0.1	B

AD antibrowning solution, *AI* atmospheric impregnation, *B* blanching

Different letters indicate significant differences between treatments ($p<0.05$)

Creep/Recovery Behavior

Treatments caused relevant changes in both creep and recovery response (Fig. 6). Compliance versus time curves of fresh and treated apples during the creep phase were well characterized (R^2_{adj}>0.99) by the mathematical model represented by Eq. (4). The rheological parameters obtained from the model are presented in Table 3. Creep parameters presented large standard deviations. This great variability has been frequently observed when evaluating the creep response of plant tissues (Mittal and Mohsenin 1987; Pitt 1992; Alzamora et al. 2008).

The MANOVA performed on creep data indicated high significant differences between untreated and treated apples at 0- and 7-day storage ($F_{24,\ 234}$=2.5; p<0.001). In the PCA, 82 % of total variance was explained by the first two components (PC1 71 % and PC2 22 %). The instantaneous compliance (J_0), the viscoelastic compliances (J_1 and J_2), and the steady-state viscous compliance ($1/\eta_N$) were the parameters that mainly contributed to the variance in PC1, while in PC2 were the retardation times (λ_1 and λ_2) (data not shown). Just after treatments, all samples showed an increase in J_0, J_1, J_2, and $1/\eta_N$, denoting that tissues became more deformable. The changes in compliances in apples subjected to blanching prior calcium impregnation were greater than in non-blanched-treated tissues. On the other hand, AD + AI and B + AI samples exposed to UV-C light did not show significant differences compared with treated samples without irradiation. In general, retardation times (λ_1 and λ_2) were lower in treated apples than in control. This would indicate that the viscoelastic elements in treated tissues exhibited a higher speed to reach a given level of deformation.

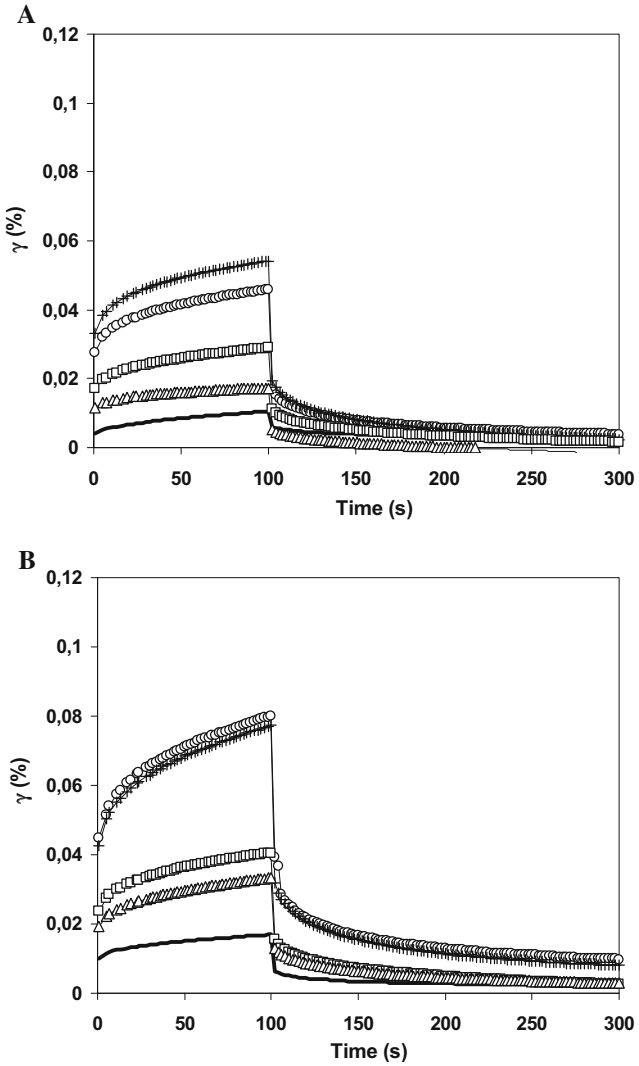

Fig. 6 Average experimental creep-recovery curves of apple samples subjected to different treatments and stored 7 days at 5 °C. **a** Day 0, **b** day 7. Control (*line*), AD + AI (*triangles*), AD + AI+20 min UV-C (*squares*), B + AI (*diamonds*), B + AI+20 min UV-C (*plus signs*). *AD* antibrowning dipping, *AI* atmospheric calcium impregnation, *B* blanching

The increase in compliances of treated apples was accentuated after storage. As on day 0, major changes were observed in tissues exposed to thermal treatment. Furthermore, there was not a significant effect due to the application of UV-C light. The retardation times remained, in general, without variations or with slight changes. Although untreated apples also showed significant variations in creep parameters during storage, these modifications were much lower than in treated samples.

The overall compliance at the end of the creep phase in stored treated apples (7 days) increased as compared to that of the fresh tissue (0 day) about 400 % in AD + AI samples (with and without UV-C) and 1000 % in B + AI apple discs (with and without UV-C). For all samples, the relative contribution of each type of compliance was in the range 41–60 % for J_0, 17–30 % for J_1, 11–13 % for J_2, and 11–19 % for $1/\eta_N$. The major contribution to overall compliance for treated and untreated apples was given by J_0, both at the beginning and at the end of storage.

Relationship Between Structure and Rheological Behavior

The major structural elements at cellular and tissue level that contribute to mechanical behavior of plant-based foods are the turgor pressure (the force exerted on the cell membrane by intracellular fluids), the cell wall rigidity, and the cell-cell adhesion, determined by the integrity of the middle lamella and the plasmodesmata. The structural elements that would mainly influence the rheological parameters related with the elastic response (G' and J_0) are the cellulose (the main component of the cell wall, which provides individuals cells with rigidity and resistance to rupture), the occluded air in the porous matrix, and the turgor pressure (Alzamora et al. 2000, 2008; Bourne 1976; John and Dey 1986; Pitt 1992). On the other hand, viscoelastic compliances (J_1 and J_2) could be attributed to time-dependent changes in pectins and hemicelluloses, respectively, and steady-state viscosity could be related

Table 3 Effect of antibrowning dipping, blanching, atmospheric calcium impregnation, and UV-C radiation on the viscoelastic parameters of cut apples derived from creep test

Storage time (day)	Treatment	J_0 (1/Pa) ($\times 10^6$)	J_1 (1/Pa) ($\times 10^6$)	J_2 (1/Pa) ($\times 10^6$)	λ_1 (s)	λ_2 (s)	η_N (Pa.s) ($\times 10^{-8}$)	
0	Control	3.6±1.2	2.7±1.2	1.1±0.4	31.5±13.8	2.5±1.4	0.7±0.4	A
	AD + AI	8.5±1.1	2.8±0.5	1.8±0.6	21.3±4.1	2.1±0.6	0.6±0.4	BC
	AD + AI+20 min UV-C	16.1±4.7	5.1±0.6	3.1±0.4	25.5±7.9	2.6±0.5	0.2±0.1	B
	B + AI	23.3±4.7	7.4±1.8	4.8±1.2	21.2±2.3	2.3±0.3	0.16±0.05	E
	B + AI+20 min UV-C	30.1±0.5	9.6±0.4	6.1±0.3	21.9±1.3	2.3±0.1	0.12±0.01	E
7	Control	8.8±6.9	2.6±1.8	1.6±1.1	23.8±7.9	2.1±0.9	0.6±0.4	C
	AD + AI	21.4±1.4	7.3±0.5	4.4±0.5	27.8±3.9	2.6±0.3	0.16±0.02	E
	AD + AI+20 min UV-C	18.9±2.6	6.1±1.4	3.7±0.6	23.6±3.5	2.4±0.3	0.17±0.03	E
	B + AI	36.6±8.6	14.4±2.9	8.5±2.1	21.1±2.7	2.3±0.2	0.07±0.02	D
	B + AI+20 min UV-C	38.1±12.1	14.4±3.2	8.5±2.9	22.3±1.1	3.1±1.7	0.10±0.1	D

Results were expressed as mean followed by the standard deviation; storage temperature: 5 °C

AD antibrowning dipping, *AI* atmospheric calcium impregnation, *B* blanching

Parameters derived by fitting Eq. (4) to compliance data from creep phase

Different letters indicate significant differences between treatments ($p<0.05$)

to cell wall fluidity arising from exosmosis and/or solubilization and degradation of polymers and less water binding capacity due to treatments (Alzamora et al. 2008; Jackman and Stanley 1995).

Dipping treatments with calcium salts in low concentration has been widely used as firming agents to improve post processing quality characteristics and extend shelf life of vegetable products (Alandes et al. 2006; Luna-Guzmán and Barrett 2000; Martín-Diana et al. 2007; Rico et al. 2007). However, the incorporation of calcium in this work was not reflected in beneficial changes in the viscoelastic behavior of apple tissue. The structural modifications provoked by treatments negatively affected viscoelastic properties. In AD + AI-treated apples, the decline in G' and the increase in J_0 would be mainly associated with the loss of turgor pressure due to plasmolysis and breakage of cellular membranes (Fig. 1b). The greater rupture of membranes provoked by the exposure of these treated tissues to UV-C light (Fig. 1c) was not reflected in additional significant changes in the viscoelastic properties. After storage, more pronounced changes in compliances J_0, J_1, J_2, and $1/\eta_N$ were observed. This would be related with the loss of turgor, accentuated due to the rupture of membranes, as well as with modifications in cell walls during storage, indicated by a more important folding and lower staining of walls (Fig. 2b, c).

In tissues subjected to blanching prior to calcium impregnation, despite the reinforcement visualized in cell walls, the decrease in the storage modulus and the increase in compliances (J_0, J_1, J_2, and $1/\eta_N$) were of greater magnitude than in non-blanched impregnated apple slices. This could be attributed to the severe internal disruption observed in the cells that provoked loss of turgidity and contraction of tissue (Fig. 1d). Subsequent irradiation of these tissues did not result in significant modifications in viscoelastic properties, although in microscopic observations, cells appeared with greater disruption of membranes and with weakened cell walls, especially at the end of storage, where some episodes of rupture could be observed (Figs. 1e and 2e).

Conclusions

The application of UV-C light was useful to reduce microbial growth in calcium-enriched apples during refrigerated storage, increasing their shelf life. AI treatment provoked a significant development of browning in apple slices throughout storage. As these changes in color were pronounced, the subsequent exposure to UV-C light did not result in important additional modifications. Blanching prior to calcium impregnation and UV-C exposure not only facilitated the mineral incorporation inside the fruit structure but also helped in reducing apple surface browning. Application of the ascorbic acid/calcium chloride solution was more effective than blanching to minimize color changes during storage but had a protective effect that diminished the impact of UV-C light on native flora. AD + AI and B + AI treatments negatively affected viscoelastic properties, being the changes more pronounced in heated tissues. However, exposure of AD + AI and B + AI apples to UV-C did not modify creep response neither at 0 day nor at day 7 and also had a negligible effect on dynamic spectra. Modifications in rheological properties and color were partially ascribed to microstructure features (breakage of cellular membranes with loss of functional cell compartmentalization and loss of turgor; modifications in cell walls).

These findings suggest that UV-C irradiation could be a valuable tool for prolonging shelf life of calcium fortified

cut apples with minimal or negligible impact on color and viscoelastic properties.

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