Relationships between rheological properties, texture and structure of apple (Granny Smith var.) affected by blanching and/or osmotic dehydration

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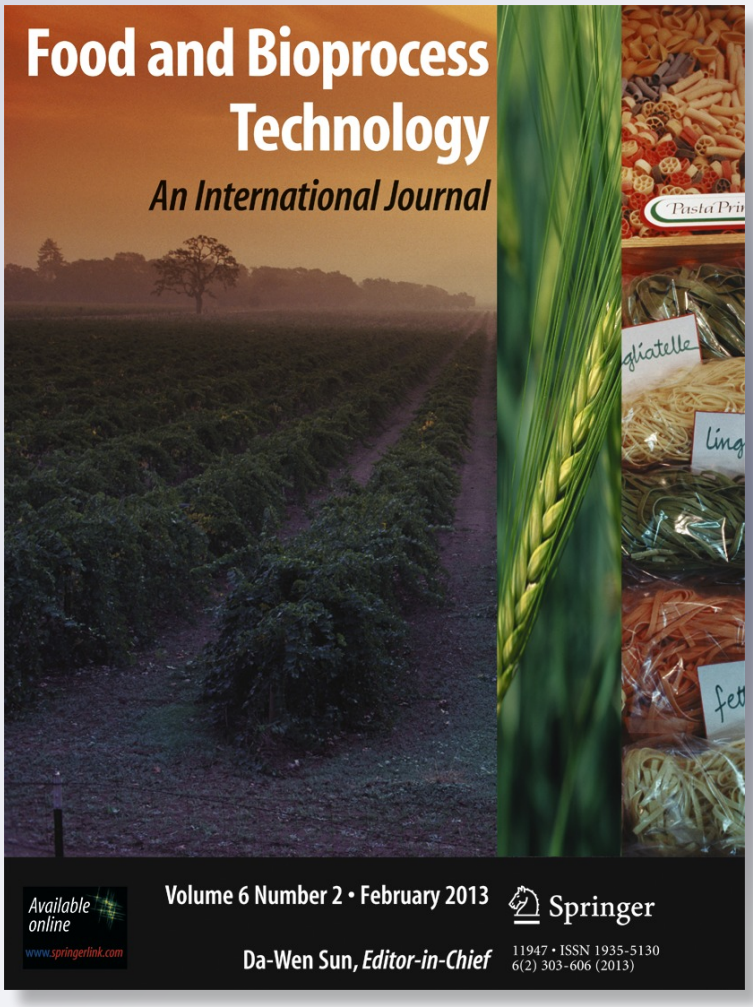
Food and Bioprocess Technology
An International Journal

ISSN 1935-5130
Volume 6
Number 2

Food Bioprocess Technol (2013)
6:475-488
DOI 10.1007/s11947-011-0701-9



Springer

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ORIGINAL PAPER

Relationships between rheological properties, texture and structure of apple (Granny Smith var.) affected by blanching and/or osmotic dehydration

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Received: 5 July 2011 / Accepted: 4 October 2011 / Published online: 26 October 2011

Abstract The objective of this work was to evaluate and correlate rheological properties (small-scale dynamic oscillatory and creep/recovery measurements and large-scale compression force-deformation testing), texture (sensory evaluation by trained panel) and structure (optical and transmission electronic microscopy observations) of apples osmotically dehydrated to water activity (a_w) 0.97 with glucose, with or without previous blanching. All apple samples showed a solid behavior ($G'>G''$) dominating the viscoelastic response, but both dynamic moduli were reduced due to processing. The instantaneous elastic compliance (J_0) and the retarded compliances (J_1 and J_2) increased for treated tissues and the steady-state viscosity (η_N) was approximately 15% to 29% of the value of fresh apple. In general, compression parameters decreased for all treated tissues. Changes in structural features were mainly evidenced in heated samples. Partial least squares regression analysis regression models revealed that texture could be well predicted by rheological properties (compression and creep parameters). Juiciness, crispness and sensory hardness were negatively correlated to J_0, J_1 and J_2, and η_N was negatively correlated to sensory fracturability. Some mechanical parameters (fracturability, hardness 2, area 2, modulus of deformability and cohesiveness) were positively related to sensory fracturability, crispness and sensory hardness; and juiciness was negatively correlated to hardness. Compression and creep parameters showed ability to evidence structure differences (rupture of membranes, swelling of cells and degradation of cell walls) and to explain texture of treated apples.

Keywords Apple · Blanching · Osmotic dehydration · Rheology · Texture · Ultra- and microstructure

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Introduction

Osmotic dehydration is a commonly used operation in processing of fruit and vegetables for obtaining high or intermediate moisture products (Alzamora et al. 1995, 1997), or as a pre-treatment in air drying (Nieto et al. 1998; Ochoa-Martínez et al. 2006) or freezing (Giangiacomo et al. 1994; Dermesonlouoglou et al. 2008) due to many advantages including better retention of color and flavor, better maintenance of cell wall selectivity and less energy requirement compared to convective hot-air drying process (Khin et al. 2007). Some techniques for obtaining high-moisture fruit products with characteristics close to fresh ones are based on a combination of blanching, a slight reduction of water activity (a_w, 0.93–0.98), control of pH (3.0–4.1) and incorporation of antimicrobial agents and other additives to improve color and texture (Alzamora et al. 1995).

In osmotic dehydration, a cellular tissue is immersed in a concentrated solution of sugars or salts and a counter-current mass transfer phenomenon occurs: water diffuses out of the tissue and sugars or salts diffuse into the tissue due to the differences in chemical potential established

between the external solution and the internal liquid phase of the cells. This concentrates the tissue with regard to some solute gain/water loss ratio, depending on process conditions (Chiralt and Fito 2003). Blanching and osmotic dehydration provoke changes in macro-, micro- and ultrastructure of tissues and water distribution, with several modifications that strongly influence the mechanical behavior and, accordingly, the perceived texture (Alzamora et al. 2008). Because of the complex connections and multivariate interdependencies, the material properties of fruit tissues, including mechanical ones, are difficult to predict and to explain (Kunzek et al. 1999).

Rheology measurements have been extensively applied to fruits and vegetables in an effort to understand the relationships between structure, texture and the mechanical changes induced by processing (Jack et al. 1995; Martínez et al. 2007). 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. In addition, the relative percentage of the different tissues, size and shape of the cells, ratio of cytoplasm to vacuoles, volume of intercellular spaces (which may contain either fluids or interstitial air), type of present solutes and presence of starch and its state are also important (Ilker and Szczesniak 1990; Alzamora et al. 2008). In general, the main structure changes affecting mechanical behavior of plant tissues induced by blanching and osmotic treatments are rupture of membranes and loss of cell turgor, degradation of cell walls with alteration of middle lamella and loss of fibrillar organization, establishment of water and solute concentration profiles, changes in air and liquid volume fractions and changes in sample size and shape (Alzamora et al. 1997; Chiralt et al. 2001).

It has been suggested that rheological parameters, and so texture perception, are associated with some structural components of the fruit tissue, reflecting changes that occur at cellular level (Jackman and Stanley 1995; Martínez et al. 2005, 2007; 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 (Bourne 1976; Alzamora et al. 2000; Alzamora et al. 2008). These structure elements, for instance, would mainly influence the values of the storage modulus (G') of dynamic spectra, and the modulus of deformability (E_d) determined in compression tests. On the other hand, Jackman and Stanley (1995) proposed an interpretation of a six-element creep mechanical model to analyze the multiple softening mechanisms in tomato pericarp tissue during ripening. This interpretation has been successfully used for explaining cooked potato and osmotically dehydrated melon and apple creep behavior (Alvarez and Canet 1998; Martínez et al. 2005, 2007). 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.

Because of the need to better understand the relationship between structure, rheology, and texture, the specific aims of this study were: (a) to analyze the linear viscoelastic behavior (as derived by from dynamic oscillatory and creep tests), the texture profile analysis (TPA) attributes, and the structure (by optical and transmission electronic microscopy observations) of osmotically dehydrated apple tissues with or without previous blanching; (b) to examine the correlation between TPA parameters and the sensory assessment of texture from a trained panel; and (c) to explore how differences in apple tissue structure were expressed by viscoelastic, TPA and sensory parameters.

Materials and methods

Preparation of apple samples

Fresh apples (*Malus pumila*, Granny Smith var.; $a_w \approx 0.98$; 10.4–12.2° Brix and pH 3.3–3.4) were obtained from a local market and stored (6–7°C) until use. This variety was chosen because it is readily available throughout the entire year at a fairly constant quality. Five hours prior to use, apples were removed from the refrigerator and left to reach room temperature (20°C). Then they were hand peeled and cut parallel to the main axis using a lathe to obtain parenchyma slabs ($0.060 \times 0.060 \times 0.010$ m for control samples and $0.060 \times 0.060 \times 0.011$ m for samples to be treated). Apple slabs were immediately dipped in distilled water (4–5°C) for 60 s to eliminate cellular fluids and dried with tissue paper. 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 less than 0.005 m were used.

The same lot of fruit was used in all the experiments in order 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.

Blanching and osmotic dehydration

Blanching was carried out by immersion of fruit slabs in saturated steam for 90 s. Samples were immediately immersed in distilled water at 4°C for 20 s and put on blotting paper three times to eliminate superficial water.

Osmotic dehydration were performed by immersing apple slabs (with or without previous blanching) into a 22% (*w*/*w*) glucose (®Cerelose, glucose monohydrate, food grade, Productos de Maíz S.A, Argentina) aqueous solution with forced convection at atmospheric pressure and 20°C to reach an equilibrium water activity (a_w) value approximately equal to 0.97 (≈6,5 h). The agitation level was chosen in order to make the surface mass transfer resistance negligible (Salvatori and Alzamora 2000). A large weight ratio of syrup/apple (60/1) was used in the osmotic treatment to minimize the dilution effect on the glucose solution by water and glucose transport. Apple samples were removed, immersed into distilled water for 20 s, and put on blotting paper three times to eliminate superficial syrup.

Blanched (B), osmotically dehydrated (OD), and blanched and osmotically dehydrated (ODB) samples were then examined for a_w and soluble solids, rheological properties, texture characteristics and structure. All treatments were compared to a control (C, fresh apple).

Determination of a_w and soluble solids

The a_w of the samples was measured with an Aqua-Lab CX-2 (Decagon Devices Inc., Pullman, WA) water activity meter at 20°C, calibrated according to Roa and Tapia de Daza (1991). Soluble solids content per cent (z_{ss}, wet basis) in the fruit homogenate was analyzed by measuring the refraction index in a refractometer (ATAGO CO., Ltd., Fukui, Japan) at 20°C. Determinations were made in triplicate.

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. Apple cylinders (0.030 m in diameter) were cut from the treated slabs of nearly 0.010 m final thickness using a cork borer. This thickness was selected based on previous experiments which indicated that for values equal or greater than 0.0060 m, viscoelastic measurements were not dependent on sample thickness (Martínez et al. 2005). 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 for 100 s. A previous stress sweep by varying the applied stress from 10 to 50 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 an additional period of 200 s. Previously to the creep assay, the sample was subjected to repeat loading and unloading cycles in order the material loss the long time memory and 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):

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

where J (t, τ) is the creep compliance ($J=\gamma\ (t)/\tau$ with γ(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 ($J_i=\eta_i \times J_i$) 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.

Instrumental texture evaluation

An Instron Universal Testing Machine model 3345 (Canton, Massachusetts, USA), with a 5,000 N compression load cell interfaced with a series data acquisition software (Bluehill 2, v. 2.17, Instron, USA), was used to conduct the TPA analysis (Bourne 1978; Bourne and Comstock 1981). A two cycle compression was set to 70% deformation.

Tests were performed with a crosshead speed of 0.001 m/s and a 0.035-m diameter cylindrical probe. The parameters 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) were obtained from the force–time curves, according to the definitions of Bourne (1978).

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

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

$$\sigma_R = F(t)[(H_0 - \Delta H)/A_0 H_0] \tag{4}$$

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

where $F(t)$ is the compression force at time t; H_o is the height of the sample before compression; ΔH difference of the height of the sample before compression and during compression; A_o cross-sectional area of the cylinder before compression. The test was replicated a minimum of 20 times and mean values for each parameter were calculated.

Sensory descriptive analysis

Nine panelists (three males and six females), all between the ages of 21–38, composed the sensory panel. For the selection of panelists, 12 persons were recruited from the 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) to recognize and measure texture attributes of hardness (SH), fracturability, cohesiveness, adhesiveness to palate, juiciness and crispness using the standard rating scales (Chauvin et al. 2008; 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 known food references and the 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. A glass of water and unsalted crackers were used for rinsing their mouth and cleaning their teeth between sample evaluations. In the beginning of each session, panelists were thoroughly refreshed with the definitions and techniques for measuring texture attributes in which they had been trained earlier. They used evaluation forms and scored intensities on the line scale (0–17, depending on the texture scale). All sessions were conducted in individual booths under white light and in an environment-controlled sensory analysis laboratory. The descriptive analysis of the samples was replicated two times in the same session.

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 then rinsed three times with distilled water, postfixed in OsO_4 solution (1.5 g/100 g) at room temperature and dehydrated in a graded acetone series prior to be embedded in low viscosity Spurr resin. Sections (1–2 μm thick) of the Spurr-embedded tissue were cut on a Sorvall MT2-B Ultracut microtome and stained with toluidine blue (1 g/100 g) and basic fuchsin (1 g/100 g) solutions (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 electronic 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 (Reynolds 1963). Sections were examined using a JEOL JEM-1200 EX II (Japan) transmission electron microscope at an accelerating voltage of 80 kV.

Statistical analysis

Instrumental and sensory data were expressed as mean ± standard deviation of the mean (mean ± SD). Two-way analysis of variance 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 $\alpha<0.05$. Turkey's test was performed in case of finding significant differences.

Multivariate analysis of variance was used detect differences among samples in rheological data (viscoelastic properties and instrumental texture profile analysis). Significance level was set at $\alpha<0.05$. Hotelling corrected for Bonferroni test was performed in case of finding significant differences. 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 (Martens and Martens 1986). Both sensory and instrumental variables were standardized previously to the PLS analysis. Osten's F test was used to determine the number of significant ($p\leq0.05$) factors. The GenStat statistical language (GenStat discovery edition 3, Oxford, UK) was used for these analyses.

Results and discussion

Dynamic moduli

The LVR ranged between 0.001% and 0.009% for fresh apples and between 0.001% and 0.1% for treated apples (Fig. 1a). Accordingly, a strain value equal to 0.005% was selected for frequency sweep test to assure linearity for all samples. The average mechanical spectra for fresh and treated apples are presented in Fig. 1b, c. All apple samples had a solid behavior with G' exceeding G'' over the entire frequency range, but both dynamic moduli were reduced due to processing, denoting that tissues became less viscous and less elastic after treatments. The values of tan δ for the different treatments ($G''/G'=0.08$–0.15) did not show any clear trend (Fig. 1d). Storage (G') and loss (G'') moduli for fresh and processed tissues showed a weaker dependence on frequency. The storage modulus G' slightly increased with increasing angular frequency, with a slope of the linear log G'–log ω behavior significant ($p\leq0.05$) greater for treated samples (n values were 0.045±0.005; 0.060±0.004; 0.055±0.003 and 0.056±0.003 for C, B, OD and ODB samples, respectively). The frequency dependence of G'' was more complex. The curves of log G'' vs. log ω consisted of one shallow negative (nearly a plateau) slope at low frequencies and a positive slope at high frequencies. The weak frequency dependence of G' and G'' would indicate a behavior similar to a flocculated gel with a three-dimensional network structure for all apple samples. However, the different dynamic mechanical spectra of raw and treated tissues revealed a change in the microstructure of the material: the decrease in the level of elastic modulus might be correlated to a loss of rigidity in the network.

The parameters of the linear regression represented by Eq. 1 showed significant differences ($p<0.05$) between fresh and treated samples but there were not significant differences between samples subjected to the different treatments. Thus, mechanical spectrum analysis was not enough sensitive for distinguishing physical differences between treatments assayed.

The pattern found for dynamic spectra (G' and G'' modules vs. frequency) were in agreement with those previously reported for apple (Martínez et al. 2007), potato

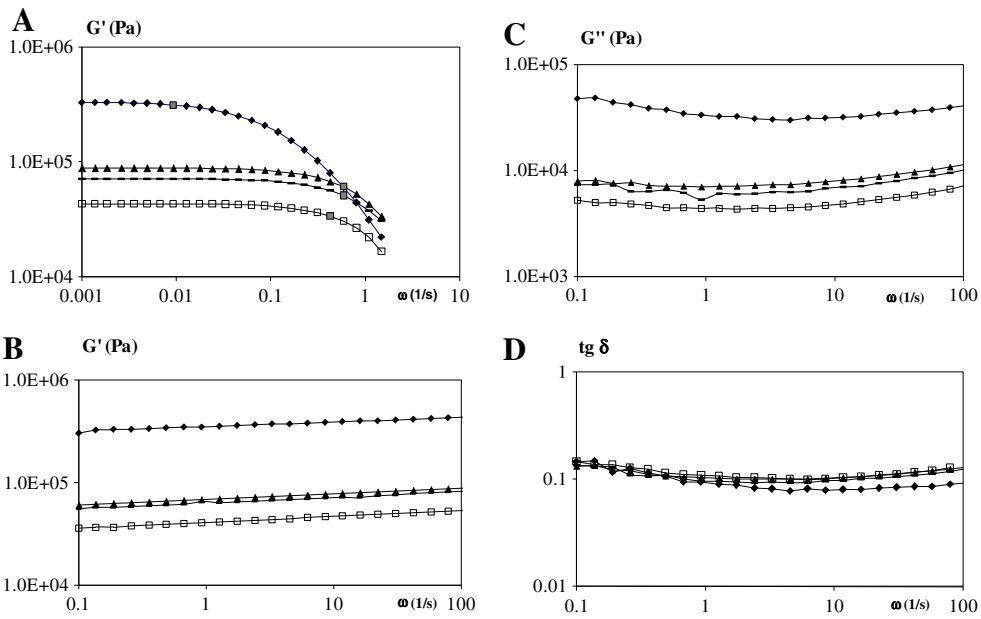

Fig. 1 Mechanical spectrum for fresh apples and apples subjected to blanching and/or osmotic dehydration. **a** Sweep amplitude; **b** storage modulus G'; **c** loss modulus G''; and **d** tan δ. Fresh (*filled diamonds*), blanched (*empty squares*), osmotically dehydrated (*filled triangles*) and blanched and osmotically dehydrated (*dashed line*), LVR (*filled squares*)

(Alvarez and Canet 1998), melon (Martínez et al. 2005) and Korla pear (Wu and Guo 2010).

Creep-recovery behavior

Representative creep/recovery curves for fresh and treated samples are presented in Fig. 2. Blanching and/or osmotic treatment caused relevant changes in both creep strain at 100 s and residual strain after an additional 200 s recovery. For the time scale of the experiments, the creep response of fresh and treated tissues 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, the weaker bonds breaking at smaller values of time than the stronger ones. 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 and the time required for them to reform is longer than the creep-recovery period; the released units will flow and part of the structure is not recovered. The relatively large variability associated with creep parameters (standard deviations) observed in Table 1 is common when evaluating viscoelastic properties of plant tissues and can be attributed to many factors, such as no homogeneity 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 ($p<0.0001$) between fresh and treated samples and also between apples subjected to OD and heated apples (subjected to ODB or B).

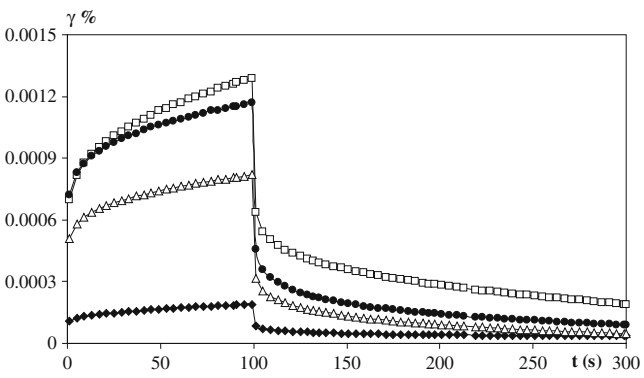

Fig. 2 Typical experimental creep/recovery curves (deformation γ vs. time t) for fresh apples and apples subjected to blanching and/or osmotic dehydration. Fresh (*filled diamonds*), blanched (*empty squares*), osmotically dehydrated (*empty triangles*) and blanched and osmotically dehydrated (*filled circles*)

The instantaneous elastic compliance J_0 and the retarded compliances (J_1 and J_2) increased in value for treated tissues relative to untreated one, revealing a decrease in the instantaneous elastic moduli E_0 ($1/J_0$) and in the elasticity or stiffness associated with Kelvin–Voigt elements. When both springs of the Voigt units are completely displaced, the constant increase of deformation is associated with the steady-state viscosity. An increase in the steady-state viscous compliance ($1/\eta_N$) occurred in the samples exposed to the different treatments; the values of η_N were approximately 15% to 29% of the value of fresh apple. For a given treatment, the relative increases in J_0, J_1 and J_2 regarding the values of the fresh fruit were similar. The lower increases in elastic and retarded compliances were exhibited by OD samples. Retardation time, λ_1, slightly decrease for treated tissues, whereas retardation time, λ_2, slightly increase for heated samples.

A multivariate approach to data analysis by PCA showed the spatial relationships of the six creep parameters for each apple sample. A two-dimensional representation is shown in Fig. 3. The first two principal components explained 85.9% and 11.7% of the variance, respectively, in the PCA of the creep data. The first contrasted J_0, J_1 and J_2 positively and η_N negatively. The second axis was positively defined by λ_1 and λ_2. Fresh apple, placed to the left of the graph, showed high η_N and λ_1. ODB and B apples, placed to the right of the graph, were mainly characterized by an increase in J_0, J_1, and J_2 and a decrease in η. On the other hand, OD sample, located in the middle of the graph, exhibited compliances greater than those corresponding to fresh apple but lower than those of heated samples (B and ODB).

There were minor changes in the contribution of each type of compliance to overall compliance because of the treatments. All samples exhibited plastic strain which remained unrecovered in the creep/recovery test. Plasticity values (the ratio of unrecoverable or permanent deformation, t/η_0, to the total deformation, $J(t,\tau)$, of treated samples were rather similar to those of untreated ones (14.6%, 18.9%, 15.6% and 15.5% for C, B, OD and ODB samples, respectively).

Instrumental texture evaluation

Figure 4 shows typical compression-time curves for fresh and treated samples. Compression curves of all apple samples exhibited the typical shape of hard materials, with abrupt rupture peaks. Table 2 shows the means and the standard deviations corresponding to the instrumental parameters of evaluated samples. Significant differences ($p<0.0001$) were observed among all samples. Hardness 2, area 1, cohesiveness, springiness, gumminess, chewiness and deformability modulus values decreased for all treated

Table 1 Means of viscoelastic parameters derived fitting Eq. 2 to compliance curves from creep phase for fresh (C), blanched (B), osmotically dehydrated (OD) and blanched plus osmotically dehydrated (ODB) apples

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	0.36±0.14 54.9[a]	0.11±0.05 16.8[a]	0.06±0.03 13.6[a]	26.8±7.5	2.29±0.76	9.4±3.9 14.6[a]	a
B	2.1±0.4 51.3[a]	0.71±0.13 18.7[a]	0.41±0.06 10.9[a]	23.7±2.7	2.7±0.3	1.42±0.38 18.9[a]	b
OD	1.4±0.2 59.1[a]	0.35±0.13 15.1[a]	0.24±0.05 10.2[a]	22.5±2.5	2.39±0.24	2.7±0.5 15.6[a]	c
ODB	2.1±0.4 55.6[a]	0.7±0.1 18.1[a]	0.41±0.06 10.7[a]	23.75±2.71	2.67±0.29	1.69±0.25 15.5[a]	b

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

[a] Percent contribution to the overall compliance at the end of the creep phase

tissues (mainly for B and ODB samples) with respect to control. But OD apple showed a slightly increase in hardness after treatment.

A two-dimensional representation of PCA is presented in Fig. 5. The first two principal components explained 84% and 9.8% of the variance, respectively. The first contrasted fracturability, hardness 2, area 1, cohesiveness, springiness, chewiness and gumminess positively. The second axis was defined positively by hardness and negatively by deformability modulus. Fresh apple showed high hardness 2, area 2, cohesiveness, fracturability and deformability module. This sample was placed to the right and in the lower half of the graph. Osmotic sample was placed to the right and in the higher half of the graph, indicating that this sample was characterized by hardness, chewiness, springiness, area 1 and gumminess. Blanching and blanching plus osmotic dehydration samples were placed to the left of the graph, indicating that they showed a decrease in all texture parameters.

These results were in partial agreement with those reported in the literature. Castelló et al. (2009) found that osmotic treatment with a glucose solution (Granny Smith var.) decreased hardness, modulus of deformability and area under the curve (area 1). Khin et al. (2007) reported a decrease in hardness, fracturability and springiness in apples (Fuji var.) osmotically dehydrated with 61.5% (*w*/*v*) sucrose solution. Matuska et al. (2006) studied the effect osmotic dehydration (61.5% (*w*/*v*) sucrose) in strawberry and found that treated sample and control had the same hardness.

Sensory texture evaluation

The means and their standard deviations for every evaluated attribute are presented in Table 3. There were no significant differences among panelists (Tukey's test, $p<0.05$) for the texture attributes hardness, cohesiveness, juiciness and adhesiveness to palate, thus showing consistency in the evaluations (data are not shown).

Sensory hardness showed significant differences ($F_{3,\ 12}=5.52$, $p=0.0063$) between C and B apple samples, and

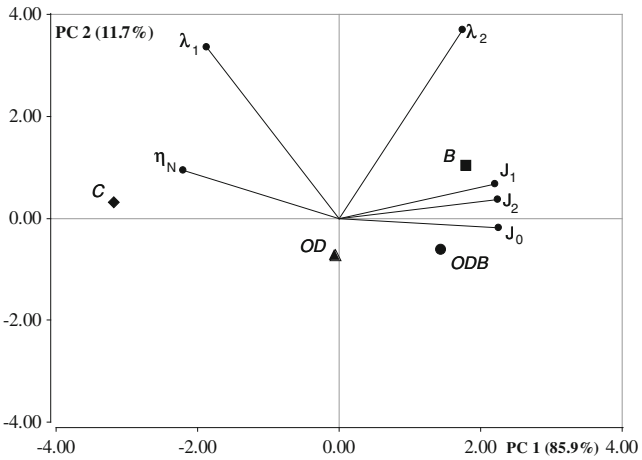

Fig. 3 Principal component analysis bi-plot of creep curve parameters used to differentiate fresh and treated apple tissues by different parameters

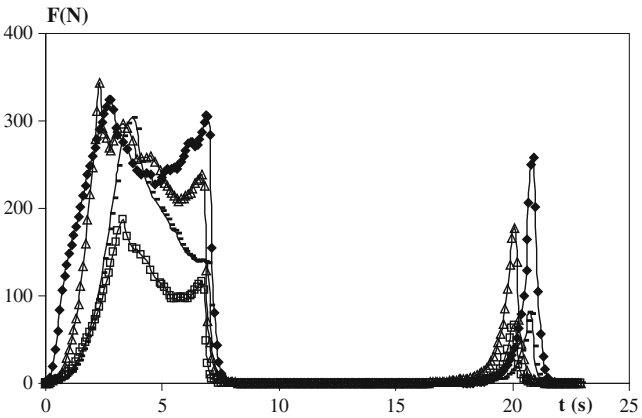

Fig. 4 Typical TPA force/time curves for fresh and treated apple tissues. Fresh (*filled diamonds*), blanched (*empty squares*), osmotically dehydrated (*empty triangles*) and blanched and osmotically dehydrated (*dashed line*)

Table 2 Mean values and standard deviation of TPA parameters and deformability modulus for fresh (C), blanched (B), osmotically dehydrated (OD) and blanched plus osmotically dehydrated (ODB) apples

Treatment	*F* (N)	*H* (N)	H_2 (N)	A_1 (J)	A_2 (J)	*S* (−)	Coh (−)	*G* (N)	Chew (N)	E_d (N/m^2)	
C	287.9±31.1	293.2±30.3	225±20	1.39±0.13	0.11±0.01	0.55±0.06	0.081±0.006	23.9±3.3	13.2±2.9	1.92±0.53	a
B	196.2±24.5	196.2±24.5	80.4±9.3	0.7±0.1	0.03±0.01	0.33±0.03	0.04±0.01	8±1	2.6±0.5	0.11±0.02	b
OD	337±30	337±30	187.4±22.7	1.31±0.13	0.08±0.01	0.53±0.06	0.064±0.007	21.3±2.6	11.4±2.4	0.17±0.03	c
ODB	nd	288.4±25.2	92.6±14.1	0.93±0.09	0.041±0.005	0.41±0.05	0.045±0.006	12.8±2.1	5.3±1.3	0.09±0.01	d

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

nd non-detected

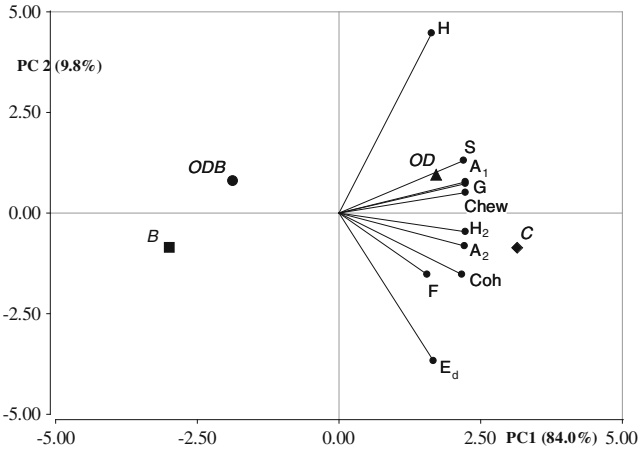

Fig. 5 Principal component analysis bi-plot of TPA instrumental parameters used to differentiate fresh and treated apple tissues by different texture attributes

between C and ODB apple samples. There were not significant differences between C and OD apple samples. Trained panel assessments perceived a decrease in hardness in the treated samples.

Sensory fracturability of treated samples decreased significantly ($F_{3,\ 24}$=52.3, p<0.05) with respect to the control. Assessor factor was significant ($F_{8,\ 24}$=10.4, p< 0.05), suggesting that panel performance was not consistent in their evaluation. The panelists showed difficulty to assess the fracturability in apple samples. However, all panelists scored the treated apples as less fractured than the control samples. Thybo and Martens (1998) reported that the trained assessors had difficulty in detecting by texture profile method differences in oral hardness and fracturability of cooked potatoes.

Cohesiveness did not show significant differences ($F_{3,\ 15}$= 1.97, p>0.05) between the samples. Treated samples (B and ODB) increased slightly its cohesiveness due to treatments. These results did not correlate with the instrumental rating. In a previous work, Garcia Loredo and Guerrero (2011) found that some sensory ratings assigned to food samples when assessors evaluated cohesiveness scales did not properly correlate with the instrumental measurements. A cohesive sample which exhibits little or no springiness (such as apple) will have very low values for A_2/A_1 because there will be scarce contact surface between the probe and the sample during the second compression. Meullenet et al. (1998) reported that the evaluation of the A_2/A_1 ratio was dependent on the evaluation of d_2/d_1 ratio (i.e. springiness of the product) and the poor correlation between instrumental and sensory cohesiveness in samples without springiness. Description of sensory perception of cohesiveness may not be well characterized by one physical measurement (A_2/A_1) when comparing samples from different food systems, but

Table 3 Mean values and standard deviation of texture parameters for fresh and treated apple tissues evaluated by the panellists

Treatment	Crispness	Juiciness	SF	SC	SH
C	7.52±0.06 a	6.02±0.29 a	6.39±2.91 a	2.33±1.85 a	7.92±0.98 a
B	5.22±0.7 b	5.11±0.42 b	5.41±1.73 b	2.86±1.41 a	6.56±0.69 b
OD	6.66±0.45 a	5.06±0.41 b	5.78±2.08 a, b	1.89±1.01 a	7.07±1.19 a, b
ODB	5.58±0.63 c	4.49±0.28 c	4.71±2.49 b, c	2.54±1.48 a	6.27±0.85 b

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

SH hardness, *SF* fracturability, *SC* cohesiveness

could be a good indicator in a limited group of foods such as gels (Meullenet et al. 1998).

Significant differences were observed in juiciness ($F_{3,\ 24}=73.7$, $p<0.05$) between control and treated samples. No significant differences were observed between OD and B apples. Juiciness of treated samples decreased during treatment. Harker et al. (2006) observed that juice release in apples was dependent on the breakdown of individual cells and varied between firm and soft apples. They commented that in firm apples (control), tissue fracture is associated with breakage of individual cells and results in the release of cytoplasm fluids. In soft apples (such as B and ODB ones), fracture occurs as a result of cell-to-cell debonding. Individual cells do not always break open and release their contents, resulting in a mealy apple. The observations of Harker et al. (2006) are supported by this study: a decrease in juiciness was observed with a decrease in hardness in heated apples.

Crispness of blanched samples (B and ODB) significantly decreased ($F_{3,\ 12}=10.9$, $p<0.05$) with respect to control samples. There were significant differences between B and ODB apples but not between C and OD samples. All assessors informed that all samples did not present adhesiveness to palate.

Chauvin et al. (2010) reported significant changes in the attributes crispness, hardness, fracturability and juiciness in apples with soft, intermediate and hard firmness levels. Hard apples showed the highest values in crispness, fracturability, hardness and juiciness. The results in this work were consistent with Chauvin et al's. findings. C and OD apples presented the highest values in hardness, fracturability, crispness and juiciness while that the lowest values were observed in blanched samples (B and ODB) with or without osmotic treatment.

PLS analysis

PLS regression analysis was performed using sensory attributes as *y*-variables and rheological parameters as *x*-variables.

Mechanical measurements

About 75% of the variability in sensory attributes could be explained by mechanical measurements using PLS regression model with two PLS factors. Approximately 59.3% of the variability was explained by PLS factor 1 and 15.3% by PLS factor 2. An inclusion of more than two PLS factors did not improve the prediction (data not shown), and therefore, the remaining PLS factors were omitted from the prediction. This was confirmed by Osten's *F* test which determinate that PLS-1 and PLS-2 were valid predictors ($p\leq0.05$).

The PLS factor 1 explained 73.5% of the *x*-variables and 59.3% of the *y*-variables and the PLS factor 2 explained 11.3% of the *x*-variables and 15.3 of the *y*-variables (data not shown).

According to this model, crispness, juiciness, sensory hardness and sensory fracturability were the best explained properties (approximately 69.5%, 57.5%, 37% and 22% of the variance explained, respectively) by PLS factor 1 while juiciness and instrumental hardness were the best explained properties (approximately 92% and 35.7% of the variance explained, respectively) by PLS factor 2 (Fig. 6). C and OD samples were located on the right along the PLS factor 1

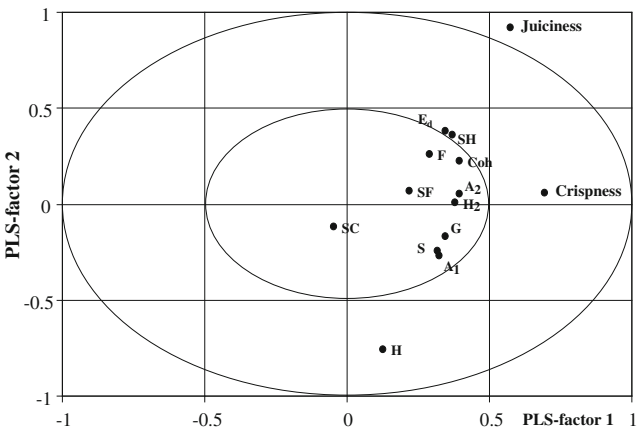

Fig. 6 Plot of *X*- and *Y*-loadings for the TPA parameters of fresh and treated apple tissues

while heated samples (B and ODB apples) were mostly located on the left. Thus, C and OD samples had higher sensory evaluation scores than B and ODB samples (data not shown). The position of hardness 2, fracturability, area 1, area 2, cohesiveness, springiness and E_d on the right side of the plot along PLS factor 1 indicated that these parameters were positively related to the sensory attributes fracturability, crispness and hardness. Along the PLS factor 2, hardness was negatively correlated to juiciness. Sensory cohesiveness had a small contribution to variability in sensory properties because of its small loadings for both PLS factors 1 and 2.

Creep curve parameters

Eighty-three percent of the variance in sensory attributes was explained by the first two PLS factors. Approximately 73% and 10.7% of the variability was explained by PLS factors 1 and 2, respectively. Osten's F test showed that PLS 1 and 2 were valid predictors ($p \leq 0.05$). The PLS factor 1 explained 58% of the x-variables and 73% of the y-variables and the PLS factor 2 explained 21% of the x-variables and 10.7% of the y-variables (data not shown). Crispness, juiciness and hardness were the best explained sensory properties (approximately 59%, 61% and 22% of the variance explained, respectively) by PLS factor 1 and sensory cohesiveness and fracturability were the best explained properties (approximately 66% and 69% of the variance explained, respectively) by PLS factor 2.

Figure 7 shows that juiciness, crispness and sensory hardness were negatively correlated to instantaneous and retarded compliances (J_0, J_1 and J_2). The steady-state viscosity (η_N) was negatively correlated to sensory fracturability and the second retarded time (λ_2) was positively correlated to sensory cohesiveness. The first retarded time (λ_1) had a small contribution to variability in sensory properties compared with other parameters because of it had small loadings for both PLS factors 1 and 2.

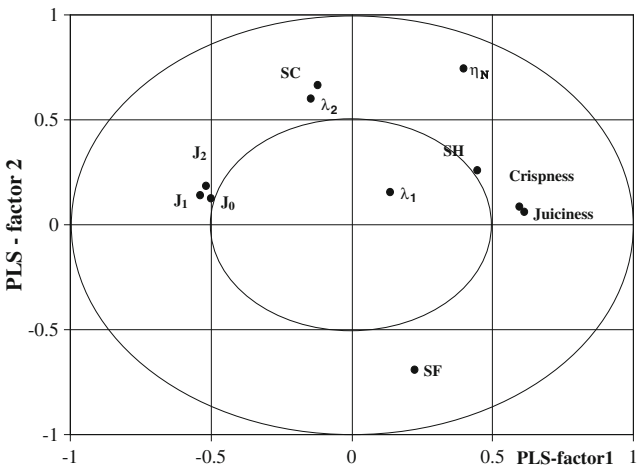

Fig. 7 Plot of X- and Y-loadings for the creep curve parameters of fresh and treated apple tissues

Structural features analysis

LM and TEM studies were performed to evaluate structure changes produced at cellular level by the different treatments. Some LM and TEM microphotographs of treated apples, as compared with fresh fruit, are shown in Figs. 8 and 9, respectively.

Cells and intercellular spaces in the fresh tissue ($a_w \cong 0.99$; 11.5±0.7°Brix) were loosely arranged in a net-like pattern that was inhomogeneous and anisotropic (Fig. 8a, b). Intercellular spaces exhibited various shapes and sizes. 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 a thin layer lining the cell surface. Cell walls appeared darkly stained, with greater intensity in the central zone of the middle lamella, and with a loose reticulate fibrilar pattern (Fig. 9a, b). Tonoplast and plasmalemma appeared intact.

Blanching of tissue caused disruption of cellular membranes (Figs. 8c, d and 9c, d). Cell walls appeared less stained than in the fresh fruit and cells became more irregular in shape. Fibrillar organization was modified, and cell walls exhibited low electronic density (except near the wall surface) as evidenced by the lack of staining (Fig. 9c, d). In some areas, the central zone appeared more electronically dense, indicating the presence of the middle lamella. In some images (not shown), fibrillar distribution became striated.

Osmotically treated tissues ($a_w \cong 0.97$; 17.1±0.0.6°Brix) showed a cell arrangement similar to control and walls appeared more smoothed (Fig. 8e, f). Cell walls showed a good electron density and a very thick middle lamella (Fig. 9e, f).

On the contrary, blanched plus osmotically dehydrated tissues ($a_w \cong 0.96$; 19.0±0.5°Brix) exhibited disruption of membranes, folding of cell walls and increased cell-to-cell contact (Figs. 8g, h and 9g, h).

Plasmodesmata appeared well conserved in fresh and treated fruits (TEM micrographs not shown).

Relationship between structure, rheological properties and texture perception

All assayed tests (instrumental texture profile analysis, oscillatory and creep-recovery tests, sensory evaluation) were sensitive distinguishing structure differences among fresh and osmotically dehydrated apples with or without previous blanching. TPA and creep parameters also allowed

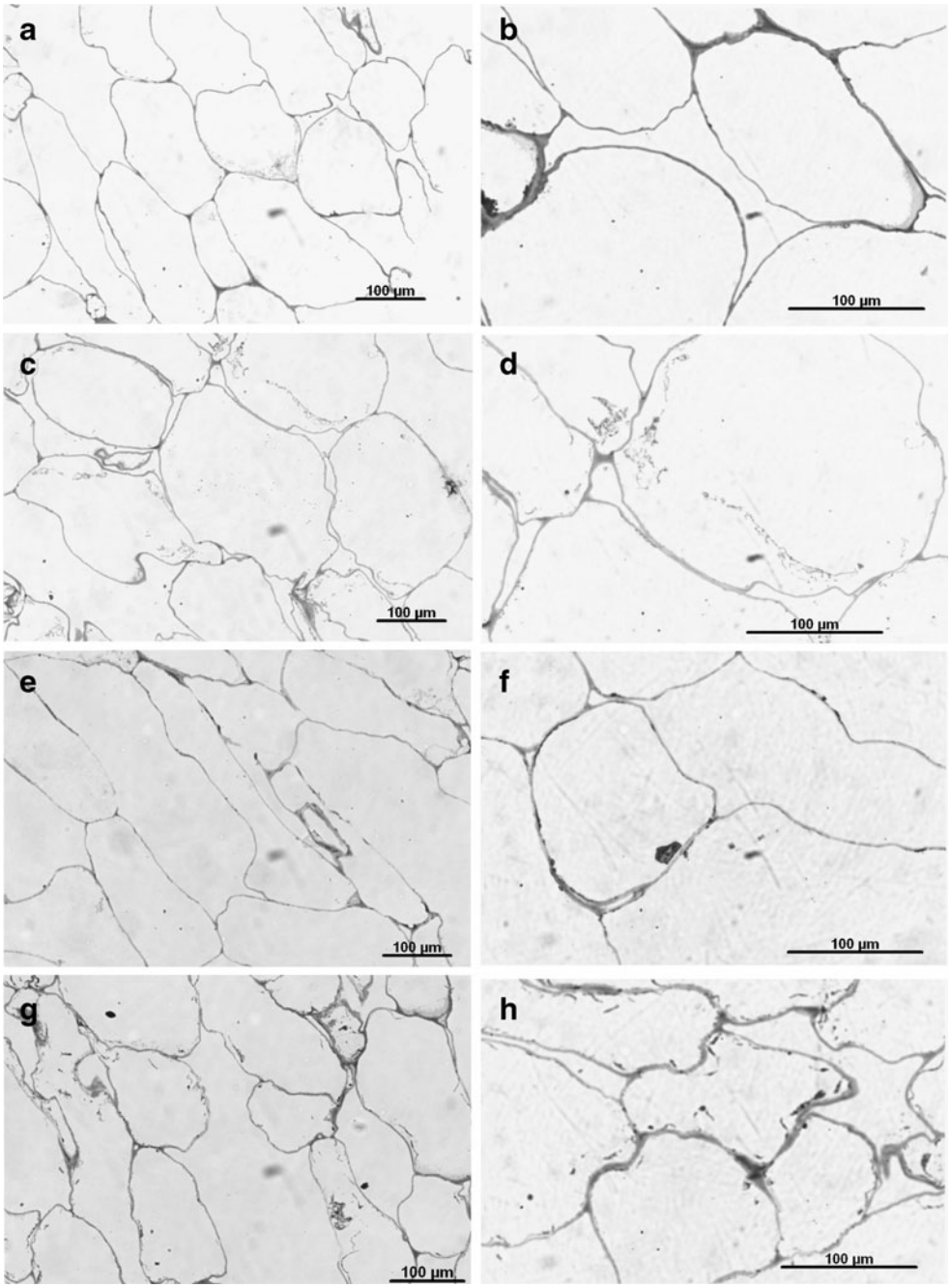

Fig. 8 LM images from fresh and treated apple tissues. **a**–**b** Fresh (control), **c**–**d** blanched, **e**–**f** osmotically dehydrated and **g**–**h** blanched plus osmotically dehydrated

discriminating between the structure of B, OD and ODB samples and OD and heated (B and ODB) samples respectively, but G' and G'' modules did not differentiate the treatments.

Intercellular adhesion seemed to be maintained by the plasmodesmata structure in all treated tissues and also by pectic substances of the middle lamella in OD apples and in some regions of B and ODB apples. Accordingly, fracture would occur as a result of cell wall breaking instead of cell-to-cell debonding.

All assayed treatments provoked a loss of cell turgidity due mainly to plasmolysis of cytoplasm and/or disruption of cellular membranes, and consequently, apple tissues showed lower E_d, lower G' and increased breaking strain and J_0 values. Besides turgor loss, alterations in cell wall structure (redistribution of fibrils, folding of walls, potential degradation and solubilisation of macromolecules), more accentuated in heated tissues, would be the main responsible in TPA parameters decrease and compliance increased. The slightly greater resistance to deformation and hardness shown by OD apples compared with C and heated apples and its smaller J_i and $1/\eta_N$ values compared with those of heated (B and ODB) samples could be explained by the greater integrity of walls and reinforced middle lamella

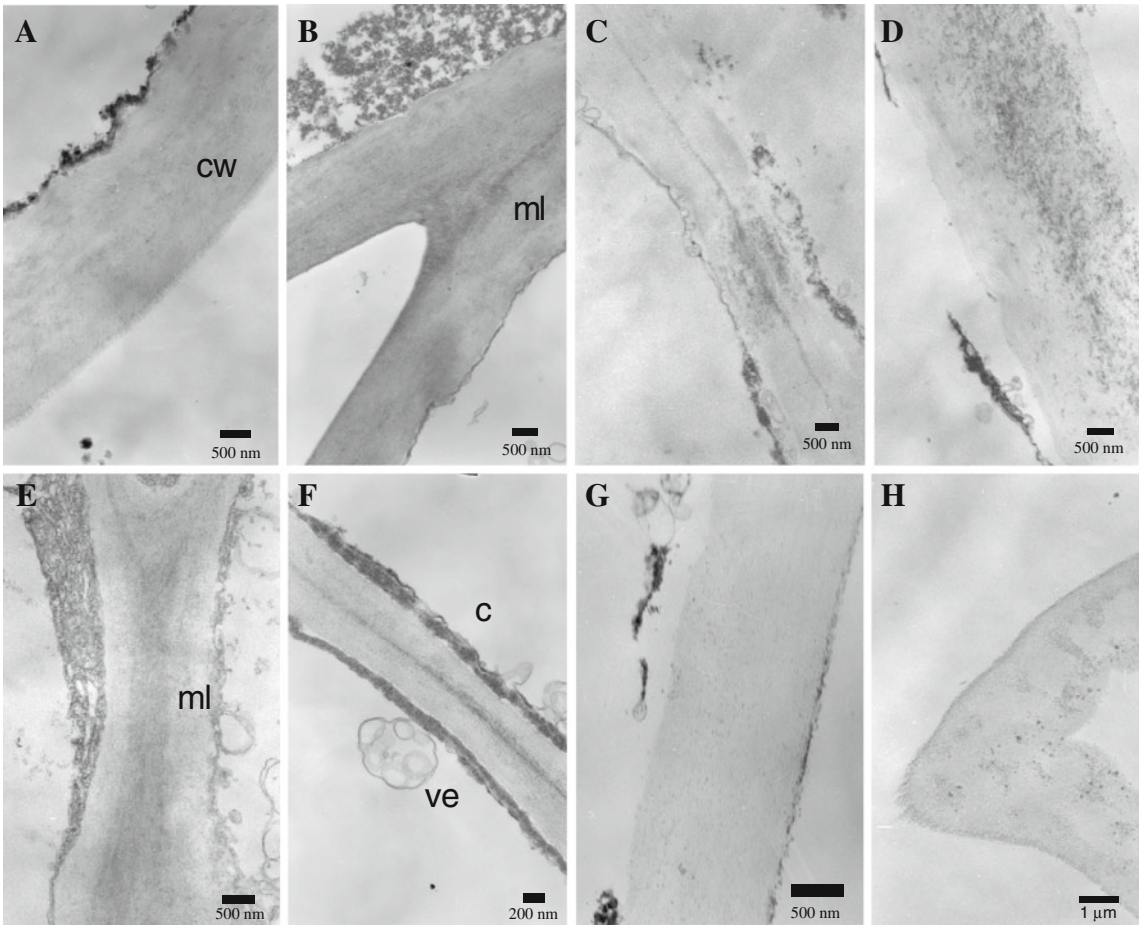

Fig. 9 TEM micrographs from fresh and treated apple tissue. **a**–**b** Fresh (control), **c**–**d** blanched, **e**–**f** osmotically dehydrated and **g**–**h** blanched plus osmotically dehydrated. *cw* cell wall, *c* cytoplasm, *ml* middle lamella, *ve* vesicle

zone observed in Fig. 9e. The severe changes in wall structure provoked by heating (B apples) were evidenced by creep parameters but the notorious additional deteriorative action of the osmotic treatment after blanching (ODB samples), traduced in folding and collapse of cell walls and fibrillar pattern modification, was only evidenced by TPA test.

PLS analysis founded clear-negative correlations between instantaneous (J_0) and retarded (J_1 and J_2) elastic compliances with the sensory attributes crispness, juiciness and hardness. These correlations were consistent with the observed changes at the cellular level, i.e. the loss of turgor and changes in the cell wall were detected at both levels, sensory and instrumental. The assessors perceived a decrease in crispness (turgor), juiciness and hardness (cell wall modification). The decrease in η_N values of treated samples (i.e. the greater fluidity of the cell wall matrix due to the greater amount of apoplastic water caused by membrane breakage and to solubilization and degradation of pectins and other wall biopolymers) was sensory traduced in lower fracturability.

Some mechanical parameters (hardness 2, fracturability, area 1, area 2, cohesiveness, springiness and E_d) were positively related to sensory fracturability, crispness and sensory hardness; and juiciness was negatively correlated to hardness. It is clear from Fig. 6 that SH, located near E_d, was thus more influenced by turgor pressure, while juiciness, correlated with hardness, was mainly dependent on wall structure.

Conclusions

Blanching and/or osmotic treatments significantly influenced compression and viscoelastic properties of apple as well as texture. Overall, a loss in tissue rigidity and a decrease in TPA parameters; an increase in creep compliances and fluidity, and a decrease in storage and loss modules, crispness, juiciness, sensory fracturability and sensory hardness were induced by the treatments. Many changes in material parameters could be partially explained by the alterations in the micro and ultrastructure of the tissues. Changes in structural features

were mainly evidenced in heated samples. PLS regression models revealed that texture could be well predicted by rheological properties (compression and creep parameters). Juiciness, crispness and sensory hardness were negatively correlated to J_0, J_1 and J_2; and η_N was negatively correlated to sensory fracturability. Some mechanical parameters were positively related to sensory fracturability, crispness and sensory hardness; and juiciness was negatively correlated to hardness.

Acknowledgments The authors acknowledge the financial support from the University of Buenos Aires, CONICET, Agencia Nacional de Promoción Científica y Tecnológica of Argentina and BID.

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