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Calcium and ascorbic acid affect cellular structure and water mobility in apple tissue during osmotic dehydration in sucrose solutions

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CHEMICAL COMPOUNDS STUDIED IN THIS ARTICLE

Ascorbic acid (PubChem CID: 54670067)
Calcium lactate (PubChem CID: 16211540)

KEYWORDS

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Mass transfer
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NMR

ABSTRACT

The effects of the addition of calcium lactate and ascorbic acid to sucrose osmotic solutions on cell viability and microstructure of apple tissue were studied. In addition, water distribution and mobility modification of the different cellular compartments were observed. Fluorescence microscopy, light microscopy and time domain nuclear magnetic resonance (TD-NMR) were respectively used to evaluate cell viability and microstructural changes during osmotic dehydration. Tissues treated in a sucrose– calcium lactate–ascorbic acid solution did not show viability. Calcium lactate had some effects on cell walls and membranes. Sucrose solution visibly preserved the protoplast viability and slightly influenced the water distribution within the apple tissue, as highlighted by TD-NMR, which showed higher proton intensity in the vacuoles and lower intensity in cytoplasm-free spaces compared to other treatments. The presence of ascorbic acid enhanced calcium impregnation, which was associated with permeability changes of the cellular wall and membranes.

1. Introduction

The concentration of plant foods by immersing solid food pieces in a hypertonic solution consisting of salt, sugar, glycerol, or other humectants is known as osmotic dehydration (OD) (Sereno, Moreira, & Martinez, 2001). This technique reduces the a_w of the product without a phase change because the flow of water from the product into the concentrated solution is compensated by the solutes migration from the solution into the product (Nieto, Vicente, Hodara, Castro, & Alzamora, 2013). This process permits the formulation of products with intermediate moisture content through dewatering and impregnation of desired solutes (Barrera, Betoret, & Fito, 2004). Because of its versatility, OD has a wide range of applications in the development of minimally processed plant foods or as pretreatment for other preservation methods such as freezing or drying (Alzamora, Cerrutti, Guerrero, & López- Malo, 1995; Garcia Loredo, Guerrero, Gomez, & Alzamora, 2013).

The addition of calcium in osmotic solutions has been widely used in plant foods as fortifier and to enhance firmness (Anino, Salvatori, & Alzamora, 2006; Barrera, Betoret, Corell, & Fito, 2009; avroudis, Gidley, & Sjöholm, 2012; Silva, Fernandes, & Mauro, 2014a). Fortification using combinations of substances such as cal- cium and iron (Barrera et al., 2004) or Ca and vitamin C (Silva, Fernandes, & Mauro, 2014b) has also been investigated.

OD causes physical modifications of cell membranes and cell walls, which affects the rheological properties and state of water (Nieto et al., 2013; Vicente, Nieto, Hodara, Castro, & Alzamora, 2012). Knowledge about the microstructure and mass transport in OD of plant tissues is fundamental for controlling production of foods fortified with vitamins and mineral salts. Mass transfer in cellular tissue is influenced by the osmotic pressure and structure properties such as permeability of the plasma membrane and vacuole membrane, cell wall porosity, or even intercellular porosity. The osmotic pressure, in turn, depends on the solute concentration and the salt and acid dissociation because each substance presents specific transport properties through plasma and vacuole membranes or cell wall pores. When the cellular structure is changed, the tissue selectivity is also modified, so that water mobility and distribution are affected.

Osmotic dehydration of plant foods is largely controlled by the cellular membranes, which have different permeabilities to different substances. Biological membranes are composed of phospholipid bilayers with intrinsic proteins. Studies have shown that water can cross plant membranes through proteinaceous channels formed by members of the aquaporin superfamily, also called water channels (Weig, Deswarte, & Chrispeels, 1997). Aquaporins are hydrophobic proteins that enhance the biological membrane's permeability to water. They belong to a group of membrane proteins, the major intrinsic proteins (MIP) family of channels, with a molar mass in the range of 26 and 30 kDa (Tyerman, Niemietz, & Bramley, 2002; Weig et al., 1997). These channels increase the permeability of biological membranes to water compared to the lipid bilayers; they are detected by the low activation energy needed to transport water across water channels (Tyerman et al., 2002).

Calcium ions that occupy spaces outside the plasma membrane (apoplast) have a structural role in the cell wall because they interact with pectic acid polymers to form cross-bridges that reinforce the cell adhesion, thereby reducing cell separation, which is one of the major causes of plant tissue softening (Roy et al., 1994). Moreover, calcium can affect water channel activity; however, the significance of the inhibition of plant aquaporins by calcium is complex and has still not been clarified (Maurel, 2007). Conversely, calcium can also cross membranes through cation channels. A vacuolar non-selective Ca^{2+} channel (Peiter et al., 2005) has been identified as a plasma membrane non-selective cation channel (Tapken et al., 2013) in plant cells.

Ascorbic acid (AA) influences the cell physiology; however, little is known about its role in plant tissue. Exposure of Arabidopsis thaliana seedlings to ascorbic acid demonstrated that exogenous AA caused grow inhibition and damage in the cellular structure by increasing the ROS (reactive oxygen species) content (Qian et al., 2014). In addition, a very low pH (2–3)

can increase the cell wall porosity (Zemke-White, Clements, & Harris, 2000), which increases diffusion of great molecules in the free spaces of the cellular tissue.

The complexity of osmotic dehydration of plant tissues rises when using a multicomponent solution because all the solutes and their respective concentrations affect the membrane permeability and cell wall. Consequently, monitoring the water distribution can be useful to clarify the behavior of the cellular microstructures as osmotic dehydration proceeds. Time domain nuclear magnetic resonance (TD-NMR) is an analytical method that allows the determination of the water content and its mobility in different cell compartments by proton relaxation times of water in foods (Hills & Duce, 1990). It is a non-invasive method suitable for large tissue samples that relates water content and water properties in different proton pools within the tissue with different transverse relaxation times (T_2) of water (Hills & Remigereau, 1997; Panarese et al., 2012; Tylewicz et al., 2011). In fruit samples, the higher the mobility of a proton bearing molecule, the higher the spin–spin (T_2) relaxation time is expected to be. The intensities of proton pools with different transverse relaxation times are a relative measure of the amount of water corresponding to a specific T_2. This technique has been used in OD of plants to evaluate water mobility and distribution within the cellular tissue (Cornillon, 2000; Panarese et al., 2012; Tylewicz et al., 2011). Microscopic techniques can also be important tools to clarify cell viability by using vital dyes. Protoplasts stained with fluorescein diacetate (FDA) allow the estimation of two types of plasma membrane injuries: lysis and the loss of semipermeability (Halperin & Koster, 2006; Koster, Reisdorph, & Ramsay, 2003). Vacuole membrane alterations can be evaluated by the capacity of intact tonoplasts to retain neutral red and provide contrast to vacuoles (Carpita, Sabularse, Montezinos, & Delmer, 1979; Thebud & Santarius, 1982).

A multianalytical approach that combines several techniques such as micro and ultrastructural microscopy, calorimetry and NMR have been successfully employed in investigations of plant foods subjected to mild processing conditions (Panarese et al., 2012; Rocculi et al., 2012; Tylewicz et al., 2011).

The main objective of this work was to investigate the effects of the addition of calcium lactate (CaLac) and ascorbic acid (AA) to sucrose (Suc) osmotic solutions on mass transfer, cell viability and structure of apple tissue, as well as the consequential water distribution and mobility modification among the different cellular compartments.

2. Materials and methods

2.1. Raw materials

Apples (Malus domestica Borkh) of the Cripps Pink variety, popularly known by the brand name Pink Lady (Castro, Barrett, Jobling & Mitcham, 2008), were provided by the local market and stored at 5 ± 1 °C for no longer than 2 weeks, during which osmotic dehydration experiments were performed. The average weight of the apples was 233.5 ± 17.7 g, and the soluble solids content was 13.4 ± 0.3 $g \cdot 100\ g^{-1}$. Apples were cut in cylinders (8-mm diameter) with a manual cork borer and cut to a length of 40 mm using a manual cutter designed for this purpose. Commercial sucrose (refined sugar, Eridania, Italy), L-ascorbic acid (Shandong Luwei Pharmaceutical Co., China) and calcium lactate (calcium-L-lactate 5-hydrate powder, PURACAL® PP Food, Corbion PURAC, Netherlands) were used in the experiments.

2.2. Osmotic dehydration

Apple cylinders were weighed (approximately 0.1 kg) in a mesh basket and immersed in the osmotic solution. Each basket corresponded to a single OD time: 0.5, 1, 2 and 4 h. The OD system consisted of a cylindrical glass vessel containing 4.5 kg of aqueous solution. The cylindrical baskets, coupled with an impeller of a mechanical stirrer, were continuously rotated. Two baskets were prepared for each process time. The syrup-to-fruit ratio was approximately 15:1 (w/w).

The OD was performed with four different aqueous solutions: 40% sucrose (Suc), 40% sucrose + 4% calcium lactate (Suc–CaLac), 40% sucrose + 2% ascorbic acid (Suc–AA) and 40% sucrose + 4% calcium lactate + 2% ascorbic acid (Suc–CaLac–AA). After the pre-established contact period, the samples were removed from the solution, rinsed with distilled water, blotted with absorbing paper, and weighed.

Immediately after the process, analyses of the total solids and soluble solids contents were performed for fresh and osmotically treated samples in triplicate. The proton transverse relaxation time (T_2) was also immediately measured for six replicates. Samples for calcium and ascorbic acid analyses were freeze-dried.

2.3. Analytical methods

The moisture content for 2 g of fresh and treated samples was determined gravimetrically, in triplicate, by drying at 70° C until a constant weight was achieved. The soluble solids content was determined at 20° C by measuring the refractive index with a digital refractometer (PR1, Atago, Japan). Water activity was measured in a water activity meter (AquaLab Series mod. CX-2, Decagon, USA).

2.3.1. Ascorbic acid

For ascorbic acid determination, an extraction was performed with 0.5 g of a freeze-dried sample added to 10 ml of meta phosphoric acid (62.5 mM) and sulfuric acid (5 mM) solution. The mixture was vortexed for 2 min and centrifuged at 10,000xg for 10 min at 4 °C. The supernatant was opportunely diluted and filtered through a 0.45 lm nylon filter. Ascorbic acid was determined according to Odriozola-Serrano, Hernàndez-Jover, and Martìn- Belloso method (2007). The HPLC system (Jasco LC-1500, Carpi, MO, Italy) was equipped with a diode array UV/Vis detector. A reverse-phase C18 Kinetex (Phenomenex Inc., Torrance, CA, USA) stainless steel column (4.6 x 150 mm) was used as the stationary phase. Samples were introduced in the column through an autosampler (Jasco AS-2055 Plus). The mobile phase was a 0.01% solution of sulfuric acid adjusted to a pH of 2.6. The flow rate was fixed at 1.0 mL/min at room temperature. Data were processed by the software ChromNAV (ver. 1.16.02) from Jasco. The ascorbic acid content was quantified at 245 nm through a standard calibration curve.

2.3.2. Calcium

The calcium concentration was determined using a flame atomic absorption spectrophotometer (Model A Analyst 400, Perkin Elmer, Santa Clara, California, USA), using a lumina hollow cathode lamp (Perkin Elmer) based on the adapted methodology of AOAC – Association of Official Analytical Chemists. (1995). Approximately 6 g of fresh samples (without treatment) and 2 g of treated samples, i.e., freeze dried and previously ground, were weighed in a 50 ml glazed, porcelain crucible placed in a muffle furnace and heated up to 550 °C until complete ignition. Then, the porcelain crucibles were cooled in desiccators, where 20 ml of chloride acid (0.1 M) was added to the capsules with fresh samples and 30 ml was added to the treated samples. The ash was dissolved, and then, an aliquot of this solution was quantitatively taken and diluted 8 times (fresh samples) or 100

times (treated samples) with 0.1 M chloride acid. Standard calcium solutions between 2 and 20 ppm were used to determine a calibration curve of absorbance versus ppm of calcium.

2.4. Mass transfer of osmotic dehydration

Mass transfer during osmotic dehydration was evaluated on the basis of mass balances. The total mass variation in relation to the initial mass during osmotic dehydration was calculated from experimental data according to Eq. (1):

$$\Delta M = \frac{(m - m_0)}{m_0} \times 100 \tag{1}$$

where m = mass and 0 = initial time (t = 0).

Water loss (WL), calcium lactate gain (ΔCaLac), ascorbic acid gain (ΔAA) and sucrose gain (ΔSuc), all calculated in relation to initial mass, are shown in the following equations:

$$WL = \frac{(w_w \cdot m) - (w_{w_0} \cdot m_0)}{m_0} \times 100 \tag{2}$$

$$\Delta CaLac = \frac{w_{CaLac} \cdot m - w_{CaLac_0} \cdot m_0}{m_0} \times 100 \tag{3}$$

$$\Delta AA = \frac{w_{AA} \cdot m - w_{AA_0} \cdot m_0}{m_0} \times 100 \tag{4}$$

$$\Delta Suc = (\Delta M - \Delta W - \Delta CaLac - \Delta AA) \times 100 \tag{5}$$

where m = mass; w = mass fraction (w/w); w = water; CaLac = calcium lactate; AA = ascorbic acid; and 0 = initial time (t = 0).

In addition, the calcium gain (ΔCa) can be calculated by:

$$\Delta Ca = \frac{w_{Ca} \cdot m - w_{Ca_0} \cdot m_0}{m_0} \tag{6}$$

To evaluate the influence of the OD parameters on the efficiency of the water removal in relation to sugar impregnation of the apples, the efficiency was defined by the following equation:

$$\text{Efficiency} = \left|\frac{WL}{\Delta Suc}\right| \tag{7}$$

2.5. Microscopic analysis

Histological techniques with vital stains, which do not cause a short-term effect on the cell physiology, were used to evaluate the influence of the osmotic dehydration on cell viability using fluorescence intensity and neutral red accumulation for vacuole integrity in preserved vacuoles. Microscopic analysis was performed on osmotic solutions in the following concentrations: Suc (20%), Suc (30%), Suc (40%), Suc–CaLac (20–2%), Suc–CaLac (30–3%), Suc–CaLac (40–4%), Suc–AA (20–1%), Suc–AA (30–1.5%), Suc– AA (40–2%), Suc–CaLac–AA (20%, 2%, 1%), Suc–CaLac–AA (30%, 3%,1.5%) and Suc–CaLac–AA (40%, 4%, 2%).

2.5.1. Fluorescein diacetate (FDA) staining

1 mm-thick apple slices were obtained using a sharp scalpel and then treated in the osmotic solutions mentioned above for 2 h. The cell viability test was performed using fluorescein diacetate (FDA, Sigma–Aldrich, USA, λ_{ex} = 495 nm, λ_{em} = 518 nm), as described by Tylewicz, Romani, Widell, and Gómez Galindo (2013) with some modifications. Apple slices were

incubated for 30 min in a 10^{-4} M FDA in an isotonic sucrose solution (13%, w/w) in the darkness at room temperature. Fluorescein diacetate is known for its ability to passively penetrate protoplast and to be hydrolyzed by cytoplasmic esterases that produce the polar product fluorescein. This charged form is accumulated intracellularly in viable cells because it is unable to cross cellular membranes that remain intact (Saruyama et al., 2013). Viable cells could be easily identified by a bright fluorescence. Observations were performed under a fluorescent light in a Nikon upright microscope (Eclipse Ti-U, Nikon Co., Japan) equipped with a Nikon digital video camera (digital sight DS-Qi1Mc, Nikon Co., Japan) at a magnification of 20x.

2.5.2. Neutral red staining

Apple tissues were stained using a neutral red dye. Neutral red is a vital stain with a relatively low molecular weight and no electric charge that penetrates the vacuole of the intact protoplast of plant cells. In vacuoles, the neutral red is transformed to an ionic state because of the low pH inside the vacuoles; in this form, neutral red is incapable of penetrating the tonoplast, so the neutral red accumulates in the vacuole. Neutral red stain has been prepared in a concentration of 0.05% (Mauro, Tavares, & Menegalli, 2003; Panarese et al., 2012) in an isotonic sucrose solution at 13% (w/w). Slices (""0.5 mm) cut manually with a sharp scalpel were stained with neutral red for 20 min. Each stained slice was immersed in an osmotic solution for a minimum of 120 min. Slices were placed on a microscopic slide accompanied by a drop of solution and covered with the slide cover. The control slices were solely washed in the isotonic solution. Slides were immediately observed under a light microscope (Optech – Optical Technology, Germany) and recorded at a magnification of 10x. RGB images were acquired using a digital camera (Camedia C-4040-ZOOM, Tokyo, Japan) and stored in JPEG format.

Additionally, slides were recorded at a higher resolution in black and white using a Nikon upright microscope (Eclipse Ti-U, Nikon Co., Japan) without a fluorescent light at a magnification of 20x.

2.6. Time domain nuclear magnetic resonance (TD-NMR)

The proton transverse relaxation time (T_2) of the samples was measured for six replicas in a Bruker The Minispec spectrometer (Bruker Corporation, Germany), operating at 20 MHz and 24 °C, using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. Fresh or osmotic dehydrated apple cylinders with an 8 mm initial diameter were cut (approximately 10-mm height, 250 mg) to not exceed the active region of the radio frequency coil and placed inside the 10-mm outer diameter NMR tubes. Each measurement comprised 16,000 echoes with a 90–180 interpulse spacing of 100 ls, with 32 scans and a recycle delay of 5 s. The specified instrumental parameters avoided heating the samples and allowed the measurement of the protons with a T_2 between 1 and 2000 ms.

The acquired CPMG curves were normalized by the sample weights and analyzed with the UPEN (uniform penalty inversion of multiexponential decay data) algorithm (Borgia, Brown, & Fantazzini, 1998) to give quasi-continuous distributions of relaxation time. The UPEN default fitting parameters were adjusted to obtain better resolved and more detailed peaks. The number of output relaxation times sampled logarithmically in the 1–2000- ms interval T_2 was set to 200, and the smoothing coefficient beta was increased to 2. However, the resulting T_2 distributions showed partially overlapped peaks. Three proton populations were found in each sample and were ascribed to cell compartment proton pools according to their T_2 and intensity values (Panarese et al., 2012): vacuole, cytoplasm-free space and cell wall. Free spaces are the spaces where the osmotic solution could interpenetrate, i.e., outside the protoplast boundaries.

To obtain quantitative information from the CMPG decay curves, sample signals were fitted using a discrete multi-exponential curve in Eq. (8):

$$S_{(\tau)} = \sum_{i=1}^{N} I_n \exp\left(\frac{-t}{T_{2,n}}\right) + E_{(\tau)} \quad (8)$$

where N is the number of the found protons populations (based on UPEN results, it was set to 3); and I and T_2 are the intensity value and average relaxation time, respectively, of the n proton pool.

The fitting was performed using the "Nonlinear Least Squared" function based on the Gauss–Newton algorithm and implemented in the "R" software (R Foundation for Statistical Computing, Austria), while the I and T_2 starting values were chosen based on the UPEN results.

2.7. Statistical analysis

The significance of the effects of the different osmotic solutions on water loss, sucrose gain, efficiency, transverse relaxation times (T_2) and intensity was evaluated by analysis of variance (ANOVA) and comparison of means using the Duncan test at a 5% probability level. The data were expressed as the mean ± standard deviation.

3. Results and discussion

3.1. Mass transfer: water loss and solid gain

Water loss (Eq. (2)) and sucrose gain (Eq. (5)) during osmotic dehydration of apples treated in different osmotic solutions are shown in Table 1. Samples treated in the sucrose solution show the smallest water loss and high sucrose uptake compared to the other treatments. When AA is added to the sucrose solution, the water loss increases but the sucrose gain also increases, especially during the first 30 min. A consequence of this relationship between the water loss and sugar gain is both the Suc and Suc–AA treatments have a lower process efficiency (Table 1) compared to the Suc–CaLac and Suc–CaLac–AA treatments. As for the water chemical potential of these solutions, the water activity measured was 0.962 in the sucrose solution (40%), 0.953 in the Suc–CaLac (40–4%), 0.954 in the Suc–AA (40–2%) and 0.944 in the Suc–CaLac–AA (40–4–2%). Consequently, the highest water loss is expected from the Suc–CaLac–AA solution followed by the Suc– CaLac and Suc–AA solutions. Indeed, both the Suc–CaLac and Suc– CaLac–AA solutions promoted greater water loss and did not have significant differences between them. However, when comparing the sucrose gain values between these two treatments, differences were found at 30 and 240 min of the process, as seen in Table 1, which were reflected in the efficiency of these processes. CaLac in solution enhances the efficiency because it is able to promote high water loss and restricts sucrose impregnation, which has been verified by other authors (Mavroudis et al., 2012; Silva et al., 2014a). However, the inhibition in sugar gain is sometimes accompanied by water loss reduction and, hence, a good efficiency is not reached, as verified by Silva et al. (2014a) who exposed pineapple tissue to high concentrations (sucrose 50% solution with 4% CaLac) for 2 h. Barrera et al. (2009) observed that for apples, osmotic dehydration assisted by vacuum impregnation favors solid gain but also reduces water removal. Restriction of solute transport has been attributed to calcium pectate formation, which decreases the cell wall porosity and limits the transport of larger molecules. However, a decrease in the water loss could also be explained by changes in the cellular membranes because calcium can affect the water channel activity (Maurel, 2007). Nevertheless, the significance of the

inhibition of plant aquaporins by calcium is complex and has not been clarified, as noted by Maurel (2007), who compared the water permeability of the Arabidopsis plasma membrane (Gerbeau et al., 2002) and Beta vulgaris roots (Alleva et al., 2006). A low sensitivity to Ca^{2+} was detected in the Arabidopsis plasma membrane, but a higher sensitivity was detected in the B. vulgaris roots. In the present work, inhibition of Ca^{2+} on water loss was not evident.

In contrast, effect of the addition of AA seems to increase the impregnation of solutes, which is the opposite effect of those promoted by calcium. This was verified by Silva et al. (2014b) and attributed to wall porosity increasing because of acidification (Zemke-White et al., 2000).

During the first 60 min of the Suc–CaLac–AA treatment, the efficiency was high probably because the calcium affected the restriction of the sucrose gain in a similar way to the behavior observed for the Suc–CaLac treatment (Table 1). Then, the efficiency decreased, which suggests that after 1 h of the process, the AA exerted an opposite influence on the sucrose transport. Silva et al. (2014b) also observed that AA positively influenced sucrose and calcium gain in pineapples treated in solutions composed of sucrose, calcium lactate and ascorbic acid. These results suggest that synergetic effects should not be ignored. Genevois, Flores, and De Escalada Pla (2014) fortified pumpkin with vitamin C and iron through a dry infusion process by sprinkling powdered sucrose on the vegetable to form a solution with the lost water from the food. The authors concluded that the addition of Fe or AA to the liquid solution increases the incorporation of sucrose into the pumpkin tissue, but the presence of both additives simultaneously produces an antagonistic effect that diminishes the solid gain.

Good impregnation of Ca and AA contents were observed during the treatments in the Suc–CaLac, Suc–AA and Suc–CaLac–AA solutions. The last solution slightly enhanced the AA and Ca impregnation; the AA content increased after 2 h of process while the Ca content increased after 4 h (Table 2). AA was not detected in the fresh samples. Indeed, very low ascorbic acid content has been previously found in the Pink Lady apples (2.3–3.0 mg/100 g, Castro et al., 2008).

In conclusion, according to mass transport evaluation, the OD efficiency was improved by CaLac, while AA presence exerted an opposite effect; when both additives were present simultaneously, AA counterbalanced an initial increase of efficiency caused by calcium, as the OD proceeded. High levels of Ca and AA contents were reached in all treatments and the impregnation of both components was slightly enhanced when they were together in the solution.

Table 1
Mean and standard deviation of water loss, sucrose gain and efficiency.

Osmotic solution	30 min	60 min	120 min	240 min
Water loss				
Suc	$-9.36^{a} \pm 0.50$	-13.00^{*}	$-15.94^{a} \pm 0.85$	$-24.66^{a} \pm 0.13$
Suc–CaLac	$-12.49^{b} \pm 0.86$	$-14.96^{a} \pm 1.00$	$-22.30^{b} \pm 0.68$	$-28.96^{b} \pm 0.63$
Suc–AA	$-10.45^{a} \pm 0.04$	$-13.61^{a} \pm 0.86$	$-18.99^{ab} \pm 0.19$	$-26.18^{a} \pm 1.25$
Suc–CaLac–AA	$-12.87^{b} \pm 0.52$	$-16.04^{a} \pm 1.48$	$-20.58^{b} \pm 2.21$	$-28.42^{b} \pm 0.02$
Sucrose gain				
Suc	$2.28^{ab} \pm 0.12$	4.11^{*}	$5.51^{a} \pm 0.26$	$6.70^{a} \pm 0.05$
Suc–CaLac	$2.19^{a} \pm 0.20$	$3.39^{a} \pm 0.27$	$4.86^{a} \pm 0.22$	$5.69^{b} \pm 0.23$
Suc–AA	$3.18^{c} \pm 0.01$	$4.11^{a} \pm 0.23$	$4.93^{a} \pm 0.06$	$6.75^{a} \pm 0.45$
Suc–CaLac–AA	$2.61^{b} \pm 0.12$	$3.20^{a} \pm 0.41$	$4.76^{a} \pm 0.69$	$7.41^{a} \pm 0.01$
Efficiency				
Suc	$4.11^{ab} \pm 0.43$	3.90^{*}	$2.90^{a} \pm 0.29$	$3.68^{a} \pm 0.05$
Suc–CaLac	$5.75^{c} \pm 0.93$	$4.44^{a} \pm 0.65$	$4.60^{a} \pm 0.35$	$5.10^{b} \pm 0.32$
Suc–AA	$3.29^{a} \pm 0.02$	$3.32^{a} \pm 0.39$	$3.85^{a} \pm 0.09$	$3.89^{a} \pm 0.44$
Suc–CaLac–AA	$4.94^{bc} \pm 0.43$	$5.08^{a} \pm 1.11$	$4.41^{a} \pm 1.10$	$3.98^{a} \pm 0.01$

The same letter on the same column means no significant difference by the Duncan test ($p < 0.05$).
* Replica not determined.

Table 2
Mean and standard deviation of calcium and ascorbic acid contents at different osmotic dehydration times and corresponding fresh apple (mg/100 g).

Osmotic solution	0 min (fresh)	30 min	60 min	120 min	240 min
Calcium content					
Suc–CaLac	$2.78^{a} \pm 0.03$	$79.80^{b} \pm 0.55$	$110.70^{c} \pm 1.88$	$142.44^{d} \pm 0.21$	$163.45^{e} \pm 5.35$
Suc–CaLac–AA	$2.78^{a} \pm 0.03$	$81.05^{b} \pm 4.00$	$108.57^{c} \pm 2.18$	$140.01^{d} \pm 12.75$	$195.20^{f} \pm 8.30$
Ascorbic acid content					
Suc–AA	Nd	$429.02^{a} \pm 13.82$	$608.88^{b} \pm 13.47$	$733.32^{c} \pm 54.61$	$1012.45^{e} \pm 2.87$
Suc–CaLac–AA	Nd	$393.09^{a} \pm 10.13$	$576.85^{b} \pm 8.20$	$779.50^{d} \pm 15.87$	$1076.53^{f} \pm 33.50$

The same letter on the same column for each component means no significant difference by the Duncan test ($p < 0.05$);
Nd: not detectable.

3.2. Microscopic analysis

3.2.1. Microphotographs of tissues stained with fluorescein diacetate

Fig. 1 presents slides of apple tissue before and after 2 h of osmotic dehydration in different solutions followed by staining with FDA. For the 20%, 30% and 40% Suc solutions (Fig. 1b.1–b.3), all slides show cell viability with an intensity that was comparable to the fresh tissues (Fig. 1a). Tissues treated in the CaLac–Suc solution presented a higher intensity for the 20% Suc–2% CaLac (Fig. 1c.1) solution. However, as the concentrations of both components increased, the viability decreased, which suggests that the solution with 40% Suc + 4% CaLac (Fig. 1c.3) affected the viability of the cells. A low fluorescence intensity was detected in tissues treated with the Suc–AA treatments in the Suc 20%, AA 1% concentrations (Fig. 1d.1) and no viability at higher concentrations was found (Fig. 1d.2 and d.3). For treatments in the Suc–CaLac–AA solutions, the apple cells did not show any viability (Fig. 1e.1–e.3). If the protoplasts did not retain the FDA, this means disruption of the plasma membrane (cell lysis) or loss of membrane semipermeability (Halperin & Koster, 2006; Koster et al., 2003). The type of membrane injury could be verified by the number of intact protoplasts without ability to retain FDA. However, apples have a poor cytosol content, which makes it difficult to distinguish the protoplasts and vacuoles using light microscopy.

Cellular injury can be caused by low water activity, but all solutions used in these experiments had a relatively high a_W (in a range of 0.944–0.986). Another cause for cellular damage could be the low pH of the osmotic solutions with AA (Zemke-White et al., 2000). For instance, Suc–AA solutions have a pH close to 2.4 and Suc–CaLac–AA solutions near 4.0. Furthermore, it has been demonstrated that AA can cause severe damage in the cellular structure (Qian et al., 2014). Hence, the AA presence in high concentrations certainly affects the cellular membrane structure of plant tissues, but the mechanisms are still not clearly delineated.

3.2.2. Microphotographs of tissues stained with neutral red

Fig. 2 presents slides of apple tissue stained with neutral red, followed by 2 h of osmotic dehydration in different solutions. Fig. 2a shows the control with no osmotic treatment that appeared completely stained. Fig. 2b.1–b.3 represent tissues treated in Suc solutions and show a broad presence of preserved vacuoles and red-stained tissue, probably because neutral red can also provide some contrast to cytoplasm (Carpita et al., 1979). Plasmolysis can be identified by the arrows.

These results agree with cell viability verified by FDA experiments with Suc solution, since, if plasma membranes remain preserved, intact vacuoles must be found.

In Fig. 2c.1–c.3, with tissues treated in Suc–CaLac solutions, vacuoles are well defined. However, the color is not spread out like it was in cells exposed to Suc solutions alone, which suggests that the cytoplasm did not retain the color despite some protoplast viability remaining even in the 30% Suc + 3% CaLac solution (Fig. 1c.2). The possibility that some plasma membranes or tonoplasts have been disrupted is based on the high calcium concentration, which can damage membranes (Wang, Xie, & Long, 2014).

Interestingly, during osmotic dehydration of thin slices previously stained with neutral red, sucrose solutions remained without color but Suc–CaLac solutions changed to red with similar tonality of the neutral red aqueous solution, Suc–AA solutions changed to pink and Suc–CaLac–AA solution changed to intense pink (registers are not shown). This confirms the loss of plasma and/or vacuole membrane permeability, thereby permitting neutral red to leave the tissue.

Effects of the pH of the Suc–CaLac solutions did not seem plausible because the pH of the solutions is nearly neutral. Conversely, tissues treated in the Suc–AA solutions had a complete absence of color, as shown in Fig. 2d.1–d.3. Very low protoplast viability and no stained vacuoles suggest that high AA concentrations and/or very low pH affect the membrane

integrity and permeability. Nevertheless, it was a surprise to be able to distinguish some vacuoles without dye (see arrows in Fig. 2d.2 and d.3), which were more visible in images captured at high resolution (Appendix A).

Whether the vacuoles contours are still visible, the membranes exist, but impermeability to a charged form of neutral red must have been lost and the stain left the vacuoles because red contrast was not observed. Moreover, it is known that the loss of plasma membrane semipermeability does not necessary mean cell lyses even though it concerns plasma membranes (Halperin & Koster, 2006), but suggests that tonoplast selectivity must have been modified without complete disruption of the vacuoles.

Finally, treatments in Suc–CaLac–AA solutions showed unexpected results. Although it is possible to visualize vacuoles in Fig. 2e.1–e.3, the cell viability was completely lost in cells that underwent this treatment (Fig. 1e.1–e.3). Because the CaLac addition elevated the pH in comparison to the AA solutions, from 2.4 (Suc–AA) to 4 (Suc–CaLac–AA), it is possible that the tonoplast semi permeability was better preserved, so the neutral red remained in some vacuoles. Conversely, plasmalemma was probably damaged due to the low pH and high CaLac and AA concentrations because no viability was detected.

These results show that plasmalemma was more sensitive to Suc–CaLac–AA solutions than tonoplast. AA caused red color absence in the vacuoles but they were visualized in images captured at high resolution, which led to the conclusion that tonoplasts maintained the vacuole content but its permeability was changed. The same inference could not be made for plasmalemma because the low cytoplasm content does not permit one to distinguish this phase.

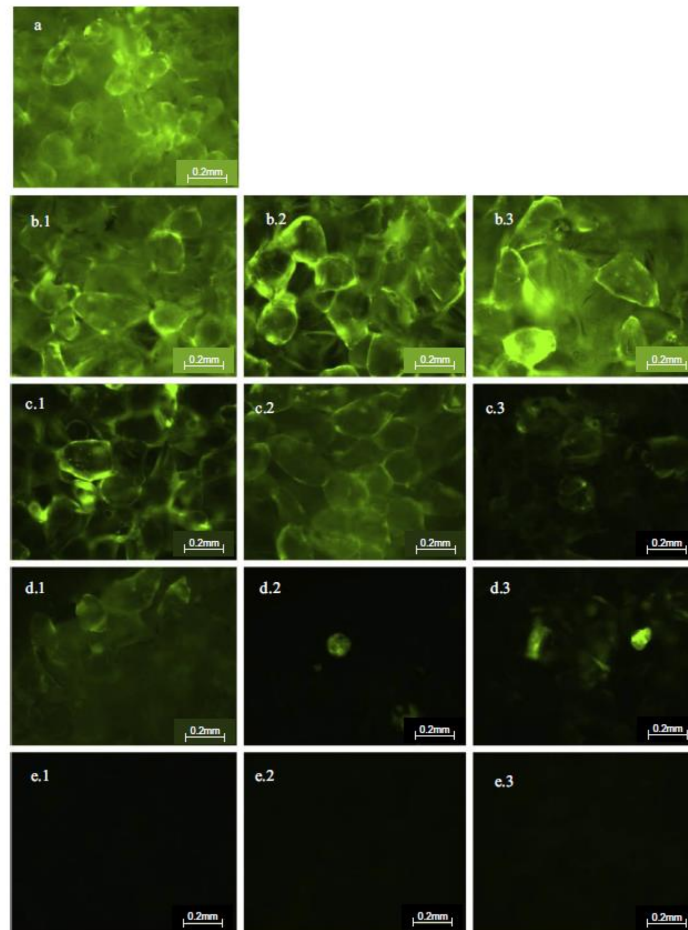


Fig. 1. Slides of parenchyma apple tissue stained with FDA after immersion in osmotic solutions for 120 min: (a) control; (b–e) osmotically dehydrated in osmotic solutions; (b.1) 20% Suc; (b.2) 30% Suc; (b.3) 40% Suc; (c.1) 20% Suc + 2% CaLac; (c.2) 30% Suc + 3% CaLac; (c.3) 40% Suc + 4% CaLac; (d.1) 20% Suc + 1% AA; (d.2) 30% Suc + 1.5% AA; (d.3) 40% Suc + 2% AA; (e.1) 20% Suc + 2% CaLac + 1% AA; (e.2) 30% Suc + 3% CaLac + 1.5% AA; (e.3) 40% Suc + 4% CaLac + 2% AA.

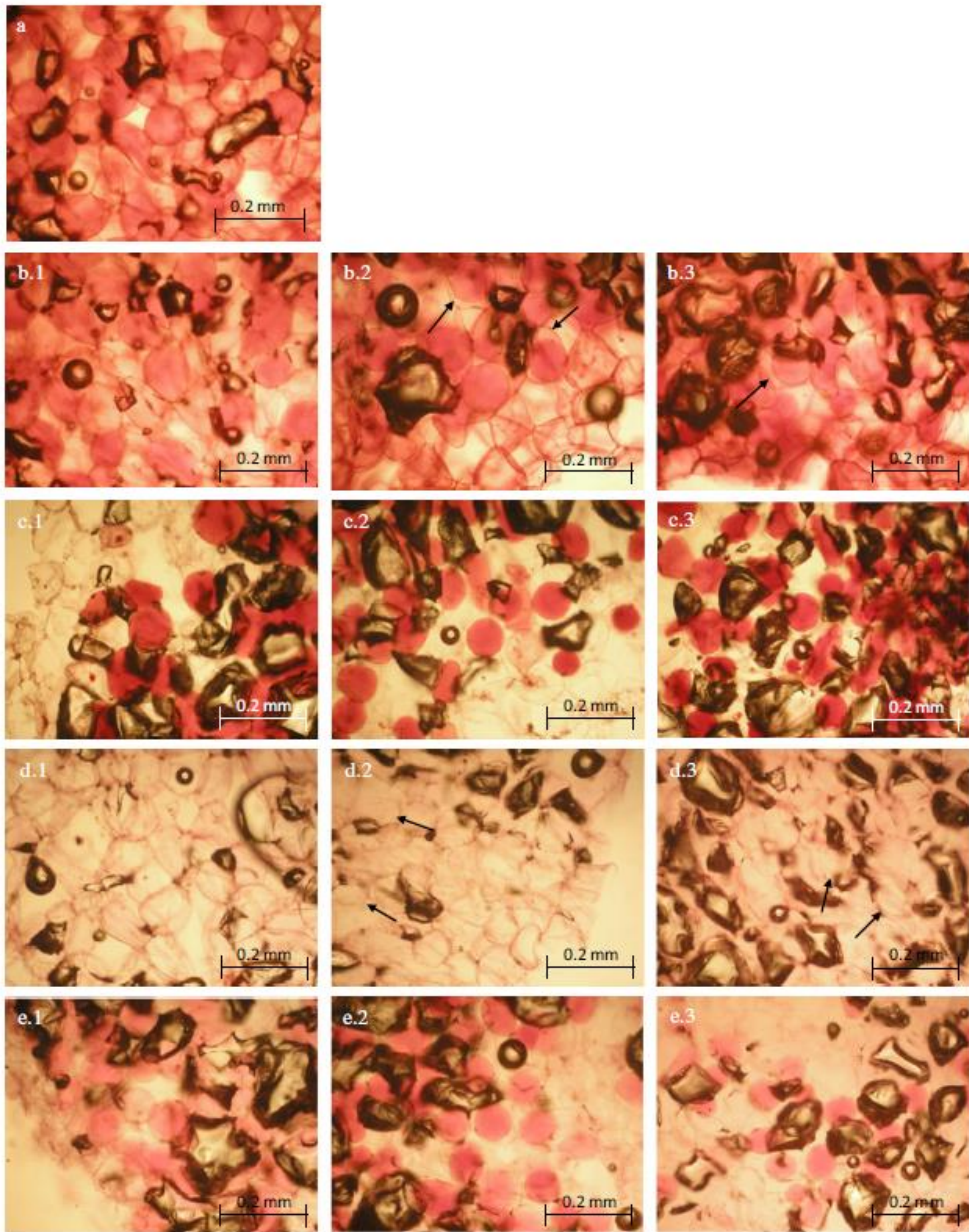


Fig. 2. Slides of apple tissue stained with neutral-red before immersion in osmotic solutions for 120 min: (a) control; (b–e) osmotically dehydrated in osmotic solutions; (b.1) 20% Suc; (b.2) 30% Suc; (b.3) 40% Suc; (c.1) 20% Suc + 2% CaLac; (c.2) 30% Suc + 3% CaLac; (c.3) 40% Suc + 4% CaLac; (d.1) 20% Suc + 1% AA; (d.2) 30% Suc + 1.5% AA; (d.3) 40% Suc + 2% AA; (e.1) 20% Suc + 2% CaLac + 1% AA; (e.2) 30% Suc + 3% CaLac + 1.5% AA; (e.3) 40% Suc + 4% CaLac + 2% AA.

3.3. Time domain nuclear magnetic resonance (TD-NMR): water mobility

Osmotic dehydration promotes important changes in cellular structure that can affect tissue selectivity and modify water mobility and its distribution through different parts of the cellular tissue.

Water mobility is related to the availability of water and, in this osmo-cellular system, could be modified by concentration of solids or by changes related to sites for hydrogen bonds because of macromolecule structure alteration.

T_2 and relative intensity results are shown in Table 3 and Fig. 3, respectively. Three protons populations were found in each sample at approximately 10, 200 and 1200 ms and were ascribed to cell compartment proton pools based on their T_2 and intensity values: cell wall, cytoplasm-free space and vacuole (Panarese et al., 2012). The free space comprises the plasmolysis space, which forms between the cell wall and plasmalemma, intraand inter-cellular spaces and interstices in the cell walls (Mauro et al., 2003).

The total signal of raw apples was considered as a reference and set at 100. The intensities of cell wall, cytoplasm-free space and vacuole signals thus corresponded to 2.8 ± 0.4, 20.5 ± 2.3 and 76.7 ± 2.5, respectively. Results related to the water distribution showed a release of water from vacuoles to the cytoplasm-free spaces (Cyt/FS), so that the vacuoles shrank while the Cyt/FS water pools swelled. A more pronounced effect was observed for the osmotic treatment with the lowest a_w solution, Sac–CaLac–AA (0.944), than by the Suc–AA (0.954) and Suc–CaLac (0.953) treatments both with similar a_w solution and, finally, by the Suc treatment with the highest a_w solution (0.962) (Fig. 3).

Regarding T2, while focusing on specific time points, most differences between treatments were insignificant. On the other hand, when T_2 was observed during the redistribution of water proton compartments, trends similar to those observed for signal intensities were registered. In this respect, some aspects should be emphasized. In the treated tissues, the transverse relaxation times T_2 assigned to cytoplasm and free spaces were, in general, very similar to those of raw apples (Table 3). During the first two hours of process in the Suc–CaLac solution, T_2 assigned to vacuoles was greater than those measured in other treatments and close to the raw value. If the water losses are the greatest for this condition (Fig. 1), it is not clear why vacuoles have the highest water mobility once concentration of the vacuole solute content would be expected. A likely explanation is that channels selectivity of the plasma and vacuole membranes for several original cell substances would be different for each osmotic treatment (Maurel, 2007; Peiter et al., 2005; Tapken et al., 2013). In addition, osmotic solutions as well as contact time can affect membrane integrity. Thus, the vacuoles and cytoplasm solute composition and consequent water interactions in these compartments could be different between treatments.

The fact that calcium can traverse both tonoplast and plasmalemma membranes is not ignored. According to Peiter et al. (2005), several classes of Ca^{2+} recently have been identified in plant cells even though not all of the ion channels that underlie these currents have been identified. These authors showed that the TPC1 ('two-pore channel 1') protein, a non-selective channel for Ca^{2+}, encodes a class of Ca^{2+}-dependent Ca^{2+}-release channel known as the slow vacuolar (SV) channel, and they demonstrated that the TPC1 protein is relatively abundant in plant vacuoles. In turn, the plasma membrane cation channels in plant cells have been related to AtGLRs (A. thaliana glutamate receptors), proteins that are members of an amino acid receptors family (Tapken et al., 2013). The authors showed that they function as ligandgated and non-selective cation channels permeable to Ca^{2+}. Consequently, compositional changes involving calcium could influence but not explain the higher water mobility because molecules with low molecular weight have a high capacity to drop water activity.

Still for Suc–CaLac treatments, T_2 assigned to cytoplasm and free spaces increased in relation to the raw material, though not significantly (Table 3). This could mean once again slower compositional changes and modifications of water interactions

because calcium limits sucrose entry into the Cyt/FS, as observed by the efficiency obtained from the Suc–CaLac treatment (Table 1). Then, it would be expected that this compartment would have a greater proportion of solutes from the original cell than sucrose arising from the osmotic solution compared to other treatments and, consequently, weaker water interactions than with sucrose during early osmosis. Of course, as osmotic dehydration proceeds, the water chemical potential in each compartment tends to equal those of the osmotic solution.

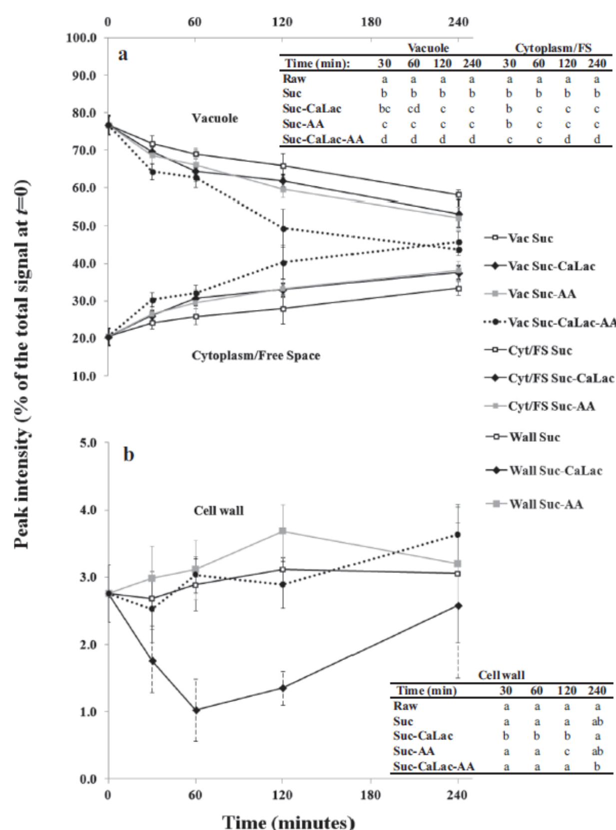
For cell walls, water mobility practically did not change. However, regarding intensity, the Suc–CaLac treatment promoted a significant reduction in the water amount associated with the wall biopolymers. Roy et al. (1994), investigating changes in the distribution of the anionic binding sites in the cell walls of apples, concluded that calcium could reduce fruit softening by strengthening the cell wall and limiting cell separation through a greater degree of cross-links with pectic acid polymers. An important observation of these authors is that these calcium bindings can restrict access of hydrolytic enzymes or the resulting increase in pH due to Ca could inhibit activity of the wall loosening enzymes that possess acidic pH optima. Nevertheless, T_2 times for the cell wall did not present a pattern, so it would be necessary for more registers because there were great variations between cells (Table 3).

Table 3
Mean and standard deviation of the transverse relaxation time (T_2).

	T_2 (ms)			
	30 min	60 min	120 min	240 min
Vacuole				
Raw (fresh)	$1215.29^{a} \pm 39.78$	$1215.29^{a} \pm 39.78$	$1215.29^{a} \pm 39.78$	$1215.29^{a} \pm 39.78$
Suc	$1134.43^{b} \pm 43.64$	$1115.06^{b} \pm 61.10$	$1091.32^{b} \pm 19.86$	$995.99^{b} \pm 15.66$
Suc–CaLac	$1203.05^{a} \pm 27.62$	$1206.24^{a} \pm 59.40$	$1124.74^{b} \pm 28.35$	$1075.15^{c} \pm 43.15$
Suc–AA	$1147.25^{bc} \pm 24.42$	$1098.85^{b} \pm 24.42$	$1086.37^{b} \pm 19.14$	$1052.85^{c} \pm 44.34$
Suc–CaLac–AA	$1183.82^{ac} \pm 52.40$	$1138.28^{b} \pm 59.85$	$1090.47^{b} \pm 38.97$	$1003.12^{b} \pm 84.20$
Cytoplasm/free space				
Raw (fresh)	$209.19^{a} \pm 23.13$	$209.19^{ab} \pm 23.13$	$209.19^{ab} \pm 23.13$	$209.19^{a} \pm 23.13$
Suc	$211.71^{a} \pm 13.89$	$196.42^{a} \pm 19.73$	$188.18^{a} \pm 15.90$	$193.85^{a} \pm 11.72$
Suc–CaLac	$206.43^{a} \pm 15.90$	$231.98^{b} \pm 21.08$	$229.91^{b} \pm 13.60$	$212.12^{a} \pm 17.92$
Suc–AA	$211.86^{a} \pm 16.19$	$210.36^{ab} \pm 22.63$	$209.23^{ab} \pm 6.96$	$197.26^{a} \pm 12.32$
Suc–CaLac–AA	$210.26^{a} \pm 12.16$	$212.01^{ab} \pm 15.43$	$208.54^{ab} \pm 17.32$	$203.61^{a} \pm 15.69$
Cell wall				
Raw (fresh)	$9.81^{a} \pm 2.42$	$9.81^{a} \pm 2.42$	$9.81^{a} \pm 2.42$	$9.81^{a} \pm 2.42$
Suc	$8.93^{a} \pm 2.41$	$12.91^{b} \pm 4.10$	$10.47^{a} \pm 1.53$	$12.98^{abc} \pm 3.44$
Suc–CaLac	$9.68^{a} \pm 5.00$	$10.90^{ab} \pm 3.09$	$9.81^{a} \pm 6.40$	$15.22^{c} \pm 6.50$
Suc–AA	$8.90^{a} \pm 0.98$	$8.95^{a} \pm 0.85$	$11.05^{a} \pm 2.22$	$10.76^{abc} \pm 3.08$
Suc–CaLac–AA	$11.57^{a} \pm 2.41$	$11.15^{ab} \pm 1.69$	$15.12^{b} \pm 1.81$	$13.52^{bc} \pm 3.22$

The same letter on the same column means no significant difference by the Duncan test ($p < 0.05$).

In conclusion, according to TD-NMR results, the Suc treatment seemed to have a lower influence on the cellular compartmentation and functionality, so that a higher vacuole water population and lower cytoplasm-extracellular spaces were observed in comparison with the other treatments. The Suc–CaLac and Suc–AA treatments resulted in similar water populations of vacuoles and cytoplasm. This highlights the presence of the vacuole compartmentation in tissues treated with Suc–AA (Appendix A), although it was not visualized by neutral red staining (Fig. 2d.1–d.3).



	Vacuole				Cytoplasm/FS			
Time (min):	30	60	120	240	30	60	120	240
Raw	a	a	a	a	a	a	a	a
Suc	b	b	b	b	b	b	b	b
Suc-CaLac	bc	cd	c	c	b	c	c	c
Suc-AA	c	c	c	c	b	c	c	c
Suc-CaLac-AA	d	d	d	d	c	c	d	d

Cell wall				
Time (min)	30	60	120	240
Raw	a	a	a	a
Suc	a	a	a	ab
Suc-CaLac	b	b	b	a
Suc-AA	a	a	c	ab
Suc-CaLac-AA	a	a	a	b

Fig. 3. Peak intensity of the proton pools in different cellular compartments as a function of the osmotic dehydration time, in different osmotic solutions. All the intensities were scaled so that the total signal from fresh samples (t = 0) equals 100. (a) Vacuole and cytoplasm plus free space; (b) cell wall. The same letter on the same column in the auxiliary tables means no significant difference by the Duncan test ($p < 0.05$).

4. Conclusions

Sucrose treatments preserved the viability and slightly affected the apple cell structure during OD, as shown by a fluorescence intensity which was comparable to fresh tissue, by a broad presence of red-stained vacuoles and by moderate changes in the water distribution within the cells, according to TD-NMR.

CaLac in the sucrose solution contributed to extended cell viability, and TD-NMR allowed detection of its influence on the cell wall as the proton intensity reduced during the first two hours of the process. In contrast, proton pools related to cell walls expanded in all other treatments. CaLac also enhanced the OD efficiency, which is associated with cell wall pore reduction due to calcium pectate formation.

Only adding AA into the sucrose solution visibly affected the cell membrane permeability by revealing the loss of viability of protoplasts and capacity of retaining vital stain in vacuoles and, simultaneously, the presence of vacuole compartmentation, which was detected by TD-NMR and also by images captured in a high resolution.

AA together with calcium strongly affect the tissue functionality, showing no viability but still some stain retention by vacuoles, and a remarkable water redistribution by vacuole shrinkage and Cyt/FS swelling verified by TD-NMR. Plasmalemma was more sensitive to Suc–CaLac–AA solutions than tonoplast. The presence of AA reduced the process efficiency and enhanced Ca impregnation in the four process hours, which were related to increase the cell wall porosity and change the membrane permeability.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.04.096.

References

Anino, S. V., Salvatori, D. M., & Alzamora, S. M. (2006). Changes in calcium level and mechanical properties of apple tissue due to impregnation with calcium salts.

Alleva, K., Niemietz, C. M., Sutka, M., Maurel, C., Parisi, M., Tyerman, S. D., et al. (2006). Plasma membrane of Beta vulgaris storage root shows high water channel activity regulated by cytoplasmic pH and a dual range of calcium concentrations. Journal of Experimental Botany, 57, 609–621.

Alzamora, S. M., Cerrutti, P., Guerrero, S., & López-Malo, A. (1995). Minimally processes fruits by combined methods. In G. V. Barbosa-Cánovas & J. WeltiChanes (Eds.), Food preservation by moisture control: Fundamentals and applications (pp. 463–492). Lancaster: Technomics Publishing.

AOAC – Association of Official Analytical Chemists. (1995). Official methods of analysis of the association of official analytical chemists (16th ed., Vol. 1, p. 4). Arlington: Association of Official Analytical Chemists A.O.A.C., chapter 3 (method 985.01).

Barrera, C., Betoret, N., Corell, P., & Fito, P. (2009). Effect of osmotic dehydration on the stabilization of calcium-fortified apple slices (var. Granny Smith): Influence of operating variables on process kinetics and compositional changes. Journal of Food Engineering, 92(4), 416–424.

Barrera, C., Betoret, N., & Fito, P. (2004). Ca^{2+} and Fe^{2+} influence on the osmotic dehydration kinetics of apple slices (var. Granny Smith). Journal of Food Engineering, 65(1), 9–14.

Borgia, G. C., Brown, R. J. S., & Fantazzini, P. (1998). Uniform-penalty inversion of multiexponential decay data. Journal of Magnetic Resonance, 132(1), 65–77.

Carpita, N., Sabularse, D., Montezinos, D., & Delmer, D. P. (1979). Determination of the pore size of cell walls of living plant cells. Science, 205, 1144–1147.

Castro, E., Barrett, D. M., Jobling, J., & Mitcham, E. J. (2008). Biochemical factors associated with a CO_2-induced flesh browning disorder of Pink Lady apples. Postharvest Biology and Technology, 48(2), 182–191.

Cornillon, P. (2000). Characterization of osmotic dehydrated apple by NMR and DSC. LWT – Food Science and Technology, 33(4), 261–267.

Garcia Loredo, A. B., Guerrero, S. N., Gomez, P. L., & Alzamora, S. M. (2013). Relationships between rheological properties, texture and structure of apple (Granny Smith var.) affected by blanching and/or osmotic dehydration. Food and Bioprocess Technology, 6(2), 475–488.

Genevois, C., Flores, S., & De Escalada Pla, M. (2014). Effect of iron and ascorbic acid addition on dry infusion process and final color of pumpkin tissue. LWT – Food Science and Technology, 58(2), 563–570.

Gerbeau, P., Amodeo, G., Henzler, T., Santoni, V., Ripoche, P., & Maurel, C. (2002). The water permeability of Arabidopsis plasma membrane is regulated by divalent cations and pH. Plant Journal, 30(1), 71–81.

Halperin, S. J., & Koster, K. L. (2006). Sugar effects on membrane damage during desiccation of pea embryo protoplasts. Journal of Experimental Botany, 57(10), 2303–2311.

Hills, B. P., & Duce, S. L. (1990). The influence of chemical and diffusive exchange on water proton transverse relaxation in plant tissues. Magnetic Resonance Imaging, 8(3), 321–331.

Hills, B. P., & Remigereau, B. (1997). NMR studies of changes in subcellular water compartmentation in parenchyma apple tissue during drying and freezing. International Journal of Food Science and Technology, 32, 51–61.

Koster, K. L., Reisdorph, N., & Ramsay, J. L. (2003). Changing desiccation tolerance of pea embryo protoplasts during germination. Journal of Experimental Botany, 54(387), 1607–1614.

Maurel, C. (2007). Plant aquaporins: Novel functions and regulation properties. FEBS Letters, 581(12), 2227–2236. and stomatal movement. Nature, 434(7031), 404–408.

Mauro, M. A., Tavares, D. Q., & Menegalli, F. C. (2003). Behavior of plant tissue in osmotic solution. Journal of Food Engineering, 56, 1–15.

Mavroudis, N. E., Gidley, M. J., & Sjöholm, I. (2012). Osmotic processing: Effects of osmotic medium composition on the kinetics and texture of apple tissue. Food Research International, 48(2), 839–847.

Nieto, A. B., Vicente, S., Hodara, K., Castro, M. A., & Alzamora, S. M. (2013). Osmotic dehydration of apple: Influence of sugar and water activity on tissue structure, rheological properties and water mobility. Journal of Food Engineering, 119(1), 104–114.

Odriozola-Serrano, I., Hernàndez-Jover, T., & Martìn-Belloso, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. Food Chemistry, 105, 1151–1158.

Panarese, V., Laghi, L., Pisi, A., Tylewicz, U., Dalla Rosa, M., & Rocculi, P. (2012). Effect of osmotic dehydration on Actinidia deliciosa kiwifruit: A combined NMR and ultrastructural study. Food Chemistry, 132(4), 1706–1712.

Peiter, E., Maathuis, F. J. M., Mills, L. N., Knight, H., Pelloux, J., Hetherington, A. M., 2+ Food Research International, 39, 154–164. et al. (2005). The vacuolar Ca-activated channel TPC1 regulates germination and stomatal movement. Nature, 434(7031), 404-408.

Qian, H. F., Peng, X. F., Han, X., Ren, J., Zhan, K. Y., & Zhu, M. (2014). The stress factor, exogenous ascorbic acid, affects plant growth and the antioxidant system in Arabidopsis thaliana. Russian Journal of Plant Physiology, 61(4), 467–475.

Rocculi, P., Panarese, V., Tylewicz, U., Santagapita, P., Cocci, E., Gómez Galindo, F., et al. (2012). The potential role of isothermal calorimetry in studies of the stability of fresh-cut fruits. LWT – Food Science and Technology, 49(2), 320–323.

Roy, S., Conway, W. S., Watada, A. E., Sams, C. E., Pooley, C. D., & Wergin, W. P. (1994). Distribution of the anionic sites in the cell wall of apple fruit after calcium treatment – Quantitation and visualization by a cationic colloidal gold probe. Protoplasma, 178(3–4), 156–167.

Saruyama, N., Sakakura, Y., Asano, T., Nishiuchi, T., Sasamoto, H., & Kodama, H. (2013). Quantification of metabolic activity of cultured plant cells by vital staining with fluorescein diacetate. Analytical Biochemistry, 441(1), 58–62.

Sereno, A. M., Moreira, R., & Martinez, E. (2001). Mass transfer coefficients during osmotic dehydration of apple in single and combined aqueous solutions of sugar and salt. Journal of Food Engineering, 47(1), 43–49.

Silva, K. S., Fernandes, M. A., & Mauro, M. A. (2014a). Effect of calcium on the osmotic dehydration kinetics and quality of pineapple. Journal of Food Engineering, 134, 37–44.

Silva, K. S., Fernandes, M. A., & Mauro, M. A. (2014b). Osmotic dehydration of pineapple with impregnation of sucrose, calcium, and ascorbic acid. Food and Bioprocess Technology, 7, 385–397.

Tapken, D., Anschütz, U., Liu, L. H., Huelsken, T., Seebohm, G., & Becker, D., et al. (2013). A plant homolog of animal glutamate receptors is an ion channel gated by multiple hydrophobic amino acids. Science Signaling, 2, 279, ra47.

Thebud, R., & Santarius, K. A. (1982). Effects of high-temperature stress on various biomembranes of leaf cells in situ and in vitro. Plant Physiology, 70, 200–205.

Tyerman, S. D., Niemietz, C. M., & Bramley, H. (2002). Plant aquaporins: Multifunctional water and solute channels with expanding roles. Plant, Cell and Environment, 25, 173–194.

Tylewicz, U., Romani, S., Widell, S., & Gómez Galindo, F. (2013). Induction of vesicle formation by exposing apple tissue to vacuum impregnation. Food and Bioprocess Technology, 6(4), 1099–1104.

Tylewicz, U., Panarese, V., Laghi, L., Rocculi, P., Nowacka, M., Placucci, G., et al. (2011). NMR and DSC water study during osmotic dehydration of Actinidia deliciosa and Actinidia chinensis kiwifruit. Food Biophysics, 6(2), 327–333.

Vicente, S., Nieto, A. B., Hodara, K., Castro, M. A., & Alzamora, S. M. (2012). Changes in structure, rheology, and water mobility of apple tissue induced by osmotic dehydration with glucose or trehalose. Food and Bioprocess Technology, 5(8), 3075–3089.

Wang, Y., Xie, X., & Long, L. E. (2014). The effect of postharvest calcium application in hydro-cooling water on tissue calcium content, biochemical changes, and quality attributes of sweet cherry fruit. Food Chemistry, 160, 22–30.

Weig, A., Deswarte, C., & Chrispeels, M. J. (1997). The major intrinsic protein family of Arabidopsis has 23 members that form three distinct groups with functional aquaporins in each group. Plant Physiology, 114, 1347–1357.

Zemke-White, W. L., Clements, K. D., & Harris, P. J. (2000). Acid lysis of macroalgae by marine herbivorous fishes: Effects of acid pH on cell wall porosity. Journal of Experimental Marine Biology and Ecology, 245, 57–68.