

EFFECT OF OSMOTIC DEHYDRATION ON KIWIFRUIT: RESULTS OF A MULTIANALYTICAL APPROACH TO STRUCTURAL STUDY UTICAJ OSMOTSKE DEHIDRACIJE NA KIVI: REZULTATI MULTIANALITIČKOG PRISTUPA PROUČAVANJA STRUKTURE

Marco DALLA ROSA*, Urszula TYLEWICZ*, Valentina PANARESE*, Luca LAGHI*, Annamaria PISI**,
Patricio SANTAGAPITA***, Pietro ROCCULI*

*Alma Mater Studiorum University of Bologna, Department of Food Science, P.zza Goidanich 60, 47521 Cesena (Italy)

**Alma Mater Studiorum University of Bologna, Department of Agroenvironmental Science and Technologies,
University of Bologna, Viale G. Fanin 44, 40127 Bologna, Italy

*** University of Buenos Aires, Faculty of Exact and Natural Sciences, Industry Department - National Council of Scientific and
Technical Research (CONICET), Buenos Aires, Argentina
e-mail: marco.dallarosa@unibo.it

ABSTRACT

This paper presents the results of the comparison of different analytical techniques (Differential Scanning Calorimetry - DSC, Low Field Nuclear Magnetic Resonance - LF-NMR, Light Microscopy - LM and Transmission Electron Microscopy - TEM) in order to evaluate the mass transfer, water status and cellular compartment modifications of the kiwifruit outer pericarp tissue during osmotic dehydration treatment (OD). Two kiwifruit species, *A. deliciosa* and *A. chinensis* were submitted to OD. OD was performed in a 61.5 % w/v sucrose solution at three different temperatures (25, 35 and 45 °C), with treatment time from 0 to 300 min. Peleg's model highlighted that the main response differences between the two kiwifruit species occurred during the initial phase of the osmotic treatment. DSC parameters appeared to be sensitive to water and solid exchange between fruits and osmotic solution. LF-NMR proton T_2 revealed the consequences of the water-solid exchange on the cell compartments, namely vacuole, cytoplasm plus extracellular space and cell wall. During OD, the reduction of the vacuole proton pool, detected by LF-NMR, suggested a shrinkage of such compartment, confirmed by LM. Cell walls of outer pericarp showed considerable changes in size, structure and stain uptake during OD observed at TEM. The proposed multianalytical approaches should enable better design of combined processing technologies permitting the evaluation of their effects on tissue response.

Key words: osmotic dehydration, cell compartments, DSC, NMR, TEM.

REZIME

U ovom radu su prikazani rezultati poređenja različitih analitičkih tehnika (Diferencijalna skeniranja Kalorimetrija - DSC, Niska Polje nuklearna magnetna rezonanca - LF-NMR, svetlost mikroskopije - LM i Prenos elektronsku mikroskopiju - TEM), u cilju određivanja prenosa mase, statusa vode i modifikacije ćelija tkiva perikarpa kivija tokom osmotske dehidracije tretmana (OD). Dve sorte kivija su bile izložene osmotskom tretmanu, *A. deliciosa* i *A. chinensis*. Osmotsko sušenje obavljeno je u 61,5% rastvoru saharoze na tri različite temperature (25, 35 i 45°C), sa vremenom trajanja sušenja od 0 do 300 minuta. Pelegovim modelom naglašene su glavne razlike između dve sorte kivija koje se događaju tokom početne faze osmotskog tretmana. DSC parametri osetljivi su na izmenu vode i čvrste materije između voća i osmotskog rastvora. LF-NMR-om otkrivene su posledice razmene vode i čvrste materije na ćeliju, odnosno vakuole, citoplazmu sa vanćelijskim prostorom i ćelijski zid. Tokom OD, smanjenje vakuole protona bazena, otkrivena je LF-NMR, predložio skupljanja takvog odeljka, potvrđeno LM. Ćelijske zidove spoljne perikarpa su pokazali značajne promene u veličini, strukturi i mrlja uzimanja u toku OD posmatrano na sistem. Predloženi multianalitički pristupi treba da omogući bolji dizajn u kombinaciji obrade tehnologija dozvoljava procenu njihovog uticaja na tkivo odgovor.

Ključne reči: osmotske dehidracije, mobilni pregrade, DSC, NMR, TEM.

INTRODUCTION

Osmotic dehydration (OD) is a partial dewatering impregnation process carried out by the immersion of cellular tissue in hypertonic solution. The difference in water chemical potential between the food and osmotic medium promotes the release of water from the tissue into the osmotic solution with a simultaneous impregnation of the product with the solutes (Ferrando, Spiess, 2001, Khin et al., 2006). OD decreases water mobility and availability, promoting the improvement of fresh vegetable tissue stability (Gianotti et al., 2001). The use of combining different analytical techniques like Differential Scanning Calorimetry (DSC) and Nuclear Magnetic Resonance (NMR) can offer different but complementary point of view in studying the water status and compartmentalization in several systems (Cornillon, 2000, Venturi et al., 2007, Tylewicz et al., 2011). DSC measurement allows to determine the unfrozen water content within the samples, which depends on the presence of small solutes, for example ions, the presence of macromolecules and membranes and viscosity of the solution (Wolfe et al., 2002). NMR meas-

urement yields an additional degree of details for the description of the embedded water. The water state and compartmentalization has been studied by NMR techniques in different fruits such as apples (Hill, Remigerau, 1997) and strawberries (Marigheto et al., 2004), showing different transversal relaxation times (T_2) for the protons located in the cell walls, vacuoles, extracellular spaces and/or cytoplasm. Cellular compartment modification can be more conveniently studied by means of light (LM) and transmission electron microscopy (TEM) techniques, already used to describe cellular organization changes during ripening and/or technological processing of vegetable tissue (Alandes et al., 2006; Salvatori, Alzamora, 2000; Hallett et al., 1992). The objective of the present study was to compare the different analytical techniques in a joint laboratory research in order to evaluate the mass transfer phenomena, water state and cellular compartment modifications of the kiwifruit outer pericarp tissue during osmotic dehydration treatment. For this purpose the DSC, LF-NMR, LM, TEM measurements were performed on fresh and osmotic dehydrated kiwifruits.

MATERIAL AND METHODS

Raw Materials

Two species of kiwifruit (*Actinidia deliciosa* var. Hayward and *Actinidia chinensis* var. Hort 16A) were bought on the local market and stored at 4 ± 1 °C until they were processed. The osmotic dehydration treatment was applied on fruit hand peeled and cut into 10 mm thick slices. The fruit had homogeneous size (major axis of 60 ± 5 mm and minor axis of 45 ± 5 mm) and refractometric index of 12.0 ± 0.4 °Brix.

Osmotic dehydration treatment

The osmotic dehydration was carried out by dipping the samples in 61.5 % (w/v) sucrose solution equilibrated at three temperatures (25, 35 and 45 °C) for pre-established contact period of 0, 15, 30, 60 and 300 min. The product/solution ratio was about 1:4 (w/w), to avoid changes in the solution concentration during the treatment. Three slices from the central part of each kiwifruit (about 180 g) were placed in mesh baskets and immersed in osmotic solution. The baskets were continuously stirred with a propeller. After that, the slices were taken from the osmotic solution and each slice face was rinsed with distilled water for 3 s and placed on blotting paper for 2 s.

Analytical determinations

Kiwifruit slices were weighted before and after osmotic dehydration process by means of technical balance (precision 0.01 g). The moisture content of kiwifruit samples was determined gravimetrically by vacuum drying (pressure ≤ 100 mm Hg) at 70 °C until a constant weight was achieved (AOAC 920.15, 2002). Soluble solids content was determined at 20 °C by measuring the refractive index with a digital refractometer (PR1, Atago, Japan).

Mass transfer parameters

Osmotic dehydration kinetics of kiwifruit were evaluated by calculating net change (Δ) of kiwifruit slices total mass (M^o), water mass (M^w) and solids mass (M^{ST}) adopting the following equations (Fito and Chiralt, 1997):

$$\Delta M_t^o = M_t^o - M_0^o = \frac{m_t - m_0}{m_0} \quad (1)$$

$$\Delta M_t^w = M_t^w - M_0^w = \frac{m_t x_{wt} - m_0 x_{w0}}{m_0} \quad (2)$$

$$\Delta M_t^{ST} = M_t^{ST} - M_0^{ST} = \frac{m_t x_{STt} - m_0 x_{ST0}}{m_0} \quad (3)$$

where: m_0 : initial weight before osmotic treatment (kg); m_t : weight after a time t (kg); x_w : water mass fraction ($\text{kg} \cdot \text{kg}^{-1}$), x_{ST} : total solids mass fraction ($\text{kg} \cdot \text{kg}^{-1}$)

DSC Measurements

DSC analysis was carried out on a Pyris 6 DSC (Perkin-Elmer Corporation, Wellesley, USA). The DSC was equipped with a low-temperature cooling unit Intacooler II (Perkin-Elmer Corporation). Temperature and melting enthalpy calibrations were performed with ion exchanged distilled water (mp 0.0 °C), indium (mp 156.60 °C), and zinc (mp 419.47 °C); heat flow was calibrated using the heat of fusion of indium ($\Delta H = 28.71 \text{ J} \cdot \text{g}^{-1}$). For the calibration, the same heating rate, as used for sample measurements, was applied under a dry nitrogen gas flux of $20 \text{ mL} \cdot \text{min}^{-1}$. Samples of about 20-30 mg were encapsulated in 50 μL hermetic aluminium pans prior to measurements. An empty pan was used as a reference. DSC curves were obtained by cooling samples to -60 °C and then heating at $5 \text{ }^\circ\text{C} \cdot \text{min}^{-1}$ to 110 °C after an isothermal hold for 5 min at -60 °C.

NMR Measurements

Samples of about 400 mg of kiwifruit were placed inside 10 mm outer diameter NMR tubes so that they did not exceed the active region of the radio frequency coil, and they were analyzed at 24 °C with the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence using a Bruker Minispec PC/20 spectrometer operating at 20 MHz. Each measurement comprised 30000 echoes, with a 2τ interpulse spacing of 80 μs and a recycle delay of 3.5 s. The number of scans was varied depending on moisture content, to obtain a S/N ratio in the range 900–1400. The CPMG decays were normalized to the sample weight, and analyzed with the UPEN program, which inverts the CPMG signal using a continuous distribution of exponential curves, according to equation (3):

$$I(2n) = \sum_{i=1}^M I_0(T_{2,i}) \exp(-2n/T_{2,i}) \quad (3)$$

where 2τ is the CPMG interpulse spacing, n is the index of a CPMG echo, and $I_0(T_{2,i})$ provides a distribution of signal intensities for each T_2 component extrapolated at $\tau = 0$ (the relaxogram), sampled logarithmically in the interval $T_{2,\min} - T_{2,\max}$ set by the user. Default values for all UPEN parameters were used throughout this work.

Neutral red staining (Light Microscopy – LM)

The kiwifruit juice, obtained by blending the raw kiwifruit in a centrifuge, thermal inactivated and filtered, was used as a medium to which neutral red stain (which penetrates the vacuole of intact protoplast of plant cells) has been added to reach a final concentration of 0.05 % (w/v) (Mauro et al., 2002). Slices, 2 mm thick, were cut manually at about 2 mm distance from the peeled kiwifruit (*A. deliciosa*) surface, parallel to its longitudinal axis and stained for 10-20 min. To obtain a real time visualization of the vacuole shrinkage occurring during the osmotic treatment, each stained slice was mounted on a microscope slide and immersed in a 61.5 % (w/v) aqueous sucrose solution drop. The slide was inserted in the microscope and viewed for 60 min at 25 °C. RGB images were acquired under the same conditions (true colour – 24 bit, 300 BPP) using a digital photcamera mod. Camedia C-4040-ZOOM (Olympus, Tokyo, Japan) and stored in JPEG format.

The images obtained using neutral red staining were processed with the software Photoshop® v. 5.0 (Adobe Systems Incorporated, USA), in order to evaluate the retraction of the vacuole after 60 min of OD treatment.

Transmission Electron Microscopy

Samples (1-2 mm cubes) of outer pericarp tissue were removed from the kiwifruit slices (*A. deliciosa*, fresh and OD at 35 °C for 120 min), parallel to the kiwifruit longitudinal axis, at about 2 mm distance from the slice surface. The choice of osmotic conditions was based on the preliminary study. Tissue was fixed in 5 % (w/v) glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. After washing in the buffer, the samples were postfixed in 1% (w/v) osmium tetroxide in 0.1 M phosphate buffer at pH 7.2, for 1 h while gently agitated. All these steps were performed at 4°C. After washing in phosphate buffer and in distilled water, these pieces were block stained in 0.5 % (w/v) aqueous uranyl acetate for 2 h at 4 °C, in the dark. All samples were washed in distilled water, dehydrated in aqueous ethanol series and embedded in Spurr's low viscosity resin (Spurr, 1969). The specimens were cut using an LKB Ultramicrotome. The ultrathin sections were double stained with uranyl acetate and lead citrate and examined under a Philips CM10 TEM, at an accelerating voltage of 60 kV. Pictures were taken on Kodak film.

Statistical analysis

Significance of the osmotic dehydration effects was evaluated by means of one-way and two-way analysis of variance (ANOVA, 95 % significance level) using the software STATISTICA 6.0 (Statsoft Inc., Tulsa, UK).

RESULTS AND DISCUSSION

Mass transfer parameters

For both *A. chinensis* and *A. deliciosa* kiwifruit species, the highest water loss rates occurred during the first treatment hour as shown in Figure 1. During that time water loss in *A. chinensis* was 18, 20 and 35 % of the initial fresh weight and in *A. deliciosa* 15, 21 and 29 %, for 25, 35 and 45 °C, respectively. After 300 min of osmotic treatment, the percentage of water loss varied on average close to 38, 45 and 62 % in *A. chinensis* and 27, 50 and 58 % in *A. deliciosa* at 25, 35 and 45 °C (Tylewicz et al., 2011). This agrees with the literature; in fact Kowalska and Lenart (2001) showed noteworthy water content decreasing during the first 30 min of the osmotic process (30 °C, 61.5 % sugar solution). After this period the dehydration of carrots, apples and pumpkins appears slower.

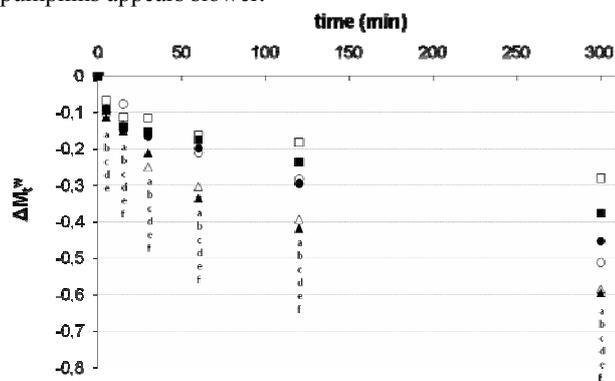


Fig. 1. Water loss during osmotic dehydration of *Actinidia chinensis* kiwifruit at 25 °C (■), 35 °C (●) and 45 °C (▲) and *Actinidia deliciosa* kiwifruits at 25°C (□), 35 °C (○) and 45 °C (△). Different letters within the same sampling time indicate statistical differences ($p < 0.01$).

Statistical analysis of the values represented in Figure 1 shows that, for both *A. deliciosa* and *A. chinensis*, the osmotic solution temperature positively influenced the water loss rate. It is well recognized that diffusion is a temperature-dependent phenomenon. Higher process temperatures seem to promote faster water loss through swelling and plasticising of cell membranes, faster water diffusion within the product and better mass (water) transfer characteristics on the surface due to lower viscosity of the osmotic medium (Lazarides et al., 1995). Kiwifruits have a porous structure, so high temperature would release trapped air from the tissue structure, resulting in more effective removal of water by osmotic pressure (Cao et al., 2006).

The kiwifruits solid gain during the osmotic dehydration is shown in Figure 2. Statistical analysis of the values represented in Figure 2 shows different solid gain behaviors for the two species. The solutes gain of *A. chinensis* seemed to be positively influenced by the temperature less than the one of *A. deliciosa*. Tylewicz et al. (2009) also observed a low temperature effect on solute gain of *A. chinensis* slices during osmotic dehydration but, to the contrary, Cao et al. (2006) showed a notably enhanced of solute gain, in *A. deliciosa* kiwifruits, by increasing osmotic dehydration temperature and time.

Moreover the Peleg's model was used to fit mass transfer parameter data over processing time ($0.83 < R^2 < 0.99$). This kinetic model confirmed and highlighted that the main response differences between the two kiwifruit species occurred during the initial phase of the osmotic treatment (data not shown).

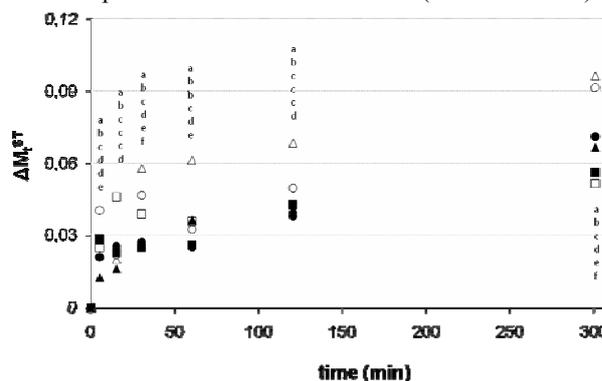


Fig. 2. Solid gain during osmotic dehydration of *Actinidia chinensis* kiwifruit at 25 °C (■), 35 °C (●) and 45 °C (▲) and *Actinidia deliciosa* kiwifruits at 25 °C (□), 35 °C (○) and 45 °C (△). Different letters within the same sampling time indicate statistical differences ($p < 0.01$).

Multianalytical approach was used in order to study the water state and compartmentalization in raw and osmotic dehydrated kiwifruit. DSC measurements permitted to evaluate the changes of initial point of ice melting ($T_{f, onset}$) and frozen water content (x_w^F), that are related to product stability. The frozen water is water having enough mobility to freeze, as weakly bound to the macromolecular matrix. During the osmotic treatment, the kiwifruit slices thermo-physical properties ($T_{f, onset}$ and x_w^F) progressively changed as shown in Table 1.

Table 1. $T_{f, onset}$ (initial point of ice melting) and x_w^F (frozen water content) average values obtained during the osmotic dehydration of *A. deliciosa* and *A. chinensis* kiwifruit, as reported by Tylewicz et al., 2011. Statistical significance was assessed by one-way ANOVA. Different letters within the same column indicate statistical differences ($p < 0.01$).

Kiwifruit species	Temperature (°C)	Time(min)	$T_{f, onset}$ (°C)	X_w^F (g/g _{fw})
<i>Actinidia chinensis</i>	raw	0	- 1.8 ^a	0.75 ^a
	25	60	- 4.3 ^{bc}	0.61 ^b
		120	- 4.4 ^{bc}	0.62 ^b
		300	- 4.8 ^c	0.60 ^b
	35	60	- 4.2 ^{bc}	0.61 ^b
		120	- 4.6 ^{bc}	0.60 ^b
		300	- 8.7 ^e	0.42 ^d
	45	60	- 4.1 ^b	0.61 ^b
		120	- 6.7 ^d	0.59 ^b
300		- 9.1 ^e	0.47 ^c	
<i>Actinidia deliciosa</i>	raw	0	- 2.7 ^a	0.66 ^a
	25	60	- 4.9 ^b	0.60 ^b
		120	- 4.5 ^b	0.67 ^a
		300	- 5.4 ^{bc}	0.65 ^{ab}
	35	60	- 4.6 ^b	0.60 ^b
		120	- 6.6 ^c	0.49 ^c
		300	- 7.8 ^c	0.48 ^{de}
	45	60	- 5.2 ^b	0.52 ^c
		120	- 7.0 ^c	0.42 ^{de}
300		- 12.6 ^d	0.36 ^e	

In agreement with Cornillon (2000) the depletion of the initial ice melting temperature ($T_{f,onset}$) progressively increased along with the proceeding of the osmotic treatment and with the increase of the treatment temperature, following the trend of water loss and solids gain results. With the proceeding of the osmotic treatment tendentially all the samples showed a decrease of frozen water content; this behaviour was particularly evident for samples treated at high temperatures (35, 45 °C). For both $T_{f,onset}$ and x_w^F two-way ANOVA analysis evidenced significant effects of both time ($p < 0.001$) and temperature ($p < 0.01$).

NMR measurements allowed the identification of three proton pools in both kiwifruit species, with T_2 for raw fruits around 40, 300 and 950 ms respectively. Through a comparison with the results obtained on apples and carrots by Hills (1997, 1999), such pools were ascribed to cell wall, cytoplasm plus extracellular space and vacuole respectively.

In raw kiwifruits, the protons located inside the vacuole represented the 61 % of the total protons in *A. deliciosa*, and an even greater portion in *A. chinensis* (70 %). During the osmotic treatment the T_2 value and amount of vacuole protons of *A. deliciosa* decreased proportionally to the temperature. The reduction

could be observed also in *A. chinensis*, resulting higher than *A. deliciosa* at 45 °C and lower at 25 and 35°C (Fig. 3).

This behaviour can be explained considering that, at 20 MHz radiofrequency, T_2 value (ms) shorter than the one of pure water (≈ 1600 ms) mainly reflects the proton exchange between water and solutes (Hills and Remigereau, 1997). Thus the T_2 of the water protons pertaining to a certain compartment can decrease when the (solutes + biopolymers) / water ratio increases. The decreasing of signal values from vacuole protons suggests that this compartment can undergo shrinkage during the dehydration, due to water leakage (Aguilera et al., 2003); consequently the solutes concentration caused a shortening of the T_2 .

In order to better understand the micro and ultrastructural changes in kiwifruit outer pericarp tissue during OD the LM and TEM measurements were performed.

LM images of neutral red stained cells displayed the vacuole shrinkage subsequent to the OD treatment confirming the results obtained by NMR measurements. Figure 4 shows the outline of a stained kiwifruit vacuole before and after one hour of OD. Comparing these two micrographs, it seems that occupied volume by the fluorescent vacuole qualitatively decreased during the 60 min of the treatment.

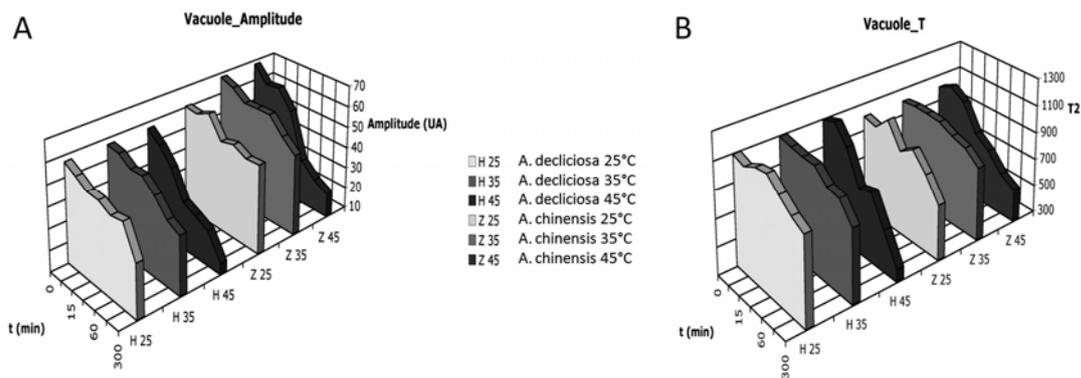


Fig. 3. Intensities (A) and transversal relaxation time (T_2) (B) of vacuole proton pools of both kiwifruit species during the osmotic treatment at 25, 35 and 45 °C.

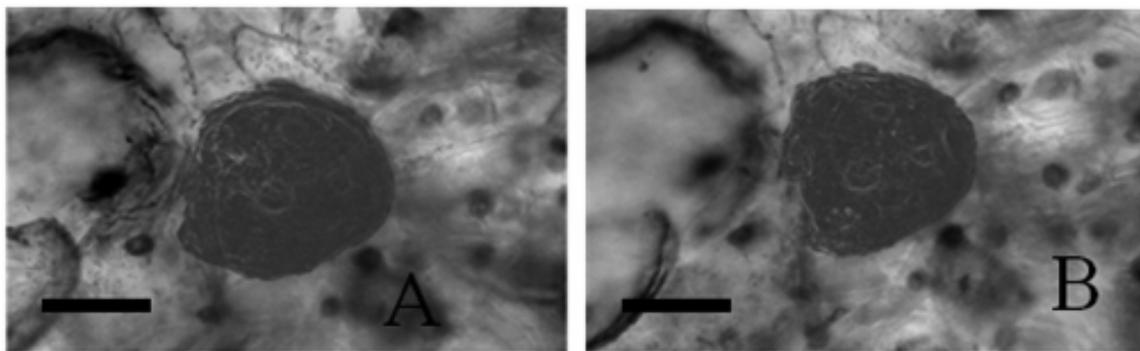


Fig. 4. LM of kiwifruit (*A. deliciosa*) outer pericarp cells. Highlighted neutral-red stained vacuole after 60 min of OD at 30 °C: (a) processed image of cell prior OD (b) processed image of cell after OD. Bar = 100 μ m.

As far as the cytoplasm / extracellular space proton pool is concerned, with the proceeding of the treatment time, the proton pool intensity increased together with a T_2 reduction (data not shown). This could be due to the intracellular space creation filled by vacuole content and osmotic solution, which contribute to the cytoplasm and the extracellular space proton pool.

The treatment seemed to increase the proton pool located in the cell walls, without changing its T_2 (data not shown). Since the NMR signal is not enough sensible to measure with accuracy the proton pools from cell wall the changes of the last were evaluated by TEM observations.

TEM observations marked a decrease of the staining together with a visible swelling of the cell wall and the loss of cell wall fibrous appearance (Figures 5A and 5B). Moreover the decrease of the plasmodesmata region staining was observed (Figures 5C and 5D). The reduction in staining intensity of cell wall material occurred predominantly in the treated kiwifruit tissues and might be partially accounted by wall expansion. The loss of staining probably reflects a change in cell wall structure and composition *in situ*, since it has not been revealed in the raw fruits.

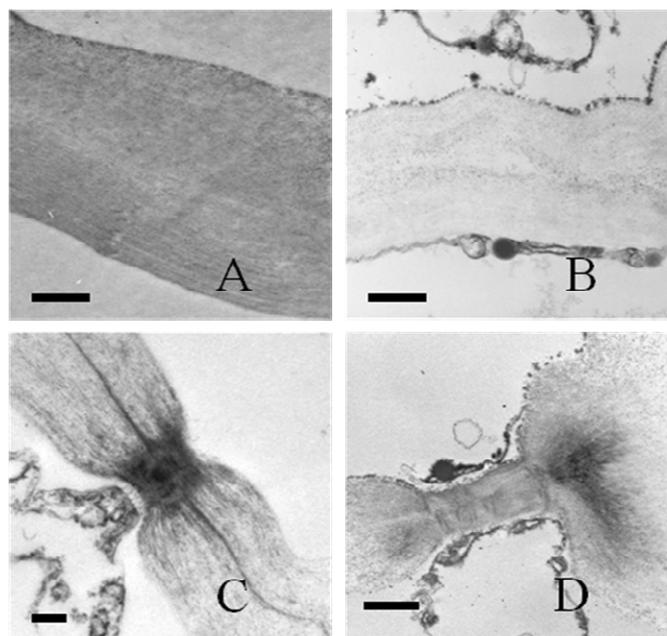


Fig. 5. TEM microscopy of outer pericarp cells from kiwifruit (*A. deliciosa*). Cell wall of raw kiwifruit (A) and 2 hours after OD-treatment (B); electron-dense plasmodesmata complex of raw (C) and two hours OD-treated kiwifruit (D). Scale (A)-(D) 1 μ m.

CONCLUSIONS

The present work shows the effect of osmotic dehydration on two kiwifruit species, *A. deliciosa* and *A. chinensis*, in terms of mass transfer phenomena, water state behaviours and cellular compartment modification.

Treatment time positively influenced water loss and solid gain of both the studied species, while temperature significantly affected only water loss. Peleg's model highlighted that the main response differences between the two species occurred during the initial phase of osmotic treatment.

Thermal properties and relaxation time measurements offered a complementary view concerning the effects of osmotic dehydration on kiwifruit. DSC parameters appeared to be sensitive to water and solid exchange between fruits and osmotic solution. During the osmotic treatment, the initial freezing temperature and the frozen water content decrease was dependent on treatment time and temperature, showing a similar tendency for both the kiwifruit species.

LF-NMR proton T_2 revealed the consequences of the water-solid exchange on the cell compartments, namely vacuole, cytoplasm plus extracellular space and cell wall. The reduction of the vacuole protons suggested shrinkage of such compartment, confirmed by LM images of neutral red stained cells. Cell walls of outer pericarp showed considerable changes in size, structure and stain uptake during OD observed at TEM.

The multianalytical approach proposed in this study represents a powerful and versatile tool with the potentiality to investigate the behaviour of vegetable tissues towards those subjected to different processing conditions.

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