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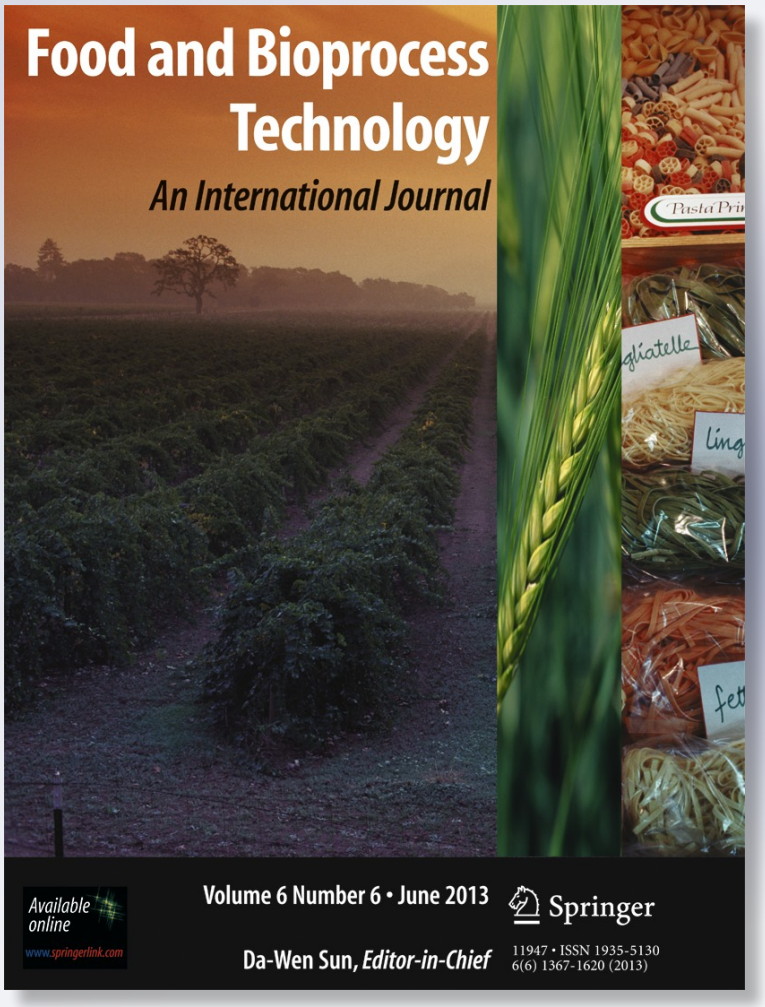

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ORIGINAL PAPER

Modification of Transverse NMR Relaxation Times and Water Diffusion Coefficients of Kiwifruit Pericarp Tissue Subjected to Osmotic Dehydration

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Abstract The objective of the present study was to evaluate cellular compartment modifications of kiwifruit (*Actinidia deliciosa*) outer pericarp tissue caused by osmotic treatment in a 61.5 % sucrose solution, through the quantification of transverse relaxation time (T_2) and water self-diffusion coefficient (D_w) obtained by low field nuclear magnetic resonance means. Raw material ripening stage was taken into account as an osmotic dehydration (OD) process variable by analyzing two different kiwifruit groups, low (LB) and high (HB) °Brix. Three T_2 values were obtained of about 20, 310, and 1,250 ms, which could be ascribed to the proton populations, located in the cell walls, in the cytoplasm/extracellular space, and in the vacuoles, respectively. According to T_2 intensity values, vacuole protons represented between 47 and 60 % of the total kiwifruit protons, for LB and HB kiwifruits, respectively. The leakage of water leading to vacuole shrinkage seemed to cause concentration of solutes, retained by the tonoplast, making the vacuole T_2 value decrease along the OD. As expected, the D_w values of raw kiwifruits were lower than the value of the free pure water. The water mobility (and hence D_w), depending on the kiwifruit distinctive cellular structures and solutes, decreased even more during OD due to water loss and sugar gain phenomena. D_w represents an average value of the diffusion coefficient of the whole kiwifruit tissue protons. In order to obtain D_w values specific for each cellular compartment, a multiple component model fitting was also used. According to these results, the vacuole water self-diffusion coefficient ($D_{w,v}$) was much higher than D_w.

Keywords Kiwifruit · Osmotic dehydration · Low field nuclear magnetic resonance (LF-NMR) · Transverse relaxation time · Diffusion coefficient

Nomenclature

m_0	Initial (fresh) weight before osmotic treatment (grams)
m_t	Weight at time t (grams)
$[g_{fw}]$	Grams of fresh weight
x_{w0} x_{wt}	Water mass fraction (g g_{fw}^{-1}) at time 0 and time t, respectively
x_{ST0} x_{STt}	Total solids mass fraction (g g_{fw}^{-1}) at time 0 and time t, respectively
M_t°	Total mass ratio at time t $m_t \cdot m_0^{-1}$
M_0°	Total mass ratio at time 0 $m_0 \cdot m_0^{-1}$
M_t^{W}	Water mass ratio at time t $m_t \cdot x_{wt} \cdot m_0^{-1}$
M_0^{W}	Water mass ratio at time 0 $m_0 \cdot x_{w0} \cdot m_0^{-1}$
M_t^{ST}	Solids mass ratio at time t $m_t \cdot x_{STt} \cdot m_0^{-1}$

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M_0^{ST}	Solids mass ratio at time 0 $m_0 \cdot x_{ST0} \cdot m_0^{-1}$
k_1 k_2	Peleg's constants
k_1^J ($k_1°$ k_1^W, or k_1^{ST}); k_2^J ($k_2°$ k_2^W, or k_2^{ST})	Mass transfer constants
$1/k_1°$	Initial rate of total mass change (1/minute)
$1/k_1^{ST}$	Initial rate of solids mass change (1/minute)
$1/k_1^W$	Initial rate of water mass change (1/minute)
$1/k_2°$	Total mass change at equilibrium (grams/gram)
$1/k_2^{ST}$	Solids mass change at equilibrium (grams/gram)
$1/k_2^W$	Water mass change at equilibrium (grams/gram)

Introduction

Osmotic dehydration (OD) is a partial dewatering-impregnation process usually carried out by immersion of vegetable tissues in hypertonic solution (Ferrando and Spiess 2001). Complex cellular structure of fruit and vegetables could be considered as semipermeable membrane; thus, the mass transfer phenomenon occurs as a consequence of the water chemical potential differences between the tissues and the osmotic medium (Ratti and Mujumdar 2004; Khin et al. 2006). The diffusion of water from cells to the solution is usually accompanied by the simultaneous counterdiffusion of solutes from the concentrated solution into the tissues (Kaymak-Ertekin and Sultanoğlu 2000; Kowalska and Lenart 2001). OD treatments could be useful to increase the shelf life of minimally processed high-moisture content products (Dalla Rosa and Torreggiani 2000).

Technological needs related to mechanically performed peeling and cutting operations require raw unripe kiwifruits with high firmness and low soluble solids content (10–11 °Bx). Hence the obtained final fresh-cut product does not present a sufficient ripening level for consumption, at least during the first part of the storage period. If unripe slices are subjected to OD, their taste and firmness can be improved (Bressa et al. 1997). Therefore, the output of OD on fresh-cut unripe vegetable tissues would be a product ready for the consumption. In a previous work, Tylewicz et al. (2011) investigated the influence of OD temperature and time on two kiwifruit species. The authors observed that the treatment time positively influenced kiwifruit water loss and solid gain while temperature significantly affected only water loss.

One of the most powerful techniques to study molecular mobility is low field nuclear magnetic resonance (LF-NMR). LF-NMR has the advantage to be a non-destructive technique and do not employ any solvent which can damage the ambient (green technique); the determinations are fast and give information about the structure and about interactions between molecules. Also, LF-NMR can be applied to a variety of biological tissues. For example, it has been applied to obtain information about a number of different physiological conditions in strawberry, carrot, apple, and kiwifruit, including changes caused by ripening, bruising, microbial infection, drying, freezing, high pressure processing, and OD (Tylewicz et al. 2011; Marigheto et al. 2004; Hills and Clark 2003; Hills and Remigereau 1997). In this direction, Panarese et al. (2011) showed that kiwifruit cellular modifications occurring along OD could be efficiently studied by means of LF-NMR. Different subcellular compartments are characterized by specific water–solutes ratio ranges, leading to different transverse relaxation time (T_2) values. These authors evidenced the shrinkage of the vacuole compartment along OD by the use of LF-NMR and light microscopy techniques and the intercellular space formations by TEM, in agreement with LF-NMR results. The measurement of water self-diffusion could enrich such observations allowing the description of barriers, interfaces, and other noteworthy features inside compartmentalized structures (Hills and Clark 2003). However, the relationship between microstructure and the attenuation of a diffusion-weighted signal is often model dependent, which is not easy to extrapolate from one fruit to another.

The information obtained by LF-NMR and its application to the OD process could help in understanding the behavior of water in the cellular tissues and its migration during processing. The purpose of the present work was to evaluate the cellular compartment modifications of kiwifruit's (*Actinidia deliciosa*) outer pericarp tissue caused by osmotic treatment in a 61.5 % (*w*/*v*) sucrose solution through the quantification of T_2 and water self-diffusion coefficient/s (D_w) obtained by LF-NMR. The maturity degree, a key factor to consider for its technological repercussions, was taken into account as an OD process variable, by analyzing two different °Brix kiwifruit groups. Diffusion-weighted LF-NMR signals were also fitted with multiple component models in order to understand the contribution of each cellular compartment to the overall water diffusion.

Materials and Methods

Raw Materials

Kiwifruits (*A. deliciosa* var *deliciosa* cv Hayward) with homogeneous size and refractometric index of 6.9±0.8 °

Bx were bought from the local market. Kiwifruits were sorted to eliminate damaged or defective fruit and were partially ripened at 4±1 °C and 90–95 % of relative humidity in air. Along the storage time, two kiwifruit groups were selected with refractometric index values of 9.5±1.1 (called as LB, low °Brix) and 14.1±0.9 (called as HB, high °Brix) ° Bx. The OD treatment was applied on the fruit, hand peeled and cut into 10-mm thick slices.

Osmotic Dehydration Treatment

OD was carried out by dipping the samples in 61.5 % (*w*/*v*) sucrose solution equilibrated at 25 °C for preestablished contact period of 0, 30, 60, 180, and 300 min, as reported by Tylewicz et al. (2011). The product/solution ratio was about 1:4 (*w*/*w*), to minimize changes in the solution concentration during the treatment as a consequence of water loss from kiwifruit to the solution and simultaneous counter-diffusion of sugar into the kiwifruit tissue. The temperature of the solution was maintained constant by a thermo-controlled water bath. OD was performed eight times on a total of 240 kiwifruit slices (30 kiwifruit slices for each time–°Brix group condition). Each slice was taken from the central part of each kiwifruit (about 60 g) and were placed in mesh baskets and immersed in osmotic solution. The baskets were continuously stirred with a propeller. The rotational speed was experimentally determined to assure negligible resistance to mass transfer. At each OD run, the osmotic solution was changed in order to guarantee the same initial concentration for the eight OD runs. 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 the OD process. The moisture content of kiwifruit samples was determined gravimetrically by difference in weight before and after drying in vacuum oven (pressure ≤100 mmHg) at 70 °C. The drying was performed until a constant weight was achieved (AOAC 920.15, 2002). Duplicate measurements were conducted for each kiwifruit slice.

The soluble solids content (SSC) was determined at 20 °C by measuring the refractive index with a digital refractometer (PR1, Atago, Japan) calibrated with distilled water. For each sample, the SSC was determined in triplicate on the juice obtained from each kiwifruit slice, after filtering through Whatman #1 filter paper. For both moisture content and SSC determinations, the average values were obtained for 30 kiwifruit slices for each time–°Brix group condition.

Mass Transfer Parameters

For each OD treatment performed, total mass change at time t $\left(\Delta M_t^{\circ}\right)$, water mass change at time t $\left(\Delta M_t^{\mathrm{w}}\right)$, and solids mass change at time t $\left(\Delta M_t^{\mathrm{ST}}\right)$ were calculated adopting the following equations (Fito and Chiralt 1997):

$$\Delta M_t^{\circ} = M_t^{\circ} - M_0^{\circ} = \frac{m_t - m_0}{m_0} \tag{1}$$

$$\Delta M_t^{\mathrm{W}} = M_t^{\mathrm{W}} - M_0^{\mathrm{W}} = \frac{m_t x_{\mathrm{w}t} - m_0 x_{\mathrm{w}0}}{m_0} \tag{2}$$

$$\Delta M_t^{\mathrm{ST}} = M_t^{\mathrm{ST}} - M_0^{\mathrm{ST}} = \frac{m_t x_{\mathrm{ST}t} - m_0 x_{\mathrm{ST}0}}{m_0} \tag{3}$$

Kinetic Model

Mass transfer data were modeled according to the equation proposed by Palou et al. (1994) and Sacchetti et al. (2001), using the Peleg's model (Peleg 1988):

$$M_t^{\mathrm{W}} - M_0^{\mathrm{W}} = -\frac{t}{k_1^{\mathrm{W}} + k_2^{\mathrm{W}} \cdot t} \tag{4}$$

$$M_t^{\mathrm{ST}} - M_0^{\mathrm{ST}} = +\frac{t}{k_1^{\mathrm{ST}} + k_2^{\mathrm{ST}} \cdot t} \tag{5}$$

In this work, the same equation rewritten as:

$$M_t^{\circ} - M_0^{\circ} = -\frac{t}{k_1^{\circ} + k_2^{\circ} \cdot t} \tag{6}$$

was also used in order to model total mass change kinetics.

As reported by Sacchetti et al. (2001) at the equilibrium condition ($t \to \infty$) the value for mass change could be calculated as:

$$P_t^{\mathrm{J}} = P_0^{\mathrm{J}} \pm \frac{1}{k_2^{J}} \tag{7}$$

where P^{J} could be M°, M^{w}, and M^{ST}, respectively.

Similarly, the initial rate (t=0) of mass transfer is:

$$1/k_1^{\mathrm{J}}$$

As shown by Sacchetti et al. (2001), the calculation of the inverse of the kinetic model constants (k_1 and k_2) enables us to obtain both the initial rate values of mass transfer parameters $1/k_1{}^{\mathrm{J}}$ ($1/k_1{}^{\circ}$, $1/k_1{}^{\mathrm{W}}$, or $1/k_1{}^{\mathrm{ST}}$) and the values of mass transfer parameters at the equilibrium condition $1/k_2{}^{\mathrm{J}}$ ($1/k_2{}^{\circ}$, $1/k_2{}^{\mathrm{W}}$ or $1/k_2{}^{\mathrm{ST}}$).

Low Field Nuclear Magnetic Resonance Measurements

One sample cylinder of about 500 mg for each kiwifruit slice was placed inside 10-mm outer diameter tubes so that they did not exceed the active region of the radio frequency coil. The samples were analyzed at 24 °C using a Bruker Minispec PC/20 spectrometer (Bruker Biospin Gmbh, Rheinstetten, Germany) with a 0.47-T magnetic field operating at a resonance frequency of 20 MHz.

Proton Transverse Relaxation Time

Proton transverse relaxation time (T_2) was measured in five samples for each osmotic treatment time by applying the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (Meiboom and Gill 1958). Each measurement comprised 30,000 echoes, with a 2τ interpulse spacing of 80 μs and a recycle delay of 3.5 s. By following the procedure setup by Panarese et al. (2011), the CPMG decays were analyzed with the UpenWin software (Borgia et al. 2000), which inverts the CPMG signal by using a continuous distribution of exponential curves, according to Eq. 8,

$$I(2\tau n) = \sum_{i=1}^{M} I_0(T_{2,i}) \exp\left(-\frac{2\tau n}{T_{2,i}}\right) \tag{8}$$

where n is the index of a CPMG echo, and I_0 ($T_{2,i}$) provides a distribution of the signal intensities for each T_2 component extrapolated at τ=0 (the relaxogram) sampled logarithmically. The so obtained relaxograms were characterized by three partially overlapped peaks, with T_2 in the ranges 8–35, 180–250, and 900–1,200 ms, respectively. To observe them separately, fittings were performed to the sum of an increasing number of exponential curves. An F test showed that the lowest ratio between error and degrees of freedom of the model was reached for the 75 % of the samples with three exponentials, as noticed in previous works (Panarese et al. 2011; Tylewicz et al. 2011), while the other samples were best represented by the sum of four exponential curves. To improve the uniformity of the fitting procedure across the samples, the first 80 experimental points were removed from each CPMG curve, as they are known to be subject to noise due to imperfections of the 180° pulses (Le Botlan et al. 1996). Each of the so pruned T_2-weighted signals was now best fit to the sum of four exponential curves. The amplitude and T_2 of the two of them showed that both described the central peak of the relaxogram and were therefore summed.

Water Self-Diffusion Coefficient

Water self-diffusion coefficient measurements were performed through pulsed magnetic field gradient spin echo (PGSE) sequence (Stejskal and Tanner 1965), consisting of a spin echo pulse sequence where two controlled magnetic field gradients are applied respectively between 90° and 180° pulses and between the 180° pulse and the acquisition.

The applied magnetic field gradient intensity (G) was calibrated in the range between 0.04 and 2.0 T/m by employing a 1.25-g/L $CuSO_4 \cdot 5H_2O$ water solution, characterized by a known D_w value ($2.3 \cdot 10^{-9}$ m^2/s at 25 °C (Holz et al. 2000)).

As described below, PGSE sequence was first applied to obtain an overall view of the system under investigation, then to separately observe water located inside the vacuoles and inside the cytoplasm/extracellular space.

Single Component Analysis

Water inside the samples was considered as characterized by a single self-diffusion coefficient, so that D_w could be measured by registering the amplitude (A) of the PGSE signal with ($A_{G(t)}$) and without ($A_{G(0)}$) an applied field gradient (G), according to Eq. 9:

$$\ln\frac{A_{G(t)}}{A_{G(0)}} = -\gamma^2 \cdot D_{w,w} \cdot \delta^2 \cdot \left(\Delta - \frac{1}{3}\delta\right) \cdot G^2 \tag{9}$$

where δ is the gradients length, Δ is the time between the gradients, and γ is the proton gyromagnetic ratio.

The kiwifruit samples were analyzed setting the magnetic field gradient amplitude to 0.7 T/m, τ (the time between 90° and 180° pulses) to 7.5 ms, δ to 0.5 ms, and Δ to 7.5 ms.

Multiple Component Analysis

The amplitude of the PGSE signal was registered for 15 magnetic field gradient values, linearly spaced from 0.04 to 0.6 T/m. To focus on water located in the vacuoles and cytoplasm/extracellular space, τ was set to 15 ms, so to remove the contributions to the signal from cell wall protons, with T_2 lower than a few milliseconds.

Self-diffusion coefficient of the water located in the two considered spaces was then obtained from the Eq. 10,

$$\begin{aligned} Y = \text{offset} &+ P_v \cdot \exp\left(-\left(\Delta \cdot q^2\right) \cdot D_{w,v}\right) \\ &+ P_c \cdot \exp\left(-\left(\Delta \cdot q^2\right) \cdot D_{w,c}\right) \end{aligned} \tag{10}$$

where P_v and P_c identify the fractions of water located inside the vacuole and cytoplasm/extracellular space, respectively; $D_{w,v}$ and $D_{w,c}$ are their corresponding diffusion values and offset is the residual % intensity remaining after the signal decay. To avoid overfitting, P_v and P_c were obtained from the CPMG experiments described above.

Diffusion-Weighted T_2 Curves

In order to double check the results from T_2 and D_w experiments, the merge of the two sources of information was performed by registering diffusion-weighted CPMG curves. For that purpose, a CMPG pulse train was added to the end of the PGSE sequence, as described by Duval et al. (2005). CPMG signals were then registered for the same 15 magnetic field gradients chosen for the multiple component analysis above described. The signals were fitted to a continuous distribution of exponential curves through UpenWin software, thus obtaining relaxograms made up to 100 points each. Finally each point followed through the 15 experiments was fitted to Eq. 9.

Statistical Analysis

Significance of the osmotic dehydration effects on T_2 values was evaluated by means of t test (95 % significance level) using the software STATISTICA 6.0 (Statsoft Inc., Tulsa, UK). In order to estimate the kinetic model constants, nonlinear regression was carried out by means of the quasi-Newton calculus algorithm using STATISTICA 6.0.

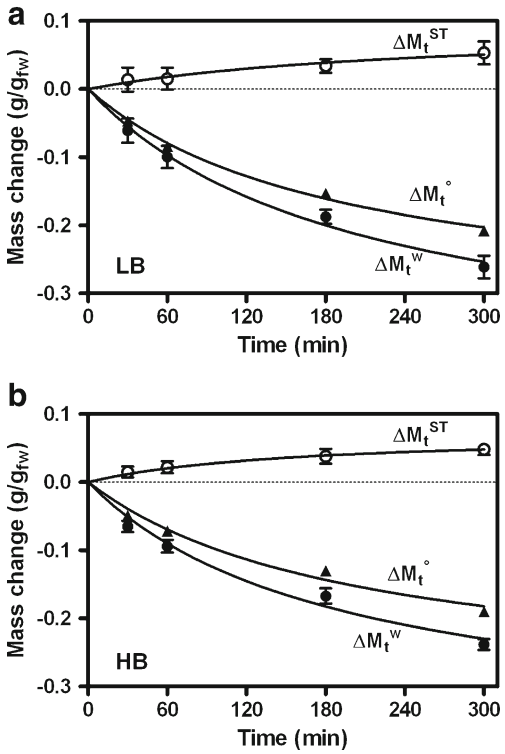

Fig. 1 Application of the Peleg's model to mass transfer data of low (**a**) and high (**b**) °Brix (LB and HB, respectively) kiwifruit subjected to OD at 25 °C. ΔM_t° total mass change, ΔM_t^{W} water mass change, ΔM_t^{ST} soluble solids mass change. Each *point* represents an average of 30 kiwifruit slices and *bars* represent SD values

Results and Discussion

Mass Transfer Parameters

OD leads to a great water loss from kiwifruit and the simultaneous counterdiffusion of solutes from the concentrated solution into the tissues. The kinetic model was used to fit mass transfer parameter data over processing time ($0.82<R^2<0.99$). Figure 1 shows the application of the kinetic model to mass transfer data of LB and HB kiwifruits. Both kiwifruit groups showed similar behavior on mass transfer data (Fig. 1a, b). The highest water loss rates occurred during the first treatment hour, enabling about the 10 and 9 % reduction of the initial fresh weight values for LB and HB kiwifruits, respectively. After 300 min, water loss reached around 26 and 24 % of the fresh weight values for LB and HB, respectively. The water loss rate was the highest at the beginning of the process because the dehydration driving force was the greatest. The observed behavior was similar to previously reported data for kiwifruit (12.0±0.4 °Bx) treated in the same osmotic conditions at 25 °C (Tylewicz et al. 2011) and for other vegetables as carrots, apples, and pumpkins (Kowalska and Lenart 2001).

The reciprocal values of the kinetic model constants (k_1 and k_2) indicating respectively the initial rate values of mass transfer parameters and the mass transfer parameters values at equilibrium condition are displayed in Fig. 2. Both the water loss initial rate value and the water loss value at equilibrium condition of LB kiwifruits are higher than HB samples, as a probable consequence of the lower availability of water in the ripe fruits. Indeed Goñi et al. (2007) found an increase of the unfrozen water along the ripening stages of cherimoya fruits. This increase may indicate a reduction in the amount of water available in the tissues of ripe and overripe fruit associated with the starch conversion and the accumulation of osmolytes. The increase of mass transfer rates from HB to LB kiwifruits is similar to the mass rate increase due to the OD solution temperature increment, reported by Tylewicz et al. (2011). Then, it could be proposed that shorter process time is needed to achieve the same water loss in LB than in HB kiwifruits.

In agreement with Vial's findings (1991), kiwifruit solid gain was minimally influenced by the increase of OD time (Figs. 1 and 2). In fact, during the first hour of OD, the solid gain was about 1.5 and 2.2 % of the fresh weight for LB and HB, respectively, and after 300 min, the percentage of solid gain was 5.3 and 4.8 % for LB and HB, respectively.

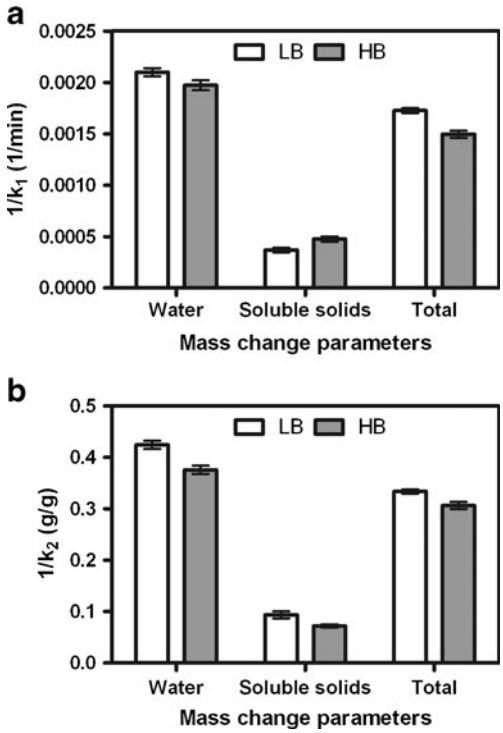

Fig. 2 Mass changes kinetic parameters: **a** initial rate values ($1/k_1$) and **b** values at equilibrium condition ($1/k_2$) of mass change parameters (water mass change, solids mass change and total mass change) during OD in low (*LB*) and high (*HB*) °Brix kiwifruits

Transverse Relaxation Time and Water Self-Diffusion Coefficient

LF-NMR analysis was performed in order to evaluate the changes on kiwifruit water mobility occurring at cellular level during the OD treatment. The kiwifruit water content is considerable high since the 85 % of its fresh weight is water, and CPMG pulse sequence enables to evaluate proton populations with different mobility. Works recently published showed that T_2-weighted curves registered through proton LF-NMR could be successfully employed to gather pieces of information about cellular structures of parenchyma tissue of kiwifruit (Tylewicz et al. 2011; Panarese et al. 2011). This prompted us to employ such technique as a first approach to highlight differences between kiwifruits with high and low °Brix. By fitting the T_2-weighted curves to a continuous and a discrete distribution of exponential curves, three proton pools were observed, with T_2 around 1,250, 310, and 20 ms that were ascribed through the literature to vacuole, cytoplasm plus extracellular space, and cell wall, respectively. Table 1 summarizes their T_2 values and the absolute intensities on raw and 300 min-treated HB and LB kiwifruits. The proton pool separation and assignment agree with Hills and Duce's findings (1990) on different fruits and vegetables.

The absolute intensities allowed to follow the partition of water across cellular structures along kiwifruit ripening groups and OD treatment time. At t_0, the signal from cytoplasm/extracellular space of LB kiwifruits is only slightly lower than the one from vacuole, while in HB kiwifruits the ratio between them is almost 1:2. Also, HB kiwifruits showed higher intensity values for vacuole proton pool than LB kiwifruits. The trends noticed in the signal intensity values seem to be reinforced by T_2 values. LB samples, characterized by a solutes/water ratio lower than HB kiwifruits, showed higher T_2 both in the vacuole and in cytoplasm/extracellular space. The water and solutes transfer promoted by osmosis can modify the original kiwifruit cellular organization and the subcellular structures. OD treatment has the effect of halving vacuole proton pool signal, with slight differences between HB and LB kiwifruits, a probable consequence of water exit from such cellular compartment. The OD shrinks the kiwifruit vacuoles, as reported previously by Panarese et al. (2011). From the decreasing T_2 of the vacuoles, it can be inferred that the water loss leading to the shrinkage of vacuoles causes a concentration of solutes, retained by the tonoplast. Bowtell et al. (1992) showed through NMR microscopy that during OD the cytoplasm sticks to the shrinking tonoplast, followed by the cellular membrane. This

Table 1 T_2 values and absolute intensities of the three proton pools identified through T_2-weighted curves for low (LB) and high (HB) °Brix, raw (t0) and 300-min osmo-treated kiwifruits

		Vacuole		Cytoplasm + extracellular space		Cell wall	
		t_0	300 min	t_0	300 min	t_0	300 min
LB	T_2 (ms)	1,321A±64	1,009 C±14	320A±21	221B±37	19±2	21±5
	Intensity	47b±9	25c±2	41a±5	36a±2	10±3	6±2
HB	T_2 (ms)	1,227B±79	991 C±92	303A±36	259AB±46	21±1	26±6
	Intensity	60a±8	31b±10	33b±3	37a±6	7±2	4±1

The intensities were scaled so that they equaled 100 in the case of raw HB fruits. The letters, capital for T_2 and lowercase for signal intensity, highlight the values which were found to be different with 95% confidence through a *t* test

creates intracellular spaces, filled with water from the vacuoles and osmotic solution, which contribute to the cytoplasm plus extracellular space proton pool. In agreement with Bowtell findings (1992), Table 1 shows that only in the case of HB kiwifruits that the vacuole T_2 decrease is paralleled by an increase of the cytoplasm and extracellular space signal. OD treatment, by removing water from such compartments and increasing their sugar content, causes a marked T_2 decrease in both kiwifruit groups, which show similar values at the end of the treatment.

The phenomena observed through T_2-weighted curves can be followed by determining the water self-diffusion coefficient (D_w) through PGSE sequence. A first overall picture can be obtained by considering the kiwifruit parenchyma tissue as homogeneous from the water distribution point of view. This oversimplification allows to quickly evaluate D_w value through one diffusion-weighted signal only. D_w value obtained through the single component analysis represented an average diffusion value (D_w) comprising the D_w coefficient contribution of each water population (vacuole, cytoplasm plus extracellular space, and cell wall). Figure 3a shows the D_w values evaluated during the OD treatment with the single component analysis.

As displayed in Fig. 3a, D_w values of raw kiwifruit resulted much lower than that of pure water ($2.3 \cdot 10^{-9}$ m^2/s at 25 °C (Holz et al. 2000)). This was not unexpected as cellular structures hinder water diffusion while solutes increase its viscosity. As confirm of such qualitative consideration, LB raw fruits, with a low sugar content, showed higher D_w values than HB ones. This agrees with Goñi's findings (2007), showing the ability of ripe tissues to hold better the water than unripe fruit. Along the OD treatment, the D_w values of both kiwifruit groups decreased (Fig. 3a) as a consequence of tissue water loss and sugar content increase as discussed above. Both kiwifruit groups showed a linear D_w decrease as a function of OD time with similar rates. An inverse correlation with R^2 >0.80 was found between D_w and dry matter for both kiwifruit groups during the OD, as shown in Fig. 3b. This could result especially interesting in view of setting up innovative and not destructive methods to monitor dry matter content in intact fruits.

In order to have a deeper insight into the consequences of the OD treatment on each cell compartment of kiwifruit, the signal from PGSE pulse sequence was analyzed through a multiple component model. Only the diffusion coefficient corresponding to the vacuole and cytoplasm/extracellular space compartments was considered, by properly choosing the PGSE τ. For both kiwifruit °Brix groups, Fig. 4 shows water self-diffusion coefficients obtained respectively for vacuole, major proton population, and cytoplasm/extracellular space. The D_w values previously obtained with the single component model are also included in the figure for comparative purpose.

The D_w values of water in the vacuoles and cytoplasm/extracellular space were respectively higher and lower than the D_w values calculated with the single component model

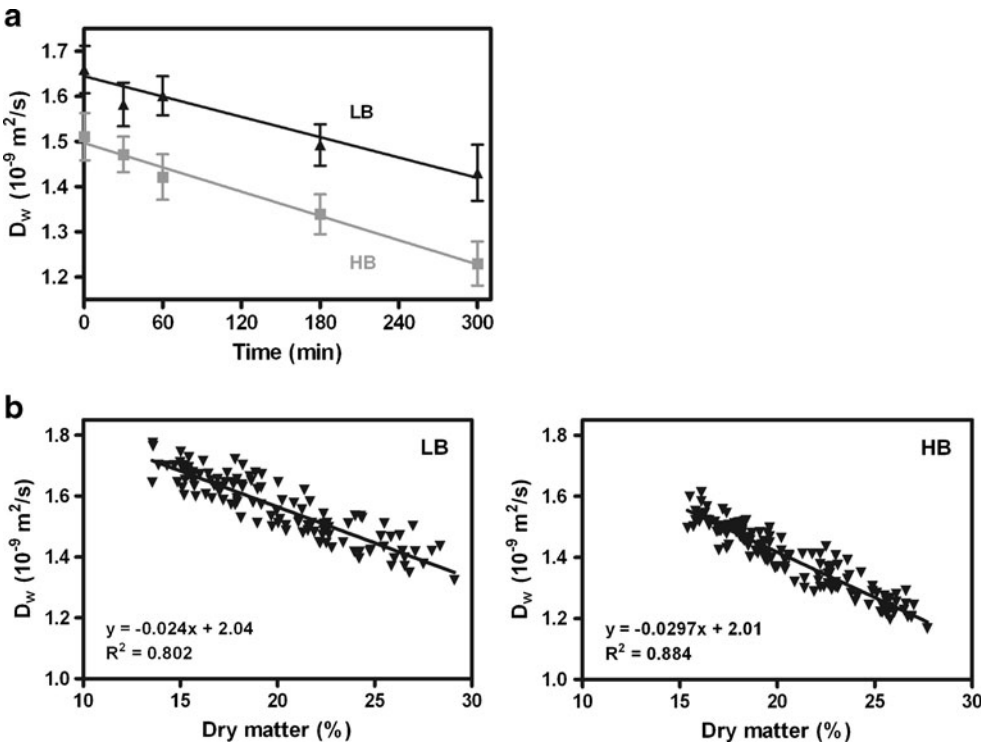

Fig. 3 **a** Average water self-diffusion coefficients of kiwifruit (D_w) as a function of OD time for both low (*LB*) and high (*HB*) °Brix groups. D_w values were obtained by applying the PGSE sequence together with the single component model. Each *point* represents an average of at least 15 kiwifruit slices of each time; *bars* represent SD values. **b** Correlation between D_w and dry matter for both kiwifruit groups

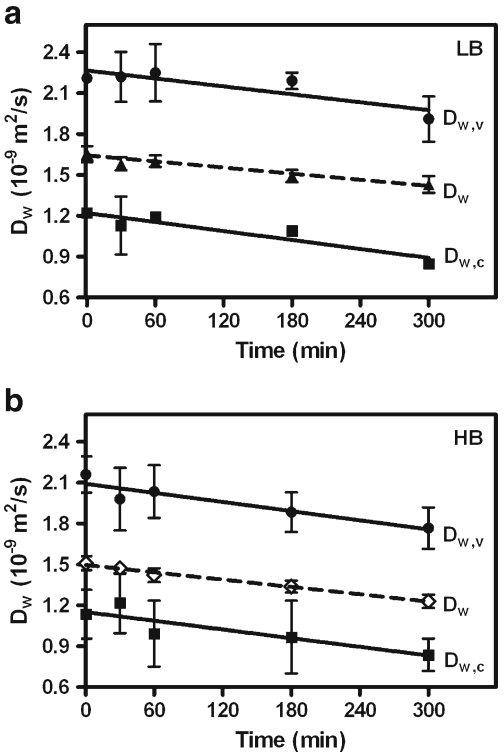

Fig. 4 Water self-diffusion coefficients (D_w) of low (*LB*) (**a**) and high (*HB*) (**b**) kiwifruits as a function of OD time. *Filled symbols* are average values obtained by applying the two components model, leading to two different D_w values for vacuole ($D_{w,v}$) and cytoplasm/extracellular space compartment ($D_{w,c}$). *Open symbols* were obtained by applying the single component model (D_w). *Lines* show the fitting obtained using linear regression. *Bars* represent SD values

for both kiwifruit °Brix groups. This confirms that the D_w obtained through the single component model gives an average value of a more complex situation. D_w value higher in the vacuole than in cytoplasm/extracellular space reflects the lower solutes/water ratio, in agreement with T_2 values. Going into deeper detail, it is possible to notice that the values obtained with the single component model are closer to the D_w values of the cytoplasm/extracellular space than to those from vacuoles. This is a consequence of the 2τ of 15 ms applied to calculate the single component values, instead of the 30 ms applied for the two components model, which does not allow the complete removal of the cell wall component (see Table 1), leading to lower average D_w values.

To have a further confirmation of T_2 and D_w values for the vacuole proton pool and cytoplasm/extracellular space, the PGSE-CPMG sequence was applied to a single sample, a raw kiwifruit pertaining to the HB group, as shown in Fig. 5. This procedure has the advantage to allow the determination of both T_2 and D_w on the same sample at the same moment, giving a 2D LF-NMR spectra (dashed line, Fig. 5). The full line was placed for comparative purposes and corresponds to T_2 spectrum (the relaxogram) of the same sample measured by CPMG, showing the proton kiwifruit populations. Cytoplasm/extracellular space showed a D_w of $1.5 \cdot 10^{-9}$ m^2/s for the considered sample, a value in qualitative agreement with the ones obtained through PGSE sequence. D_w obtained for vacuole proton pool was higher than the one obtained for cytoplasm/extracellular space,

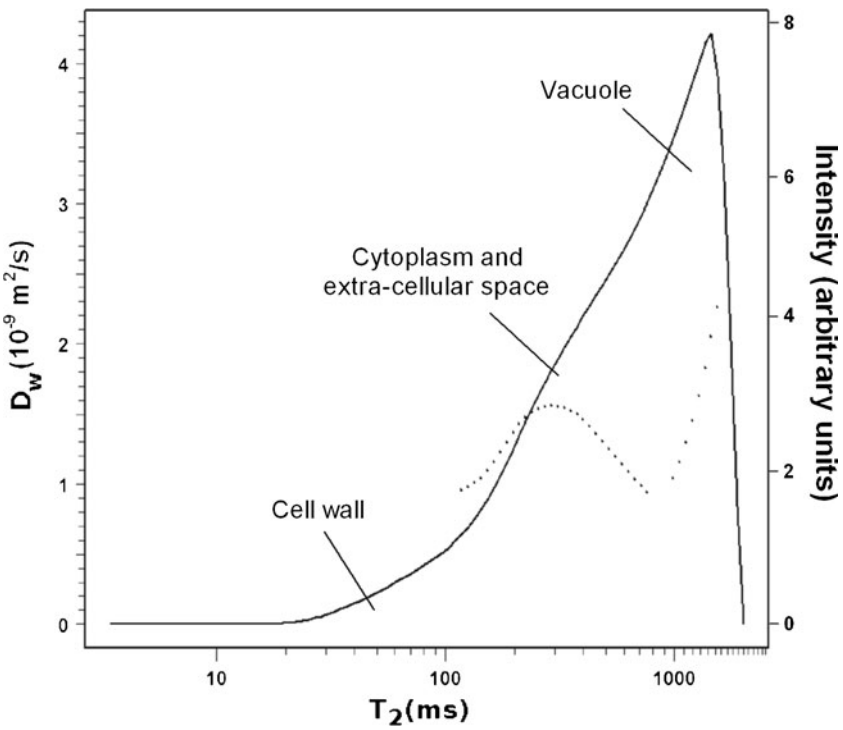

Fig. 5 Diffusion-weighted T_2 curves for HB kiwifruit: water self-diffusion coefficients (D_w) as a function of T_2 values (*left axis, dashed line*). *Full line* corresponds to T_2 spectrum (*right axis*)

again in agreement with PGSE values. Unfortunately vacuole peak appears at the side of the relaxogram, so that D_w values resulted as very noisy and it was not possible to go into deeper detail. However, it is interesting to observe qualitatively the D_w–T_2 dependency and how it is modulated. At the other side of the relaxogram, the low signal hindered the measurement of consistent D_w values for cell wall proton pool.

Conclusions

The combined application of T_2 and water self-diffusion coefficient measurements allowed having an insight of the changes in the cellular compartments of kiwifruit's outer pericarp promoted by OD. T_2 measurements enabled the quantification of the protons located in the three main cell compartments within the mobile water is located: vacuole, cytoplasm plus extracellular space, and cell wall. Water self-diffusion coefficient evaluated through a single component model was found to be a fast technique to follow OD treatment consequences along time and to highlight the different response to the treatment from fruits of different ripeness. D_w evaluated through a two components model allowed a deeper understanding of the changes in each cellular compartment at the expense of both experiment time, in the order of 5 min for each sample, and measurement precision. LF-NMR results showed that the OD process influenced water mobility characteristics within the vacuole and cytoplasm plus extracellular space compartments. The possibility to highlight how the response to OD treatment was modulated by fruit ripeness suggests that LF-NMR can represent a powerful and versatile tool to investigate the behavior of vegetable tissues during minimal processing. Then, by knowing the extent of changes on mass transfer parameters and water behavior during OD, which in turn modified the structural and textural characteristics of the final product, it is possible to exploit both raw unripe and ripe kiwifruits from a technological point of view. Thus, the extent of OD process could be modulated regarding fruit maturity to obtain products that can be used in different food products/processes.

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