Osmotic dehydration of organic kiwifruit pre-treated by pulsed electric fields and monitored by NMR

Osmotic dehydration of organic kiwifruit pre-treated by pulsed electric fields: Internal transport and transformations analyzed by NMR

Maria Victoria Traffano-Schiffo, Luca Laghi, Marta Castro-Giraldez, Urszula Tylewicz, Santina Romani, Luigi Ragni, Marco Dalla Rosa, Pedro J. Fito

PII: S1466-8564(17)30197-2
DOI: doi: 10.1016/j.ifset.2017.03.012
Reference: INNFOO 1733

To appear in: *Innovative Food Science and Emerging Technologies*

Received date: 14 February 2017
Revised date: 22 March 2017
Accepted date: 22 March 2017

Please cite this article as: Maria Victoria Traffano-Schiffo, Luca Laghi, Marta Castro-Giraldez, Urszula Tylewicz, Santina Romani, Luigi Ragni, Marco Dalla Rosa, Pedro J. Fito , Osmotic dehydration of organic kiwifruit pre-treated by pulsed electric fields: Internal transport and transformations analyzed by NMR.

This is a PDF file of an unedited manuscript that has been accepted for publication. The final published version is available online at: https://doi.org/10.1016/j.foodchem.2017.02.046

OSMOTIC DEHYDRATION OF ORGANIC KIWIFRUIT PRE-TREATED BY PULSED ELECTRIC FIELDS: INTERNAL TRANSPORT AND TRANSFORMATIONS ANALIZED BY NMR

Maria Victoria Traffano-Schiffo[a], Luca Laghi[b,c], Marta Castro-Giraldez[a], Urszula Tylewicz[b], Santina Romani[b,c], Luigi Ragni[b,c], Marco Dalla Rosa[b,c], Pedro J. Fito[a*]

[a] Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universitat Politecnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

[b] Department of Agricultural and Food Sciences, University of Bologna, Piazza Goidanich 60, 47521 Cesena, Italy

[c] Interdepartmental Centre for Agri-Food Industrial Research, University of Bologna, Via Quinto Bucci 336, 47521 Cesena, Italy

*author for correspondence: pedfisu@tal.upv.es

ABSTRACT

This work analyzes the effect of Pulsed Electric Fields (PEF) as a pre-treatment of the osmotic dehydration (OD) of kiwifruit (*Actinidia deliciosa cv Hayward*) in the internal structure and in the internal water transport. PEF pre-treatments were done using three PEF intensities (100, 250 and 400 V/cm) and analyzed by TD-NMR. The OD was carried out by immersing the samples in 61.5% sucrose solution at 25 ºC. The application of a PEF pre-treatment before the OD produces a process of plasmolysis proportional to the electric field applied. It is because the PEF removes the mobile charges of the medium, such as electrolytes, organic acids, aminoacids;

Ca^{+2} is the major culprit of the plasmolysis because it fixes some of the junctions of the microtubules between the cell wall and the membrane. Therefore, a previous plasmolysis produces an increase in the apoplastic transport increasing the rate of dehydration.

Keywords: Kiwifruit, Pulsed Electric Fields, Osmotic dehydration, TD-NMR, Water distribution, Plasmolysis.

NOTATION

x_i	mass fraction of i chemical specie ($kg_i\ kg_T^{-1}$)
T_2	spin-spin or proton transverse relaxation time
r_I	relative intensities
M	mass (kg)
S	surface of samples (m^2)
r	radius (m)
X_{w0}^{ADS}	monomolecular moisture layer ($kg_w\ kg_{dm}^{-1}$)
N_A	avogadro number ($6.022 \cdot 10^{23}$ molecules mol^{-1})
M_w	molecular weight of water (18 g mol^{-1})
t	treatment time
I	Intensity
E	electric field (V cm^{-1})

Subscripts

w	water

T	total
0	raw sample
1	sample after the pre-treatment by PEF
i	internal
j	any chemical species

Superscripts

j	specific group of water molecules j
t	treatment time (s)
0	initial time
ELP	extracellular liquid phase
ILP	intracellular liquid phase
ADS	adsorbed water

1. Introduction

Cellular structure is considered as a complex organized system where flows are carried out by different solutes or solvents transports systems. Based on the free energy gradients, commonly known as passive transports, symplastic, aplopastic and aquaporins transmembrane transports are involved. Firstly, cells are interconnected by plasmodesmatas which led to generate symplastic transports across them. In the extracellular space, the fluxes are produced by apoplastic pathways (Marcotte, Toupin, & Le Maguer, 1991) and finally, the transmembrane transport is characterized by the exchange between intra and extracellular spaces by protein channels, called aquaporins (Agre et al., 2002; Maurel & Chrispeels, 2001). In contrast, when the flows occur in the opposite direction of the free energy gradients, they are produced by protein channels and they require energy consumption as adenosine triphosphate (ATP) (Moraga, Moraga, Fito, & Martínez-Navarrete, 2009). The main active pumps are Ca^{2+}, Na^{+} and Na^{+}/K^{+}, which are the responsible of the transport of water, sucrose and electrolytes (Traffano-Schiffo et al., 2016).

Taking into account the water content, Tylewicz, Fito, Castro-Giráldez, Fito, & Dalla Rosa (2011) explain that the water distribution in fresh kiwifruit can be segregated in adsorbed water (solid matrix) and liquid water. In turn, for parenchymatic tissue, the liquid water can be divided into intra and extracellular liquid phases. During osmotic dehydration (OD) treatment, the water distribution of the cellular structure changes due to mass transfer phenomena, which are generated as a consequence of the water and sucrose chemical differences between the tissue and the osmotic solution (Traffano-Schiffo et al., 2016; Khin, Zhou, & Perera, 2006, Castro-Giráldez, Tylewicz, Fito, Dalla Rosa, & Fito, 2011; Castro-Giráldez, Fito, Dalla Rosa, & Fito,

2011). From the contact of the fruit tissue with the osmotic solution, the semipermeable membranes, plasma membrane and the tonoplast are forced to separate, due to the water losses from the vacuoles, starting the process known as plasmolysis, characterized by the loss of the turgor pressure (Lang, Sassmann, Schmidt, & Komis, 2014; Lang, Barton, & Overall, 2004). Plasmolysis occurs in two stages: partial and the complete plasmolysis. Partial plasmolysis is produced when the plasma membrane starts to detach from the cell wall and the complete plasmolysis occurs when the complete separation of the protoplast is produced (Seguí, Fito, & Fito, 2012).

For many years, OD has been extensively studied around the world for the partial dehydration of fruits and vegetables; however, it presents some limitations such as the low dehydration rate and the high solute content in the final product. Therefore, the use of pre-treatment such as Pulsed Electric Fields (PEF) has been reported to facilitate water removal and to improve the quality of the dried products (Dermesonlouoglou, Zachariou, Andreou, & Taoukis, 2016; Dellarosa et al., 2016; Barba et al., 2015).

PEF is a non-thermal technology which involves the application of short and repeated voltage pulses to a biological tissue placed between two electrodes (Gürsul, Gueven, Grohmann, & Knorr, 2016); it induces changes and reorganization in the electric conformation of the cell membrane (Baier, Bußler, & Knorr, 2015), modifying the normal fluxes during drying process when it is used as a pre-treatment.

Time Domain Nuclear Magnetic Resonance (TD-NMR) is a non-destructive technique able to determine the spin-spin or transverse relaxation (T_2) of protons and the intensity of the signal, differentiating extracellular space, vacuoles and solid matrix (Santagapita et al., 2013). TD-NMR is considered as one of the most powerful techniques able to follow the water distribution in cellular tissues, thus, it is necessary for describe the driving forces that promote the water fluxes.

The aim of this research was to determine the effect of PEF pre-treatment in the microstructure and in the internal water transport throughout an osmotic treatment of kiwifruit in hypertonic sucrose solution.

2. Material and methods

Organic kiwifruits (*Actinidia deliciosa* cv ‘‘*Hayward*’’) with the same ripeness and similar size were bought on a supermarket located in Cesena (Italy) and kept refrigerated at 4 ± 1 ºC until use. The fruits were tempered at 25 ºC, peeled and cylinders (8 mm diameter and 10 mm length) were obtained from the outer pericarp, avoiding the core, the inner pericarp and the seeds. The initial solute content of the fruits used were 13 ± 1 º Brix.

Fresh kiwifruits were characterized by mass, volume, solutes (º Brix), water activity (a_w), moisture (x_w) and TD-NMR by quadruplicate. 12 sample cylinders were used for each treatment (total number of treated samples of 576). They were placed inside the PEF chamber avoiding free spaces between them and subjected to different electric fields strengths. Immediately after, the samples were weighed and introduced into the osmotic dehydration solution. According to previous results, the selected OD treatment times were 0, 10, 20, 30, 60 and 120 min (Traffano-Schiffo et al., 2016).

Due to the fact that the samples after treatments show concentration profiles, another batch of samples were treated and equilibrated at 4 ºC during 24 hours in decagon containers closed with parafilm®. Finally, mass, volume, solute content (º Brix), a_w, x_w and TD-NMR were measured as final determinations for treated and equilibrated samples. In addition, at each osmotic time, an aliquot of sucrose solution was taken to measure a_w and solute content.

2.1. PEF treatment

Pulsed electric field treatments were applied to the samples using monopolar pulse generator equipment based on MOSFET technology and capacitors as energy tanks (Dellarosa et al., 2016). The cylinders of organic kiwifruit were placed in a rectangular treatment chamber avoiding free spaces between them. The chamber was equipped with two stainless steel electrodes (20 x 20 mm^2) with a separation between them of 30 mm and filled with 5 mL of tap water with an electrical conductivity of 328 ± 4 μS/cm at 25 ºC.

PEF pre-treatments were done by applying three different pulsed electric fields (100, 250 and 400 V/cm at 50 Hz) with near-rectangular shape pulses, a train of 60 pulses, a fixed pulse width of 100 ± 2 μs and a repetition time of 10.0 ± 0.1 ms.

2.2. Osmotic dehydration treatment

The osmotic solution at 61.5% (w/w) was prepared with commercial sucrose and distilled water at 25 ºC. Samples were immersed into the sucrose solution maintaining a relationship of 1:4 (w/w) between the fruit and the solution.

2.3. Analytical determinations

A dew point Hygrometer Decagon (Aqualab™, series 3 TE) was used for measuring the water activity, with a precision ± 0.003. Mass was determined by using a Kern balance ABS 320-4N (± 0.0001) (KERN & SOHN GmbH, Germany).

The analysis of the moisture was accomplished following the AOAC Method 934.06, 2000.

Sugar content was determined by measuring the refractometric index with a digital refractometer (KRÜSS Optronic© GmbH, Germany) calibrated with distilled water at 25 ºC. The sample was pressed in order to extract the external liquid phase. Solute content was measured by refractometry of both kiwifruit samples and osmotic solution after the treatments.

Analytical determinations described above were obtained by quadruplicate.

2.4. TD-NMR measurements

Proton transverse relaxation time (T_2) decay was measured for each sample by applying the CPMG pulse sequence (Meiboom & Gill, 1958), using a Bruker 'The Minispec' spectrometer (Bruker Corporation, Germany) operating at 20 MHz, as described by Dellarosa et al. (2016). Each measurement comprised 32000 echoes, with an interpulse spacing of 0.08 ms and a recycle delay of 10 s, which allowed the measurement of proton decays included between 1 and 3000 ms and avoided sample overheat. Each acquisition was performed over 8 scans giving rise to a total time of analysis around 90 s. The registered spectra were normalized to unitary area and analyzed by UpenWin software (Borgia, Brown, & Fantazzini, 1998) to give quasi-continuous distributions of relaxation time. The number of output relaxation times,

sampled logarithmically in the 1–3000 ms range, was set to 100. To obtain quantitative information from the T_2-weighted decay curves, signals were fitted using a discrete multi-exponential curve. The fitting was run using the 'Levenberg–Marquardt nonlinear least squares' algorithm implemented in 'R' software (R Foundation for Statistical Computing, Austria). Unlike Santagapita et al. (2013), the optimum number of exponential curves for each tested treatment was found to be three, without removing any initial T_2 weighted point.

2.5. Low-temperature scanning electron microscopy (Cryo-SEM)

Microstructure was analyzed by Cryo-SEM. A Cryostage CT-1500C unit (Oxford Instruments, Witney, UK), coupled to a Jeol JSM-5410 scanning electron microscope (Jeol, Tokyo, Japan). The sample was immersed in N_2 slush (-210 ºC) and then quickly transferred to the Cryostage at 1 kPa where sample fracture took place. Sublimation (etching) was carried out at -95 ºC. The final point was determined by direct observation under the microscope, working at 5 kV. Then, once again in the Cryostage unit, the sample was coated with gold in vacuum (0.2 kPa) applied for 3 min, with an ionization current of 2 mA. The observation in the scanning electron microscope was carried out at 20 kV, at a working distance of 15 mm and temperature ≤ -130 ºC.

3. Results and discussion

Fruit tissue, such as kiwifruit, is composed by parenchymatic and vascular tissues, where cells with big vacuoles coexist with vascular cells. This system is full of chemical species charged, with capacity to orientate and move with an external

electric field. Moreover, these compounds, with high electric activity, are responsible of part of the cell pathways and metabolisms. Therefore, any electric disturb in the fruit tissue produces high disorder in the cell functionality. Moreover, biological cells are delimited by a phospholipid bilayer membrane (protoplast and tonoplast), which separates the internal liquid phase from a completely different external medium. Despite there is an electric equilibrium at both sides of the bilayer, the concentration and nature of the charged compounds are different (Bezanilla, 2008). When a biological tissue is subjected to an external electric field, it induces changes in the electric conformation of the bilayer and in the involved chemical species (Traffano-Schiffo et al., 2016). Therefore, the different liquid mediums and the solid matrix suffer transformations with the application of external electric fields.

Table 1 shows the main chemical species of kiwifruit with capability to orientate and migrate when an external electric field at low frequency is applied. These species are: electrolytes with biological activity, responsible of the active transport pumps and bonds of the middle lamella; aminoacids, part of proteins channels or passive transport pumps; chlorophylls, carotenoids and anthocyanins, greatly charged antioxidant compounds with functional activity and organic acids, which regulate de pH of the fruit and play an important role in the respiration pathway. These quantities of different chemical species, with different electric nature, shows the complexity in the explanation of the effects in the kiwifruit tissue affected by an external electric field.

In order to understand the correct functionality of the biological tissue under the effect of an external electric field, it is necessary to know the involved metabolic

transports. Figure 1a shows a schematic representation of the cellular transports. Inside the cellular tissue, fluxes occur by different pathways: apoplastic, symplastic and transmembrane (aquaporins) transports or passive transports, produced by free energy gradients and the transmembrane transport or active transport, which is based on proteins channels and with ATP consumption (Johansson, Karlsson, Johanson, Larsson, & Kjellbom, 2000). Previously, it has been demonstrated that the application of an external electric field through the vegetal tissue induces compositional changes (removing part of the native electrolytes such as Ca^{2+} and Na^{+}) and, as a consequence, the transmembrane active transports are affected. The main affected transmembrane active transports are: Ca^{2+} pump which is the responsible to maintain the homeostatic cellular system (water transport) and Na^{+} pump, responsible of sucrose transmembrane transport (Traffano-Schiffo et al., 2016).

Figure 1a shows the schematic diagram of a plant cell tissue where it is possible to observe the different active and passive transports that can be produced. These transports are classified by the extracellular transport (apoplastic), the transport between cells or intracellular (symplastic) and the transmembrane that communicates both phases. These spaces are described by their corresponding liquid phases, extracellular (ELP) and intracellular (ILP). Figure 1b shows two micrographs obtained by Cryo-SEM, the upper picture shows raw parenchyma tissue of kiwifruit and the lower one shows partially dehydrated parenchymal tissue with symptoms of plasmolysis.

Figure 1c shows examples of the distribution of T_2-weighted signals obtained by TD-NMR in parenchyma tissue of kiwifruit in the present research. The three protons populations that were observed in the non pre-treated samples had T_2 or relaxation times of 1170, 425 and 53. The smaller the $T_{2,}$ the lower the mobility of the molecule with the induced proton is, so that it is possible to determine the origin of each group of molecules in function of the different motion capacity of water molecules in the tissue. The lower value of T_2 corresponds to water molecules with less mobility, water molecules subjected to electrical adsorption forces. This group is adsorbed on the cell wall and on the cell membrane (protoplast and tonoplast), thus it can be considered the entire adsorbed water of tissue. The remaining groups might correspond to the different liquid phases that make up the parenchyma, from the interior of the cell mostly occupied by the vacuole (higher T_2 and higher intensity), named ILP, and external liquid phase (intermediate T_2), named ELP. Therefore, the intensities measured in NMR, can be divided in three groups of water molecules; Adsorbed, ELP and ILP. They are shown in Table 2.

As previously described by different authors (Nakashima, 2001; Muller, Scrivener, Gajewicz, & McDonald, 2013; Traffano-Schiffo et al., 2017), it is possible to divide the water molecules into groups according to their situation in the tissue using a value of proportionality to the intensity measured by NMR as it is shown in Table 2. The relative intensities (r_I^j) of a specific group of water molecules (j) along OD treatment can be defined as the relationship between the intensity of each group of water molecules and the overall measured intensity. This parameter let to obtain the water distribution (x_w^j) of a specific group of water molecules (j) as follows:

$$x_w^j = x_w \cdot r_I^j \quad (1)$$

Where x_w^j is the water mass fraction of water group j ($kg_{w\ in\ j}/kg_T$) and x_w is the water mass fraction (kg_w/kg_T).

In order to understand the structural transformations induced by the PEF pre-treatment it is possible to estimate the water mass variation of each liquid phase, considering the initial state as non PEF pre-treated samples and the state after the PEF pre-treatment. In this sense, the next equation estimates the water mass variation after the PEF:

$$\Delta M_w^j = \frac{M_1 x_{w1}^j - M_0 x_{w0}^j}{M_0} \quad (2)$$

Where M represents the mass (g), x the mass fraction (kg/kg), the subscripts w represents the water, 0 and 1 represent the samples before and after the pre-treatment by PEF (V/cm) respectively, and the superscript j corresponds to a specific group of molecules (ELP, ILP, ADS or overall).

Figure 2a shows the water mass variation in the intracellular space and in the extracellular space after the PEF treatment, where it is possible to observe how the water mass variation of intracellular space decreases proportionally to the increase of the water mass variation of extracellular space when the electric field grows. Considering that the composition of both phases is different, since the relaxation times are different, the only possible explanation for this liquid phase exchange is that the electric field induces a process of plasmolysis. In interphase cells, plasmolysis (which is the disruption of the cell wall—plasma membrane—cortical cytoskeleton continuum) is expected to exert the strongest impact on cortical microtubules since they are closely linked to the plasma membrane (Ambrose, Allard, Cytrynbaum & Wasteneys, 2011). These microtubules are formed by

different microfilaments, and one of the most important microfilament is the actin (Wen & Janmey, 2011). Actin microfilament links the microtubules with the membrane by using Ca^{2+}, maintaining the membrane mechanical integrity (Lang et al., 2014). Therefore, any perturbation in the calcium bonding between microtubules and membrane can initiate a plasmolysis process.

Figure 2b shows how the overall water mass variation, induced by the electric field applied (Traffano-Schiffo et al., 2016) is smaller (external transport) than the water mass variation of internal and external liquid phase (internal transport). Moreover, Figure 2b shows the water adsorbed mass variation, where the greater the electric field applied the greater the amount of adsorbed water.

In order to understand the behaviors involved in the increasing of water adsorption, the internal surface of samples (m^2) where estimated as follows (Farroni, 2011; Condon, 2006):

$$S_i = \frac{\pi\, r_w^2\, M\,(1-x_w)\, X_{w0}^{\mathrm{ADS}} N_A}{M_w} \quad (3)$$

Where r_w is the radius of the water molecule based in Lewis model ($1.375 \cdot 10^{-10}$ m) (Pierotti, 1965), M corresponds to the mass of the sample (g), x_w is the moisture of the sample (g_w/g_T), X_{w0}^{ADS} represents the moisture of the monomolecular layer obtained by BET model (g_w/g_{dm}), N_A corresponds to the Avogadro constant ($6.022 \cdot 10^{23}$ molecules/mol) and M_w is the molecular weight of water (18 g/mol).

Figure 3a shows the internal surface variation of the samples according to the intensity of the applied PEF pre-treatment, where at higher intensities of the electric pulses, the internal surface of the sample increases. The internal surface, calculated from the moisture of the monomolecular layer (equation 3), covers the entire surface

with capacity to adsorb water. In this sense, any process of surface release or surface availability will cause an increase in the amount of adsorbed water, previously observed by Traffano-Schiffo et al., 2017. As described above, the electric field applied on the tissue produces a partial plasmolysis associated with the displacement of calcium ions in the fixations of the actin microfilaments (cell wall-plasma membrane bond). It induces a water transport from inside to the outside of the cells and increases the availability of surface, as Figure 3a shows, and as was corroborated in Figure 3b in which the mass of adsorbed water increases proportionally to the internal surface.

In order to understand the movement of water across the kiwifruit tissue affected by the PEF pre-treatment throughout OD treatment, the water mass flow was estimated as follows:

$$m_w^j = \frac{M^t x_{wi}^t - M^0 x_{wi}^0}{t} \quad (4)$$

Where m_w^j represents the water mass flow (g_w/s), M represents the mass sample (g), x_w the water mass fraction of specific phase (g_w/g_T), t is treatment time (s), the superscript j corresponds to a specific water phase (ELP, ILP or total), t to the treatment time and 0 to the initial time.

Figure 4 shows schematically the directions that water flows can take inside the fruit tissue. The water mass flow produced in the ILP can only leave, and it can be produced towards the external liquid phase or towards the osmotic solution through the symplastic pathways, whereas the water mass flow in the ELP can be coming in

from the ILP or can be leaving to the osmotic solution. Finally, total water mass flow represents the water molecules that cross the interface sample/osmotic solution.

Water mass flow from ILP, ELP and whole tissue (total) was estimated by using equation 4, where positive value of water mass flow represents a water flux coming in the phase and negative value represents a water flux leaving the phase. Samples pre-treated with PEF show an increment of ELP as Figure 4 shows, induced by a plasmolysis process.

Figure 5 shows the evolution of each water mass flow (ILP, ELP and total) throughout the OD treatment per PEF pre-treatment. As it can be appreciated, the water mass flow in ELP shows positive values in non pre-treated samples (Figure 5a). Taking into account that the water activity in the osmotic solution is lower than in the ELP, the mass of water has to come from the ILP (as Figure 4 shows). However, negative values of water mass flow in samples pre-treated (Figure 5 b, c and d) indicate that the water leaves from ELP to the osmotic solution. With regard to ILP, the water mass flow decreases the higher the applied PEF. Nevertheless, the overall water mass flow increases the higher the applied PEF, because water from the ELP is joined with the water from the ILP. Finally, the water mass flow of the ELP increases with PEF pre-treatment with negative values (negative values mean that the water mass flow is leaving the liquid phase), therefore only the non-pretreated samples show the effect of plasmolysis. In conclusion, PEF treatment produces a partial plasmolysis in parenchymatic kiwifruit tissue, and this plasmolysis process depends on the intensity of PEF pre-treatment (as was observed in Figure 2).

4. Conclusions

It has been proven that the application of an electric field prior to the osmotic dehydration produces a process of plasmolysis proportional to the applied electric field. The induction of the plasmolysis process is caused by the elimination of mobile charges of the medium, such as electrolytes, organic acids, etc.; among them Ca^{+2} is the major culprit because it is not available to fix some of the junctions of the microtubules between the cell wall and the membrane.

In addition, the process of plasmolysis induced by the electric field changes the behavior of kiwifruit tissue during the OD process. In a normal OD, the main transport is the symplastic, whereas if previously treated with PEF, the apoplastic transport is as important as the symplastic, considerably increasing the rate of dehydration.

5. Acknowledgements

The authors Urszula Tylewicz and Marco Dalla Rosa want to thank for the financial support provided by funding bodies within the FP7 ERA-Net CORE Organic Plus, and with cofounds from the European Commission. The author Maria Victoria Traffano Schiffo wants to thank the FPI Predoctoral Program of the Universidad Politécnica de Valencia for support her PhD studies, ERASMUS PRÁCTICAS program to finance her mobility to Italy. The authors Pedro J. Fito, Marta Castro-Giraldez and M. Victoria Traffano-Schiffo acknowledge the financial support from the Spanish Ministerio de Economía, Industria y Competitividad, Programa Estatal de I+D+i orientada a los Retos de la Sociedad AGL2016-80643-R, Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (FEDER).

6. References

Agre, P., King, L. S., Yasui, M., Guggino, W. B., Ottersen, O. P., Fujiyoshi, Y., ... & Nielsen, S. (2002). Aquaporin water channels–from atomic structure to clinical medicine. *The Journal of Physiology*, 542(1), 3-16.

Ambrose, C., Allard, J. F., Cytrynbaum, E. N., & Wasteneys, G. O. (2011). A CLASP-modulated cell edge barrier mechanism drives cell-wide cortical microtubule organization in Arabidopsis. *Nature Communications*, 2, 430.

Ampomah-Dwamena, C., McGhie, T., Wibisono, R., Montefiori, M., Hellens, R. P., & Allan, A. C. (2009). The kiwifruit lycopene beta-cyclase plays a significant role in carotenoid accumulation in fruit. *Journal of Experimental Botany*, 60(13), 3765-3779.

AOAC. (2000). AOAC, Association of Official Analytical Chemist Official Methods of Analysis. Washington, D.C.

Baier, A. K., Bußler, S., & Knorr, D. (2015). Potential of high isostatic pressure and pulsed electric fields to improve mass transport in pea tissue. *Food Research International*, 76, 66-73.

Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., Saraiva, J. A., Raso, J., Martin-Belloso, O., Witrowa-Rajchert, D., Lebovka, N., & Lebovka, N. (2015). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Research International*, 77, 773-798.

Barboni, T., Cannac, M., & Chiaramonti, N. (2010). Effect of cold storage and ozone treatment on physicochemical parameters, soluble sugars and organic acids in Actinidia deliciosa. *Food Chemistry*, 121(4), 946-951.

Bezanilla, F. (2008). How membrane proteins sense voltage. *Nature Reviews Molecular Cell Biology*, 9(4), 323-332.

Bohn, T., Walczyk, T., Leisibach, S., & Hurrell, R. F. (2004). Chlorophyll-bound Magnesium in Commonly Consumed Vegetables and Fruits: Relevance to Magnesium Nutrition. *Journal of Food Science*, 69(9), S347-S350.

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.

Cano, M. P., & Marin, M. A. (1992). Pigment composition and color of frozen and canned kiwi fruit slices. *Journal of Agricultural and Food Chemistry*, 40(11), 2141-2146.

Cassano, A., Donato, L., Conidi, C., & Drioli, E. (2008). Recovery of bioactive compounds in kiwifruit juice by ultrafiltration. *Innovative Food Science & Emerging Technologies*, 9(4), 556-562.

Castaldo, D., Lo Voi, A., Trifiro, A., & Gherardi, S. (1992). Composition of Italian kiwi (Actinidia chinensis) puree. *Journal of Agricultural and Food Chemistry*, 40(4), 594-598.

Castro-Giráldez, M., Fito, P. J., Dalla Rosa, M., & Fito, P. (2011). Application of microwaves dielectric spectroscopy for controlling osmotic dehydration of kiwifruit (*Actinidia deliciosa cv Hayward*). *Innovative Food Science & Emerging Technologies*, 12(4), 623-627.

Castro-Giráldez, M., Tylewicz, U., Fito, P. J., Dalla Rosa, M., & Fito, P. (2011). Analysis of chemical and structural changes in kiwifruit (*Actinidia deliciosa cv Hayward*) through the osmotic dehydration. *Journal of Food Engineering*, 105(4), 599-608.

Condon, J. B. (2006). *Surface area and porosity determinations by physisorption: measurements and theory*. (1st ed.) Amsterdam, The Netherlands: Elsevier.

Dellarosa, N., Ragni, L., Laghi, L., Tylewicz, U., Rocculi, P., & Dalla Rosa, M. (2016). Time domain nuclear magnetic resonance to monitor mass transfer mechanisms in apple tissue promoted by osmotic dehydration combined with pulsed electric fields. *Innovative Food Science & Emerging Technologies*, 37, 345-351.

Dermesonlouoglou, E., Zachariou, I., Andreou, V., & Taoukis, P. S. (2016). Effect of pulsed electric fields on mass transfer and quality of osmotically dehydrated kiwifruit. *Food and Bioproducts Processing*, 100, 535-544.

Dorai, M., Papadopoulos, A., & Gosselin, A. (2001). Influence of electric conductivity management on greenhouse tomato yield and fruit quality. *Agronomie*, 21(4), 367-383.

Famiani, F., Battistelli, A., Moscatello, S., Cruz-Castillo, J. G., & Walker, R. P. (2015). The organic acids that are accumulated in the flesh of fruits: occurrence, metabolism and factors affecting their contents–a review. *Revista Chapingo Serie Horticultura*, 21(2).

Farroni, A. E. (2011). Transformaciones estructurales y físico-químicas de maíces argentinos en la producción de alimentos obtenidos por procesos de gelatinización-laminación (Doctoral dissertation, Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires).

Gürsul, I., Gueven, A., Grohmann, A., & Knorr, D. (2016). Pulsed electric fields on phenylalanine ammonia lyase activity of tomato cell culture. *Journal of Food Engineering*, 188, 66-76.

Havaux, M. (1998). Carotenoids as membrane stabilizers in chloroplasts. *Trends in Plant Science*, 3(4), 147-151.

Johansson, I., Karlsson, M., Johanson, U., Larsson, C., & Kjellbom, P. (2000). The role of aquaporins in cellular and whole plant water balance. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1465(1), 324-342.

Keutgen, A. J., & Pawelzik, E. (2008). Contribution of amino acids to strawberry fruit quality and their relevance as stress indicators under NaCl salinity. *Food Chemistry*, 111(3), 642-647.

Khan, S. A., Beekwilder, J., Schaart, J. G., Mumm, R., Soriano, J. M., Jacobsen, E., & Schouten, H. J. (2013). Differences in acidity of apples are probably mainly caused by a malic acid transporter gene on LG16. *Tree Genetics & Genomes*, 9(2), 475-487.

Khin, M. M., Zhou, W., & Perera, C. O. (2006). A study of the mass transfer in osmotic dehydration of coated potato cubes. *Journal of Food Engineering*, 77(1), 84-95.

Lang, I., Barton, D. A., & Overall, R. L. (2004). Membrane–wall attachments in plasmolysed plant cells. *Protoplasma*, 224(3-4), 231-243.

Lang, I., Sassmann, S., Schmidt, B., & Komis, G. (2014). Plasmolysis: Loss of Turgor and Beyond. *Plants*, 3(4), 583-593.

Ma, T., Sun, X., Zhao, J., You, Y., Lei, Y., Gao, G., & Zhan, J. (2017). Nutrient compositions and antioxidant capacity of kiwifruit (Actinidia) and their relationship with flesh color and commercial value. *Food Chemistry*, 218, 294-304.

Marcotte, M., Toupin, C. J., & Le Maguer, M. (1991). Mass transfer in cellular tissues. Part I: The mathematical model. *Journal of Food Engineering*, 13(3), 199-220.

Marsh, K. B., Boldingh, H. L., Shilton, R. S., & Laing, W. A. (2009). Changes in quinic acid metabolism during fruit development in three kiwifruit species. *Functional Plant Biology*, 36(5), 463-470.

Maurel, C., & Chrispeels, M. J. (2001). Aquaporins. A molecular entry into plant water relations. *Plant Physiology*, 125(1), 135-138.

Meiboom, S., & Gill, D. (1958). Modified spin-echo method for measuring nuclear relaxation times. *Review of Scientific Instruments*, 29(8), 688-691.

Montefiori, M., McGhie, T. K., Costa, G., & Ferguson, A. R. (2005). Pigments in the fruit of red-fleshed kiwifruit (Actinidia chinensis and Actinidia deliciosa). *Journal of Agricultural and Food Chemistry*, 53(24), 9526-9530.

Moraga, M. J., Moraga, G., Fito, P. J., & Martínez-Navarrete, N. (2009). Effect of vacuum impregnation with calcium lactate on the osmotic dehydration kinetics and quality of osmodehydrated grapefruit. *Journal of Food Engineering*, 90(3), 372-379.

Muller, A. C. A., Scrivener, K. L., Gajewicz, A. M., & McDonald, P. J. (2013). Use of bench-top NMR to measure the density, composition and desorption isotherm of C–S–H in cement paste. *Microporous and Mesoporous Materials*, 178, 99-103.

Nakashima Y. (2001) Pulsed field gradient proton NMR study of the self-diffusion of H_2O in montmorillonite gel: Effects of temperature and water fraction. *American Mineralogist*, 86, 132–138.

Nishiyama, I., Fukuda, T., Shimohashi, A., & Oota, T. (2008). Sugar and organic acid composition in the fruit juice of different Actinidia varieties. *Food Science and Technology Research*, 14(1), 67-73.

Park, Y. S., Ham, K. S., Park, Y. K., Leontowicz, H., Leontowicz, M., Namieśnik, J., Katrich, E., & Gorinstein, S. (2016). The effects of treatment on quality parameters of smoothie-type 'Hayward' kiwi fruit beverages. *Food Control*, 70, 221-228.

Park, Y. S., Im, M. H., Ham, K. S., Kang, S. G., Park, Y. K., Namiesnik, J., Leontowicz, H., Leontowicz, M., Katrich, E., & Gorinstein, S. (2013). Nutritional and pharmaceutical properties of bioactive compounds in organic and conventional growing kiwifruit. *Plant Foods for Human Nutrition*, 68(1), 57-64.

Park, Y. S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Suhaj, M., Cvikrová, M., Martincová, O., Weisz, M., & Gorinstein, S. (2011). Comparison of the contents of bioactive compounds and the level of antioxidant activity in different kiwifruit cultivars. *Journal of Food Composition and Analysis*, 24(7), 963-970.

Pierotti, R. A. (1965). Aqueous Solutions of Nonpolar Gases1. *The Journal of Physical Chemistry*, 69(1), 281-288.

Santagapita, P., Laghi, L., Panarese, V., Tylewicz, U., Rocculi, P., & Dalla Rosa, M. (2013). Modification of transverse NMR relaxation times and water diffusion coefficients of kiwifruit pericarp tissue subjected to osmotic dehydration. *Food and Bioprocess Technology*, 6(6), 1434-1443.

Seguí, L., Fito, P. J., & Fito, P. (2012). Understanding osmotic dehydration of tissue structured foods by means of a cellular approach. *Journal of Food Engineering*, 110(2), 240-247.

Sivakumaran, S., Huffman, L., Sivakumaran, S., & Drummond, L. (2016). The nutritional composition of Zespri® SunGold Kiwifruit and Zespri® Sweet Green Kiwifruit. *Food Chemistry*. http://dx.doi.org/10.1016/j.foodchem.2016.08.118

Snowden, C. J., Thomas, B., Baxter, C. J., Smith, J. A. C., & Sweetlove, L. J. (2015). A tonoplast Glu/Asp/GABA exchanger that affects tomato fruit amino acid composition. *The Plant Journal*, 81(5), 651-660.

Tarchevsky I.A., Marchenko G.N. (1991). *Cellulose: Biosynthesis and Structure*. Berlin, Germany: Springer-Verlag, Berlin Heidelberg, (Chapters 1 & 8).

Traffano-Schiffo, M. V., Laghi, L., Castro-Giraldez, M., Tylewicz, U., Roculi, P., Ragni, L., Dalla Rosa, M., & Fito, P. J. (2017). Osmotic dehydration of organic kiwifruit pre-treated by pulsed electric fields and monitored by NMR. *Food Chemistry*. http://dx.doi.org/10.1016/j.foodchem.2017.02.046

Traffano-Schiffo, M. V., Tylewicz, U., Castro-Giraldez, M., Fito, P. J., Ragni, L., & Dalla Rosa, M. (2016). Effect of pulsed electric fields pre-treatment on mass transport during the osmotic dehydration of organic kiwifruit. *Innovative Food Science & Emerging Technologies*, 38, 243-251.

Tylewicz, U., Fito, P. J., Castro-Giráldez, M., Fito, P., & Dalla Rosa, M. (2011). Analysis of kiwifruit osmodehydration process by systematic approach systems. *Journal of Food Engineering*, 104(3), 438-444.

Wen, Q., & Janmey, P. A. (2011). Polymer physics of the cytoskeleton. *Current Opinion in Solid State and Materials Science*, 15(5), 177-182.

Table 1. Main chemical species of fruit tissue that can be affected by the PEF.

Chemical group	Specie	Biological activity	Position	Macroscopic changes	Reference
Electrolytes	K^+	Secondary signal, active transmembrane pump	Intracellular, Extracellular liquid phase and membrane	Nutritional	Traffano-Schiffo et al., 2016 Sivakumaran, Huffman, Sivakumaran, & Drummond, 2016
	Na^+				
	Mg^{2+}	Part of the chlorophyll complex		Textural and nutritional Functional activity	Park et al., 2011 Bohn, Walczyk, Leisibach & Hurrell, 2004 Tarchevsky & Marchenko, 1991 Lang et al., 2014
	Ca^{2+}	Calcium bridges in the middle lamella Bridges in microtubules (actin microfilaments)			
Aminoacids	Arg	Protein channel	Free, cell membrane and tonoplast	Maillard browning, nutritional value and taste	Ma et al., 2017; Snowden, Thomas, Baxter, Smith, & Sweetlove, 2015; Keutgen, & Pawelzik, 2008; Castaldo et al., 1992
	Asp	Products of the metabolic γ-aminobutyrate conversion			
	Glu	Microtubules in cell structure			
Chlorophylls	Chlorophyll a	O_2 transporter, main role in photosynthesis	Liquid phase and Chloroplast	Color, functional activity	Park et al., 2013; Park et a., 2016; Montefiori McGhie, Costa, & Ferguson, 2005; Cano & Marin, 1992
	Chlorophyll b				
Carotenoids	Neolutein	Secondary role in photosynthesis	Chloroplast and chromoplast	Color, chloroplast membrane stabilizer, source of vitamin A, antioxidant capacity	Park et al., 2013; Havaux, 1998; Ampomah-Dwamena et al., 2009; Cano & Marin, 1992; Dorai, Papadopoulos, & Gosselin, 2001
	β-carotene				
Organic acids	Citric acid	Krebs cycle intermediate; part of cytosolic pyruvate metabolism	Extra and Intracellular liquid phases	Antioxidant	Sivakumaran et al., 2016; Famiani, Battistelli, Moscatello, Cruz-Castillo, & Walker, 2015 Khan et al., 2013; Nishiyama, Fukuda, Shimohashi, & Oota, 2008 Marsh, Boldingh, Shilton, & Laing, 2009 Barboni, Cannac, & Chiaramonti, 2010; Cassano, Donato, Conidi, & Drioli, 2008
	Malic acid			Acidity, flavor	
	Quinic acid	Fruit maturity		Sugar/acid balance, flavor	
	Ascorbic acid	Antioxidant, enzyme Cofactor, electron transport, chloroplast activity		Bioactive compound	

Table 2. Intensity values of the samples obtained by TD-NMR during OD treatment (I^{ADS}: intensity of water adsorbed; I^{ELP}: intensity of extracellular liquid phase; I^{ILP}: intensity of internal liquid phase).

E (V/cm)		OD time (min)					
		0	10	20	30	60	120
0	I^{ADS}	31 ± 4	41.78 ± 0.91	45 ± 2	50 ± 8	59 ± 2	74 ± 2
	I^{ELP}	25 ± 8	58 ± 5	103 ± 6	132 ± 5	120 ± 9	131 ± 11
	I^{ILP}	251 ± 4	183 ± 12	165 ± 9	125 ± 11	124 ± 12	107 ± 7
100	I^{ADS}	33 ± 2	49 ± 3	59 ± 1	61 ± 3	73 ± 5	77 ± 5
	I^{ELP}	91 ± 7	101 ± 12	115 ± 8	126 ± 11	126 ± 14	123 ± 6
	I^{ILP}	208 ± 7	172 ± 13	146 ± 7	131 ± 13	96 ± 8	104 ± 8
250	I^{ADS}	39 ± 2	48 ± 2	56 ± 3	54 ± 6	57 ± 4	70 ± 12
	I^{ELP}	163 ± 5	175 ± 9	174 ± 9	184 ± 5	165 ± 10	169 ± 6
	I^{ILP}	125 ± 2	101 ± 7	91 ± 10	71 ± 8	70 ± 2	57 ± 8
400	I^{ADS}	39 ± 3	50.8 ± 0.4	53 ± 6	54 ± 5	64 ± 2	60 ± 6
	I^{ELP}	167 ± 4	183 ± 9	174 ± 9	183 ± 6	181 ± 2	170 ± 5
	I^{ILP}	107 ± 14	86 ± 5	79 ± 8	75 ± 7	61 ± 6	68 ± 7

FIGURE CAPTIONS

Figure 1. a) Schematic representation of system and the cellular transports during osmotic dehydration treatment (adapted from Traffano-Schiffo et al., 2016), b) Cryo-SEM observation of fresh (750x) and 30 min osmodehydrated kiwifruit tissue (1000x), c) T_2-weighted signal distribution, normalized to unitary area, registered on fresh samples treated by PEF at 0 (black solid line) and 250 V/cm (black dashed line) samples before OD treatment (adapted from Traffano-Schiffo et al., 2017). PT: Passive transport, AT: Active transport, V: Vacuole, IT: Internal transport, ST: Symplastic transport, AP: Apoplastic transport, AQ T: Aquaporins transmembrane transport (water) and TT: Transmembrane transport.

Figure 2. Water mass variation a. (●) extracellular liquid phase (ELP), (▲) intracellular liquid phase (ILP) and b. (□) adsorbed water (ADS) and (♦) total mass (T) variation at different PEF treatments (V/cm).

Figure 3. a. Internal Surface of the solid matrix; b. relationship between the water mass variation of the adsorbed water and the internal surface of the solid matrix.

Figure 4. Schematic representation of cell structure with and without PEF pre-treatment.

Figure 5. Water mass flow during osmotic dehydration treatment of the samples pre-treated with PEF. a. 0 V/cm, b. 100 V/cm, c. 250 V/cm and d. 400

V/cm, where: (●) extracellular liquid phase (ELP), (▲) intracellular liquid phase (ILP) and (♦) total water mass flow.

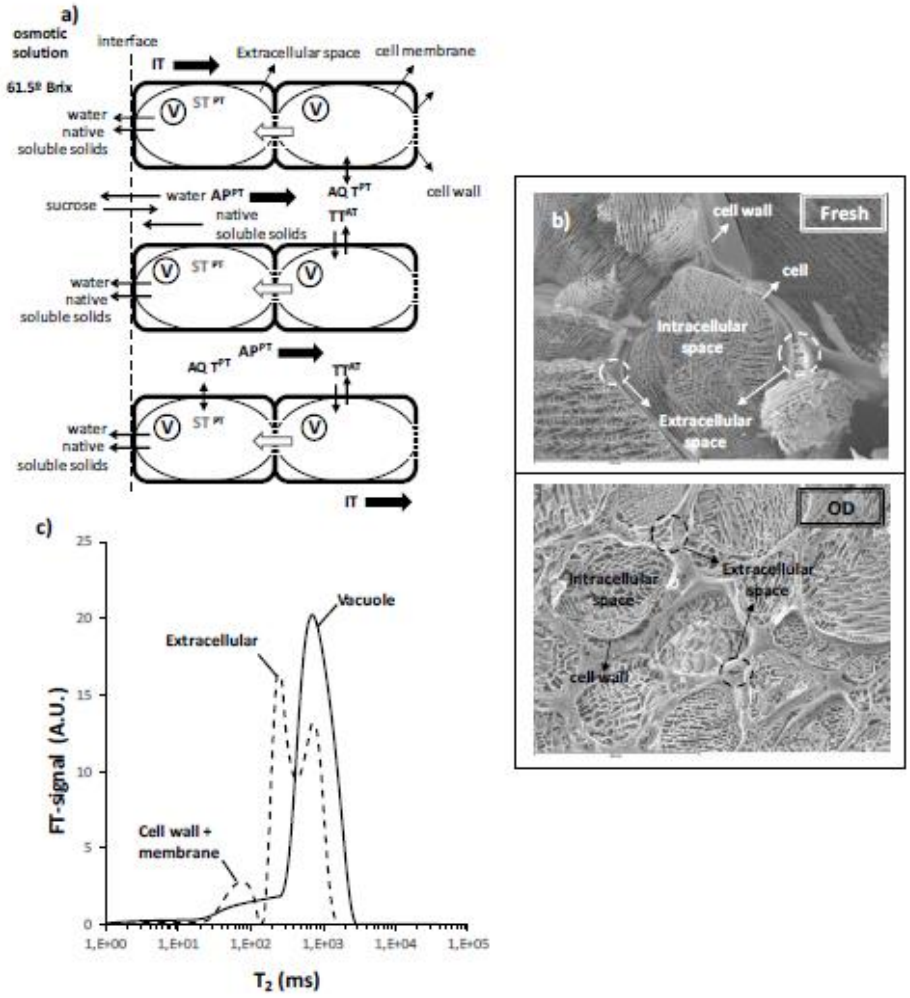

Figure 1.

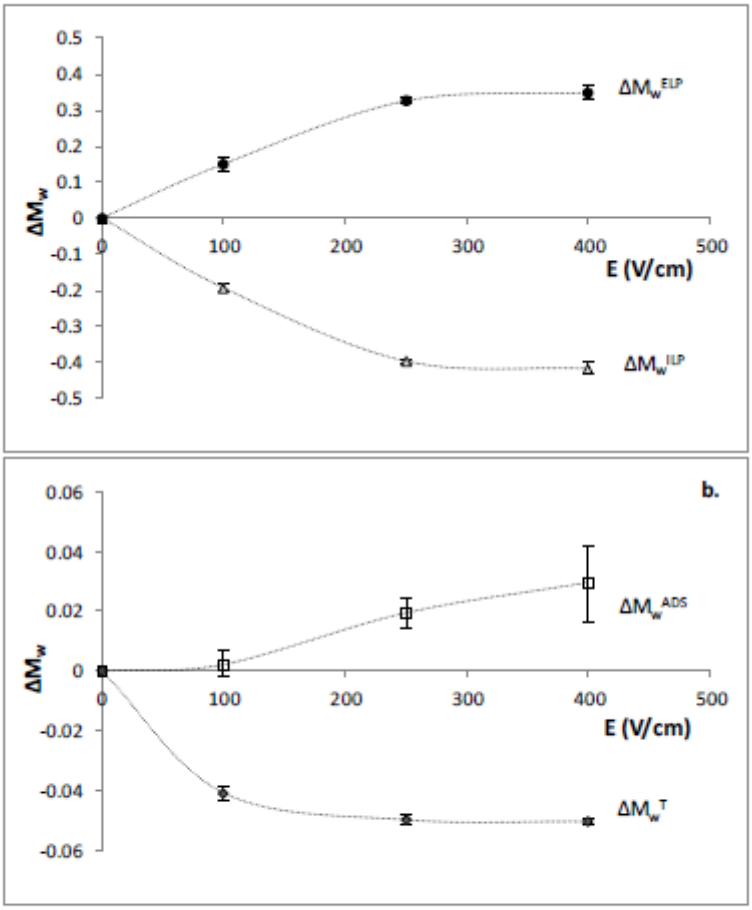

Figure 2.

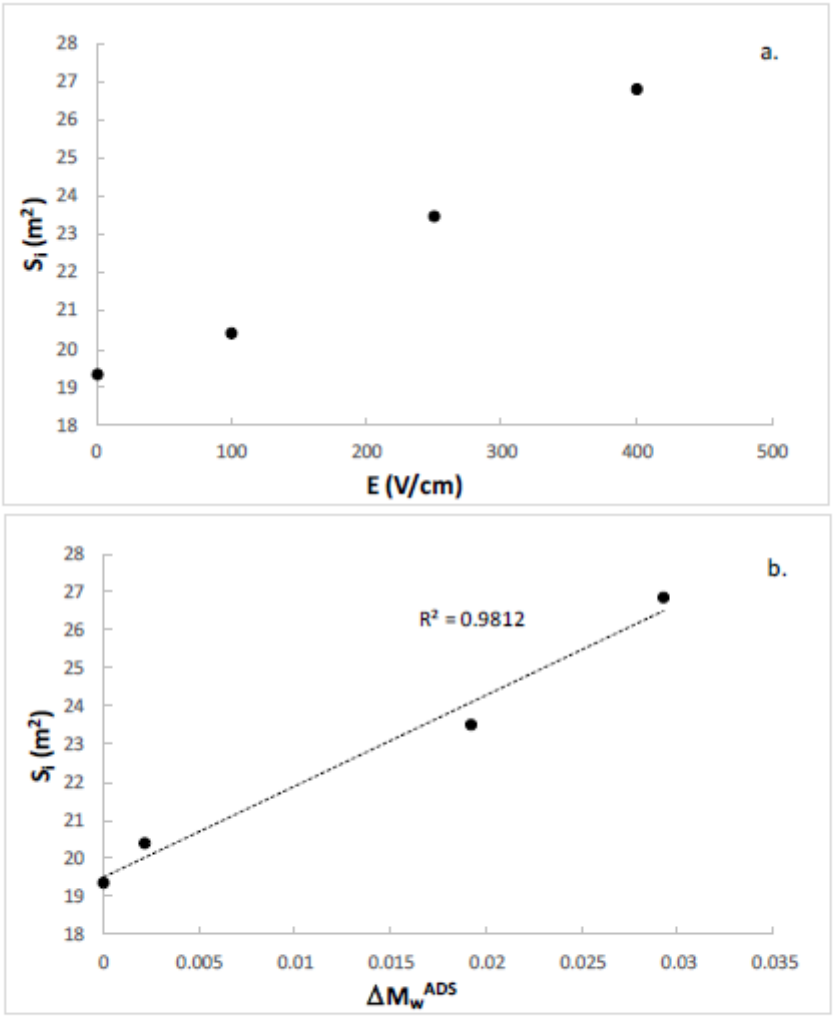

Figure 3.

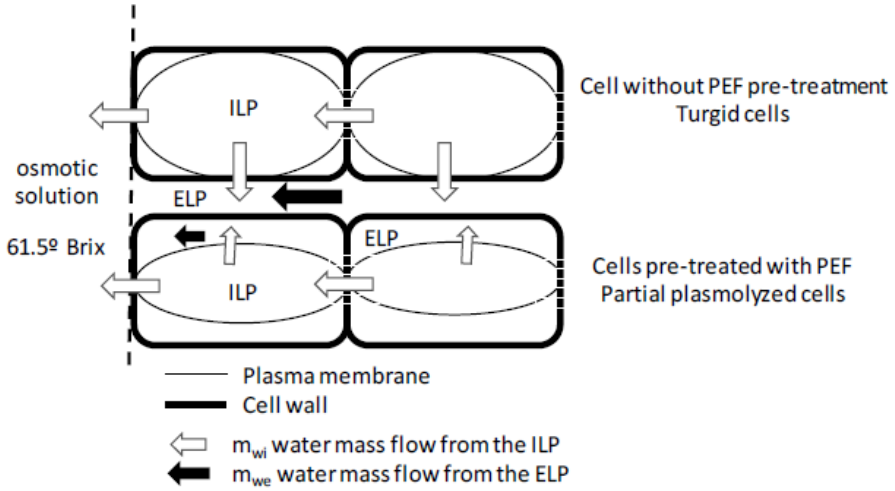

Figure 4.

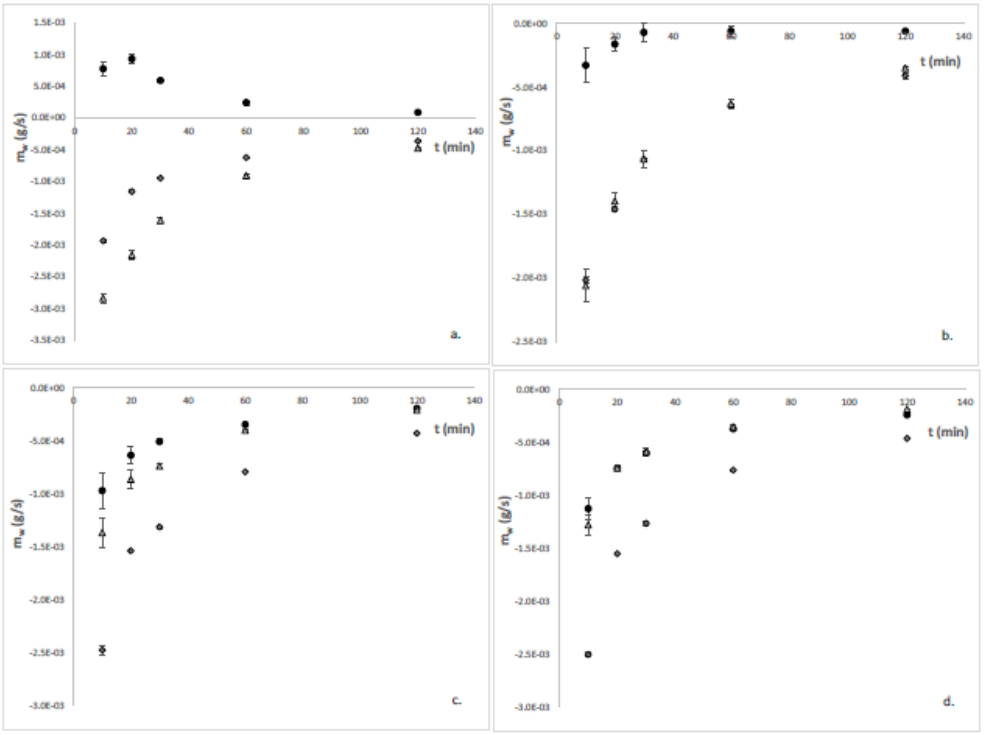

Figure 5.

Industrial relevance

This research describes the effect of the PEF pre-treatment of osmotic dehydration in the microstructure and in the internal water liquid phases transports of the kiwifruit tissue by using TD-NMR. The results of this research have demonstrated that the application of an electric field prior to the osmotic dehydration produces a process of plasmolysis more the greater the electric field applied, due to the elimination of the mobile charges of the medium and mainly affecting the internal transports. Therefore, PEF pre-treatment accelerate the OD treatment preserving the structure and consequently remaining the life cycles, necessaries to obtain a long shelf life of the product. It has been demonstrated the useful of the TD-NMR as a technique able to analyzed the effect of PEF at a microstructural degree.

RESEARCH HIGHLIGHTS

- The application of PEF as OD pre-treatment removes the mobile charges of the tissue.
- PEF pre-treatment produces a greater plasmolysis, the greater the electric field applied.
- In samples pre-treated with PEF, calcium is the major culprit of the plasmolysis.
- PEF pre-treatment affects the normal behavior of the internal transports.
- After PEF, apoplastic transport is as important as the symplastic.