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

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## 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 $4 \pm 1$ 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 $13 \pm 1$ 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).

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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 $328 \pm 4$ 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 $100 \pm 2$ µs and a repetition time of $10.0 \pm 0.1$ 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[TM], 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 $\leq$ -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

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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:

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

Where $x_w^j$ is the water mass fraction of water group j (kg$_{\text{w in j}}$/kg$_{\text{T}}$) and $x_w$ is the water mass fraction (kg$_{\text{w}}$/kg$_{\text{T}}$).

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:

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

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

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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):

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

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 ($g_w/g_T$), $X_{w0}^{\mathrm{ADS}}$ 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

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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:

$$m_w^j = \frac{M^t x_{wi}^t - M^0 \, x_{wi}^0}{t} \tag{4}$$

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

## 6. References

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**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 | $K^+$ <br><br> $Na^+$ <br><br><br> $Mg^{2+}$ <br><br><br> $Ca^{2+}$ | Secondary signal, active transmembrane pump <br><br> Part of the chlorophyll complex <br><br> Calcium bridges in the middle lamella Bridges in microtubules (actin microfilaments) | Intracellular, Extracellular liquid phase and membrane | Nutritional <br><br><br><br> Textural and nutritional Functional activity | Traffano-Schiffo et al., 2016 <br> Sivakumaran, Huffman, Sivakumaran, & Drummond, 2016 <br> Park et al., 2011 <br> Bohn, Walczyk, Leisibach & Hurrell, 2004 <br> Tarchevsky & Marchenko, 1991 <br> Lang et al., 2014 |
| Aminoacids | Arg <br><br> Asp <br><br> Glu | Protein channel Products of the metabolic γ-aminobutyrate conversion Microtubules in cell structure | 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 |
| Chlorophylls | Chlorophyll a <br><br> Chlorophyll b | $O_2$ 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 |
| Carotenoids | Neolutein <br><br> β-carotene | 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 |
| Organic acids | Citric acid <br><br> Malic acid <br><br> Quinic acid <br><br><br> Ascorbic acid | Krebs cycle intermediate; part of cytosolic pyruvate metabolism <br><br> Fruit maturity <br><br><br> Antioxidant, enzyme Cofactor, electron transport, chloroplast activity | Extra and Intracellular liquid phases | Antioxidant <br><br> Acidity, flavor <br><br> Sugar/acid balance, flavor <br><br> Bioactive compound | Sivakumaran et al., 2016; Famiani, Battistelli, Moscatello, Cruz-Castillo, & Walker, 2015 <br> Khan et al., 2013; Nishiyama, Fukuda, Shimohashi, & Oota, 2008 <br> Marsh, Boldingh, Shilton, & Laing, 2009 <br> Barboni, Cannac, & Chiaramonti, 2010; Cassano, Donato, Conidi, & Drioli, 2008 |

**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.
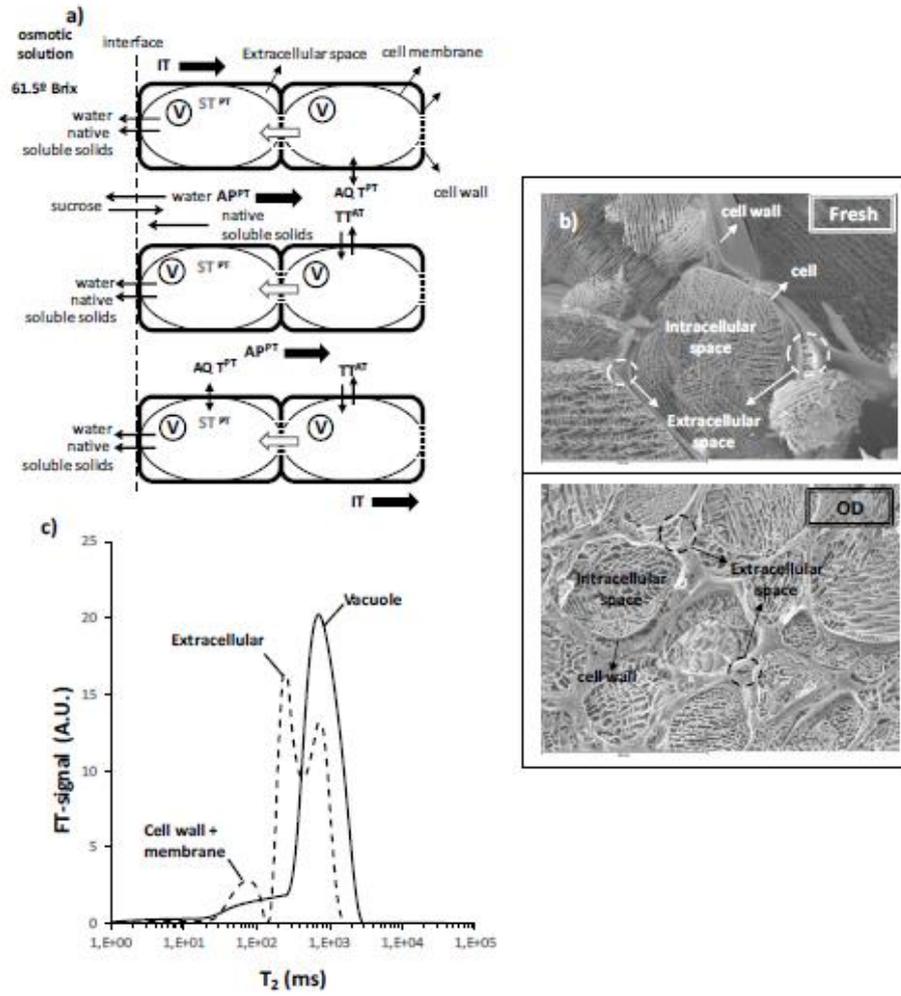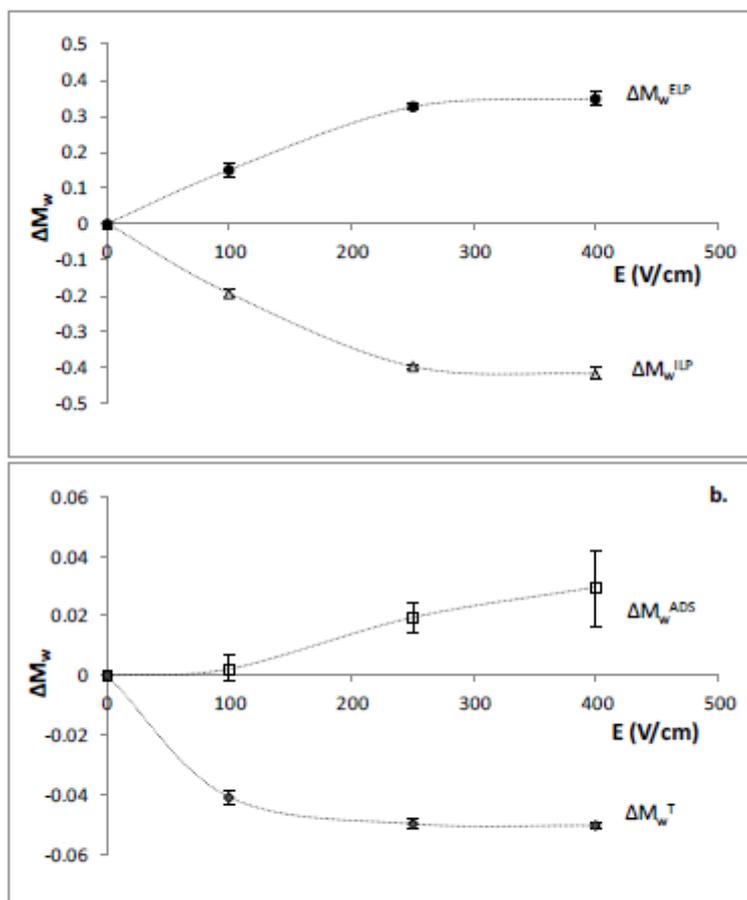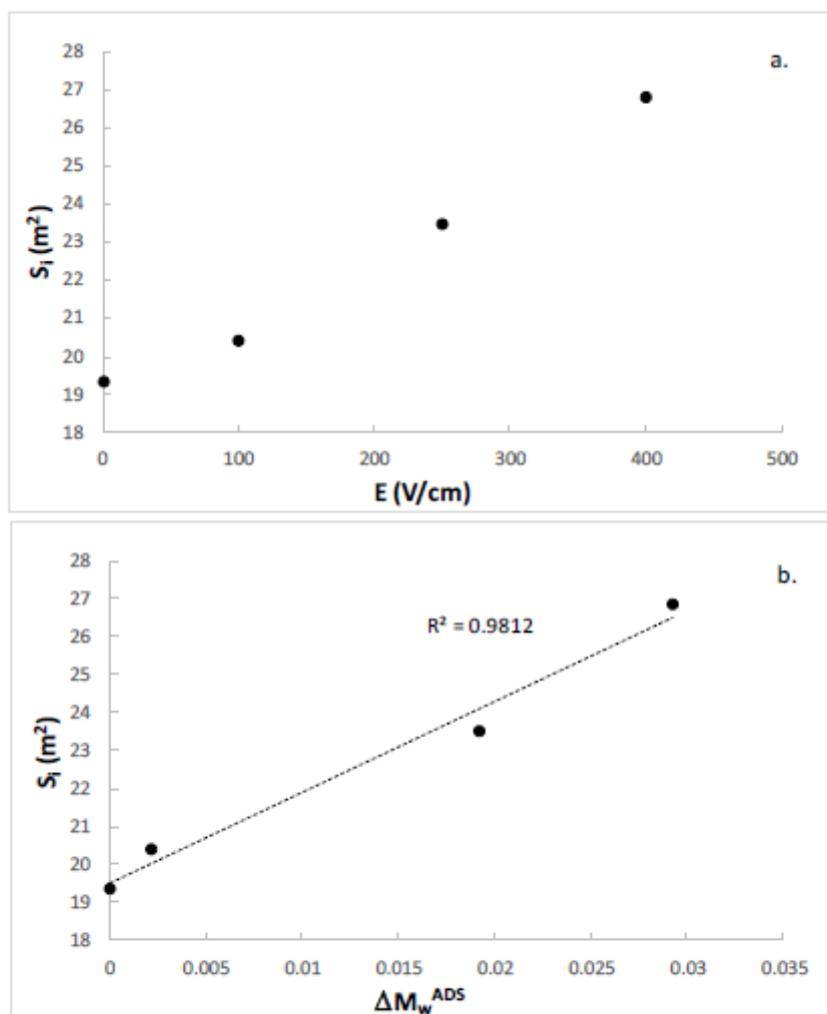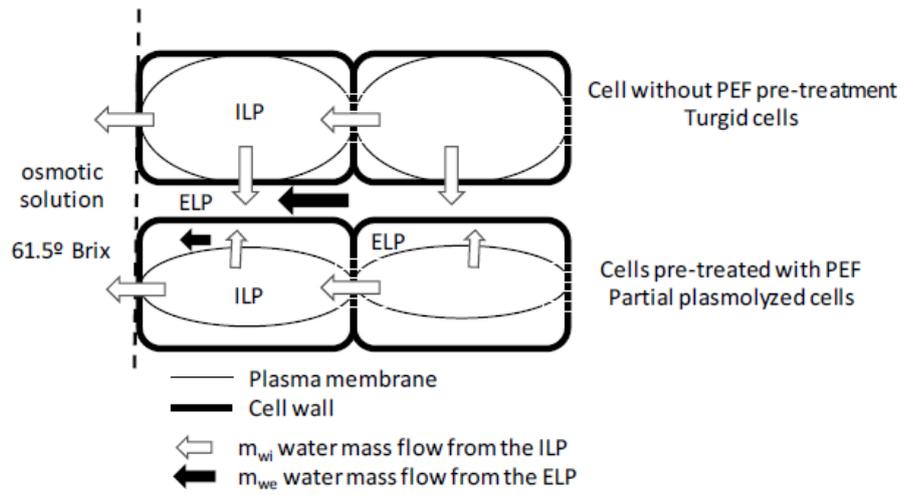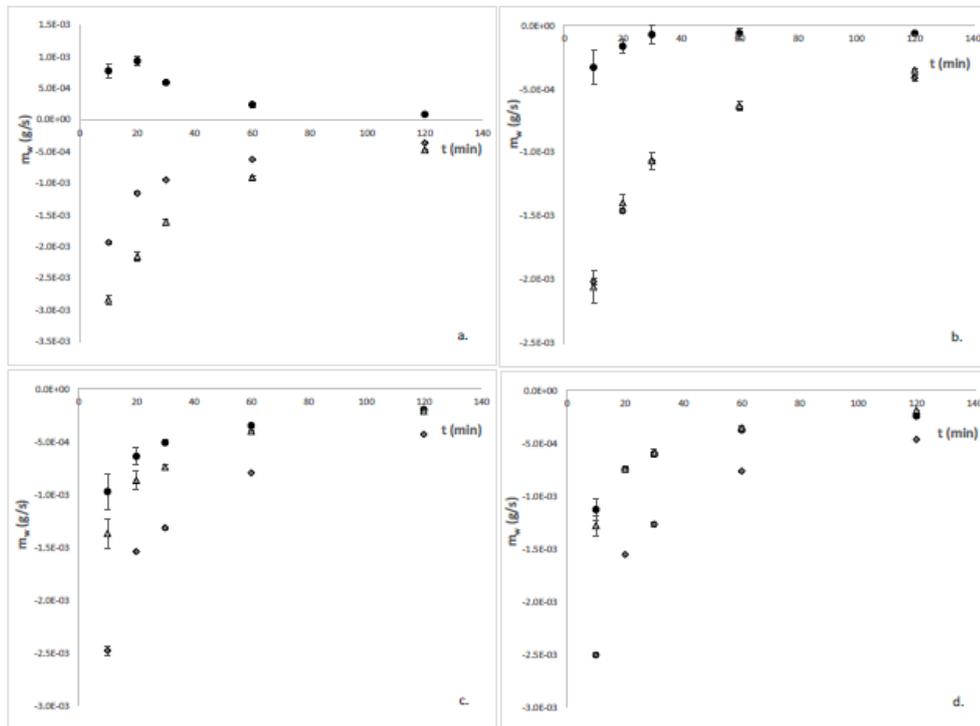
Figure 1.

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.