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Title

Time domain nuclear magnetic resonance to monitor mass transfer mechanisms in apple tissue promoted by osmotic dehydration combined with pulsed electric fields

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PEF; osmotic dehydration; mass transfer; TD-NMR; water distribution; water self diffusion

ABSTRACT

Pulsed electric fields (PEF) technology is gaining momentum as a pre-treatment to enhance mass transfer of vegetable tissues obtained by further processing. In this study PEF pre-treatment increased osmotic dehydration (OD) effectiveness, in terms of water loss and solid gain in apples, as a function of electric field strength and number of pulses. Mass transfer was particularly high when average electric fields of 250 and 400 V/cm were applied. Time domain nuclear magnetic resonance (TD-NMR), with the use of a contrast agent, clarified structural changes that drive mass transfer. Treatments at 100 V/cm redistributed water between vacuole, cytoplasm and extracellular space, while at 250 and 400 V/cm the membranes breakages caused the loss of cellular compartmentalization. Two non-destructive and fast acquirable parameters, the longest measured relaxation time (T_2) and water self diffusion coefficient (D_w), allowed the separate and accurate observation of PEF treatment and osmotic dehydration effects.

Industrial relevance: The developed non-destructive method, here described, allows the measure of the effects of PEF treatment on apple tissue which can be exploited to have reliable control of the process within minutes. Since mass transfer parameters depend on subcellular water redistribution, the present work provides a tool to boost the development and optimization of agri-food processes on fresh vegetable tissues.

1. Introduction

Pulsed electric fields (PEF) is an innovative non-thermal technology which delivers short pulses to food products, placed between two electrodes, generating electric fields, that usually span from 0.1 to 5 kV cm^{-1}. When coupled to extraction techniques, its application leads to an enhancement of mass transfer phenomena, which can be exploited to increase extraction yields from vegetable tissues (Donsì, Ferrari, & Pataro, 2010). In addition, its effectiveness has been demonstrated by combining PEF together with osmotic dehydration (Ade-Omowaye, Angersbach, Taiwo, & Knorr, 2001; Amami, Vorobiev, & Kechaou, 2006; Wiktor, Śledź, Nowacka, Chudoba, & Witrowa-Rajchert, 2014), air drying (Ade-Omowaye, Rastogi, Angersbach, & Knorr, 2003; Wiktor, Iwaniuk, Śledź, Nowacka, Chudoba, & Witrowa-Rajchert, 2013), compression (Bazhal, Lebovka, & Vorobiev, 2001) and thermal treatments (Lebovka, Praporscic, Ghnimi, & Vorobiev, 2005; Parniakov, Lebovka, Bals, & Vorobiev, 2015).

The application of PEF on vegetable tissue acts on the membrane permeability, inducing electroporation of cells (Teissie, Eynard, Gabriel, & Rols, 1999). The mechanism of electroporation includes different steps: polarization of membranes, creation of pores, expansion of pore radii and resealing of pores (Donsì, et al., 2010; Vorobiev & Lebovka, 2008). In addition to the type of fruit and vegetable tissue, the extent of electroporation, especially the resealing of pores, which can last from seconds to hours, depends on the applied electric field strength, duration, number and shape of pulses, interval between pulses. It is of practical importance that the application of electric fields lower than 1 kV cm^{-1}, and a total treatment time in the order of milliseconds, does not significantly contribute to a temperature increase, which would alter membrane permeability caused by heat related damages (Lebovka, Bazhal, & Vorobiev, 2002) and the quality of the obtained products.

In mass transfer applications, PEF effects on vegetable tissues are generally evaluated by the extraction yields, or by the release of some target compounds (Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009). The measurement of the apparent diffusion coefficient, often compared to untreated and totally destroyed samples, is another index of macroscopic changes. This method has the drawback of being indirect and

invasive, leading to inconsistent results due to possible modification of the structure of the tissue (Vorobiev, Jemai, Bouzrara, Lebovka, & Bazhal, 2005). Alternatively, changes of colour and texture are also controlled as side effect of PEF treatment, being even desirable, for instance, when the material softening is the objective of the study (Lebovka, Praporscic, & Vorobiev, 2004). Direct effects on membrane permeabilization can be qualitatively observed by staining of plant tissues followed by microscope visualization (Fincan & Dejmek, 2002). However, the most commonly applied method to measure cell disintegration is based on changes in electrophysical properties, i.e. the impedance, that gives information on the damage degree of a sample when compared to both an untreated and a totally destroyed sample (Angersbach, Heinz, & Knorr, 1999; Lebovka, et al., 2002).

Time domain nuclear magnetic resonance (TD-NMR) is a fast, non-destructive analytical technique that allows to evaluate spatial features in vegetable cellular compartments by the indirect measurement of water distribution inside and outside cells. Recently, the measurement of transverse relaxation time (T_2) curves has been successfully applied to study the subcellular water redistribution upon osmotic dehydration, its combination with ultrasound in kiwifruit (Nowacka, Tylewicz, Laghi, Dalla Rosa, & Witrowa-Rajchert, 2014; Tylewicz, et al., 2011) and the addition of calcium and ascorbic salts to the osmotic solution in apple tissue (Mauro, et al., 2015). Furthermore, through the evaluation of the water self diffusion coefficient, an overview of water possibility to explore the surrounding space can be achieved. Santagapita et al. (2013) found that water loss and solid gain, during the osmotic treatment of kiwifruit, were in good agreement with the reduction of the water self diffusion coefficient.

The present work evaluated the effect of PEF on apple tissue as preliminary treatment to osmotic dehydration, at three different electric field strengths (100, 250 and 400 V cm^{-1}) and total number of pulses (20 and 60 train series). Beside the control of the mass transfer parameters water loss and solid gain, a subcellular level observation was applied by means of TD-NMR to understand, in-depth, PEF-induced mechanisms that affect mass balances. Differently from previous works, transverse relaxation time (T_2) of the osmotic solution was selectively dropped by the addition of a contrast agent. This eased the discrimination of three characteristic cellular compartments, namely vacuole, cytoplasm and extracellular

space, respectively delimited by plasma membrane and tonoplast. Moreover, once the membranes permeability was altered due to electroporation, the contrast agent was a key element to observe the external solution diffusing through the inner compartments of apples. In addition, the average water self diffusion coefficient (D_w) of water contained in apple tissue was evaluated as a non-destructive control tool for the osmotic dehydration process.

2. Material and methods

2.1 Material

Apples (*Malus domestica*) of the Cripps Pink variety, also known by the brand name Pink Lady®, were purchased at a local marked and stored at 2 ± 1 °C for no longer than a month, within which experiments were run. Average moisture and soluble solid contents were, respectively, 83.5 ± 0.5 g and 14.0 ± 0.5 g per 100 g of fresh product (g_{fw}). Apples were cut with a manual cork borer and cutter to obtain cylinders of 8 mm diameter and a length of 10 mm.

2.2 Pulsed electric field (PEF) treatment

Pulsed electric field (PEF) treatments were applied to apple cylinders using an in-house developed pulse generator equipment based on MOSFET technology and on capacitors as energy tank. The PEF generator provides monopolar pulses of near-rectangular shape at different voltages, adjustable repetition time between pulses and variable total treatment duration which lead to a variable number of delivered pulses. Treatments were run at 20 °C in a 30 × 20 × 20 mm (length × width × height) chamber equipped with two stainless steel electrodes (active contact surface = 20 × 20 mm^2) with a distance between them fixed at 30 mm. For each treatment 12 apple cylinders (approximately 5 g) were inserted into the chamber with the two circle sides parallel to the electrodes (Fig. 1). The chamber was filled up with tap water, with an electrical conductivity of 328 ± 4 µS cm^{-1} at 25 °C, with product-to-water ratio around 1:1 (v/v).Table 1 shows the experimented pulse series and the average applied electric field strengths in the chamber of trials conducted at fixed pulse width (100 ± 2 µs) and repetition time (10.0 ± 0.1 ms) with a voltage of 300 V, 750 V, and 1200 V to the electrodes. The current and voltage values were registered by using a digital oscilloscope (PicoScope 2204a, Pico Technology, UK) connected to a personal computer.

FIGURE 1 and TABLE 1

2.3 Osmotic dehydration (OD) and mass transfer control

Immediately after PEF application, treated apple cylinders were removed from the PEF treatment solution and placed into 7 different beakers containing a continuously stirred 30% (w/w) sucrose osmotic solution, in a product-to-solution ratio of approximately 1:20 (w/w), to avoid changes in the concentration of the solution during the treatment. The rotational speed was experimentally determined to assure negligible resistance to mass transfer. Besides, control samples were prepared by directly placing apple cylinders into the osmotic solution without PEF pre-treatment. Iron (III) chloride (Sigma Aldrich -Steinheim, Germany) was employed as contrast agent for NMR analysis and added to the osmotic solution to obtain a final concentration of 0.01 M. Samples were collected 0 (fresh control), 15, 30, 60 and 120 minutes after the immersion, blotted with absorbing paper, weighted and analysed. The moisture content of 3 apple cylinders (weighing approximately 1.5 g) of fresh and treated samples was determined gravimetrically by drying at 70 °C until a constant weight was achieved, as recommended for fruit products by AOAC International (2002). In parallel, the same experimental plan (Table 1) was run by replacing the osmotic treatment with an isotonic solution, to gain insight of mass transfer phenomena caused by PEF only, without an external osmotic driven force.

Mass transfer was evaluated by calculating the mass balances, in terms of mass variation, water loss and solid gain. The total mass variation (ΔM) in relation to the initial mass during osmotic dehydration was calculated from experimental data according to Eq. (1):

$$\Delta M = \frac{(m - m_0)}{m_0} \qquad (1)$$

where m = mass and m_0 = mass at initial time (t = 0).

Water loss (ΔM_w) and solid gain (ΔM_s) were calculated in relation to the initial mass according to Eq. (2) and Eq. (3), respectively:

$$\Delta M_w = \frac{(w \cdot m - w_0 \cdot m_0)}{m_0} \qquad (2)$$

$$\Delta M_s = \Delta M - \Delta M_w \quad (3)$$

where w = water content, w_0 = water content at initial time (t = 0).

2.4 Time domain nuclear magnetic resonance (TD-NMR)

2.4.1 Transverse relaxation time curves measurement

Proton transverse relaxation time (T_2) decay was measured for each sample immersed in both the isotonic and osmotic solutions by applying the CPMG pulse sequence (Meiboom & Gill, 1958) using a Bruker 'The Minispec' spectrometer (Bruker Corporation, Germany) operating at 20 MHz. Apple cylinders were collected from the solutions, placed into 10-mm diameter NMR tubes and directly analysed. Each measurement comprised 6000 echoes, with an interpulse spacing of 0.3 ms and a recycle delay of 10 s which allowed the measurement of protons 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 analysed 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), according to Eq. (4):

$$S_{(t)} = \sum_{i=1}^{N} I_n \exp\left(\frac{-t}{T_{2,i}}\right) + E_{(t)} \quad (4)$$

where N = number of protons populations, which was set at 3 (vacuole, cytoplasm and extracellular space) according to UPEN results, I = signal intensity, T_2 = average relaxation time of each protons population (n) and E = residuals error.

2.4.2 Water self-diffusion coefficient measurement

Water self-diffusion coefficient (D_w) was measured by means of pulsed magnetic field gradient spin echo (PGSE) sequence (Stejskal & Tanner, 1965). The sequence implemented in the Bruker 'The Minispec' spectrometer software allowed to apply a magnetic field gradient spanning from 0.04 to 2.00 T m^{-1} which was calibrated by using pure water with a known D_w value of 2.3 10^{-9} m^2 s^{-1} at 25 °C (Holz, Heil, & Sacco, 2000). To allow a comparison between samples treated using different applied energies, water inside apple tissue was considered as characterized by a single self diffusion coefficient (D_w) according to Eq. (5) (Santagapita, Laghi, Panarese, Tylewicz, Rocculi, & Dalla Rosa, 2013):

$$\ln \frac{A_G}{A_{G0}} = -\gamma^2 D_w \delta^2 \left(\Delta - \frac{1}{3}\delta\right) G^2 \qquad (5)$$

where A_G =amplitude of PGSE with the applied gradient (G = 1 T m^{-1}), A_{G0} =amplitude of PGSE without the gradient, γ = proton gyromagnetic ratio, δ = gradient length set at 0.5 ms, Δ = time between the gradients fixed at 7.5 ms.

2.5 Statistical analysis

To evaluate whether PEF pre-treatment significantly enhanced mass transfer during osmotic treatment, the analysis of variance (ANOVA) and Tukey multiple comparisons were applied, by accepting the significance level of 95 % ($p < 0.05$). All the experiments were conducted in triplicate and results were expressed as mean ± standard deviation of replications.

3. Results and Discussion

3.1 Mass transfer

Immediately after PEF application, treated apple cylinders appeared similar to raw material. No loss of material was noticed, although the samples treated at 250 and 400 V cm^{-1} seemed to partially lose the original hardness of the apple tissue. Nevertheless, pulsed electric fields applied as a pre-treatment for osmotic dehydration overall enhanced the mass transfer between apple tissue and the osmotic solution. Fig. 2 shows, indeed, water loss and solid gain of samples treated at different voltages with trains of 60 pulses in comparison with control untreated samples. Numeric scores and statistical analysis for each treatment are shown in Table 2.

The measured initial water content of untreated apples (0.835 ± 0.005 g g_{fw}^{-1}) decreased by a minimum of 3.2 ± 0.1 % (after 15 minutes) to a maximum of 15.8 ± 0.4 % (after 120 minutes). During the first 15 minutes from the beginning of the osmotic treatment, water loss significantly increased when either the highest field strength (400 V cm^{-1}) or the combination medium field strength (250 V cm^{-1}) and 60 pulses were applied. As an example, treatment with 400 V cm^{-1} 20 pulses led to a water loss of 4.6 ± 0.6 %, while 60 pulses boosted this value to 6.7 ± 0.1 %. Taking into account the whole osmotic process, both voltage and number of pulses positively influenced the water loss. Indeed, at the end of the experimental trial (120 minutes), untreated apple samples reached the water loss of 15.8 ± 0.4 % while 20 pulses at 250 and 400 V cm^{-1} resulted in higher values, spanning from 20.1 to 20.2 % and 60 pulses at the same field strengths led to the highest water removal, around 20.9-22.2 %. This shows, in agreement with previous works (Amami, et al., 2006; Parniakov, et al., 2015; Wiktor, et al., 2014), that the initial electroporation effect caused by PEF lasted for several minutes after application (Ade-Omowaye, Talens, Angersbach, & Knorr, 2003).

Similarly to water loss, solid gain showed an increased rate when PEF was applied. In detail, while control samples gained 2.8 ± 0.4 % of solid content in 2 hours treatment, each PEF pre-treated sample reached a 4-5 % gain. Interestingly, samples treated at the lowest field (100 V cm^{-1}) showed a similar behaviour to the samples treated at higher fields, especially when a 60 pulses train was choose. Indeed, application of 60

pulses increased both solid gain and water loss after 120 minutes (Fig. 2 and Table 2) when compared to control even though the water removal was lower than sample which underwent to 250 and 400 V cm^{-1} treatments. In the same way, some authors found that 100 V cm^{-1} were also able to improve mass transfer, in terms of juice extraction yields during the compression of apple tissue (Bazhal, et al., 2001) and diffusion coefficient measured in apple discs (Jemai & Vorobiev, 2002). Although some studies on apple tissue highlighted that the number of pulses do not affect the mass transfer (Taiwo, Angersbach, & Knorr, 2003; Wiktor, et al., 2014), the present study showed the number of pulses had a significant effect. This difference can be probably ascribed to the different electric field strengths which were applied in the present work, lower than the other studies.

FIGURE 2 and TABLE 2

3.2 Water distribution and self diffusion

To gain insight into the mechanisms which drive mass transfer phenomena, TD-NMR was employed by registering T_2-weighted curves. Since the T_2 of protons depends on chemical exchange among water, solutes and biopolymers (B. P. Hills & Remigereau, 1997; Santagapita, et al., 2013), this allowed the separate observation on raw apple tissue of extracellular space, cell wall, cytoplasm and vacuole, together with their modifications upon technological treatments (Mauro, et al., 2015). In raw material, water was found to be distributed as follows: 77.5 ± 1.7 % in vacuole (T_2 1391 ± 45 ms), 18.5 ± 1.5 % (T_2 282 ± 25 ms) in cytoplasm/extracellular space and 4.0 ± 0.4 % (T_2 17 ± 3 ms) was ascribed to structural water of cell wall.

A preliminary study on osmotic dehydration showed that the T_2 of osmotic solution entering the extracellular space was similar to the one of cytoplasm. In order to observe the two compartments separately this prompted us to lower the T_2 of the osmotic solution by means of iron (III) chloride, which therefore acted as contrast agent. A concentration of 0.01 M was chosen in order to equal the T_2 of cell wall, typically non-sensitive to technological treatments (Nowacka, et al., 2014; Santagapita, et al., 2013).

Higher iron (III) chloride concentrations were discarded because leading to T_2 lower than the instrument limits (Van Duynhoven, Voda, Witek, & Van As, 2010).

The continuous line in Fig. 3a shows T_2-weigthed signal distribution of untreated apple tissue upon 120 minutes of dipping in isotonic solution. Beside the peak ascribable to the extracellular space solution, set to 12 ms with the addition of the contrast agent, vacuole and cytoplasm signals, centred around 200 and 1200 ms as in the raw apples, demonstrated that the contrast agent itself was not able to passively diffuse through the native intact plasma membranes. The osmotic dehydration without any PEF pre-treatment (continuous line in Fig. 3b) led to a partial water removal from the inner cellular compartments toward the external space so that, after 120 minutes, the T_2 of the extracellular space was slightly increased, resulting in a peak around 50 ms. This was due to the partial dilution promoted by the shrinkage of the inner compartments, which have a higher T_2, leading to a higher mean value of the extracellular population. As expected, the relative area of the vacuole peak, i.e. its water content, also showed a marked reduction after osmotic treatment.

FIGURE 3

The non-continuous lines of Fig. 3a allow to appreciate the water redistribution caused only by the application of the external electric field, in absence of any osmotic driven force, while Fig. 3b shows the joint contribution of PEF and OD treatments on water redistribution. Taking into account PEF treatments alone, the application of medium and high voltages (250 and 400 V cm^{-1}) led the extracellular space, cytoplasm and vacuole signals to collapse into a single broad protons population. This highlighted that the membranes were electrically damaged with the consequent loss of any compartmentalization. After PEF treatment at 100 V cm^{-1}, conversely, the structure was still apparent. Nevertheless, the vacuole/extracellular ratio was lower than the NoPEF sample as a consequence of the reduction of the vacuole population shown in Fig. 3a-b. This behaviour suggested that electroporation took place but its effect was probably reversible. Fine tuning of the applied voltage allowed finding the no-reversibility threshold at around 150 V cm^{-1} with 60 train pulses (data not shown).

TABLE 3

Table 3 offers a complete view of water distribution among cell compartments in case of osmotic dehydration, with and without a 100 V cm^{-1} PEF pre-treatment. Electrical pre-treatment using 60 pulses led to significantly higher vacuole shrinkage than control throughout the entire osmotic process, increasing the relative water content of both cytoplasm and extracellular spaces. This water rearrangement caused a vacuole T_2 decrease, that can be qualitatively visualized from Fig. 3b, which was far less pronounced than what expected in case of contrast agent entrance. The two joint pieces of information strongly suggest that the treatment at 100 V cm^{-1} did not induce the permeabilization of the plasma membrane, but led to damages to tonoplast, which surrounds vacuole, probably because more sensitive to electric fields. This water migration from the internal cellular compartments can explain the increase of mass transfer phenomena which were noticed both in the present work and in previous studies which applied moderate electric fields (Sensoy & Sastry, 2004; Vorobiev, et al., 2008).

From an industrial point of view, it is important to highlight that after each of the tested treatments water distribution among cell compartments showed that PEF effects were time-dependent. This is in agreement with previous studies based on a macroscopic evaluation of mass transfer (Ade-Omowaye, Talens, et al., 2003; Angersbach, Heinz, & Knorr, 2002). This suggests that time after treatment can be a fundamental factor to be considered in order to optimize PEF application in a combined multi step manufacturing process.

The present investigation showed that the three compartments model, which is typically applied to describe raw apple tissue relaxation curves (Mauro, et al., 2015), can be effectively used to model the NMR signals of apple treated at 100 V cm^{-1} but it is, unfortunately, inadequate when a voltage above the irreversible electroporation threshold is applied. Efforts were therefore made to find a characteristic of T_2 curves which could be universally applied to estimate the electric cell damage. In this respect, the longest relaxation time, which can be directly obtained from the UpenWin software output, was found to be tailored to the goal. In the present experimental conditions, this T_2 could be ascribed to the water protons

located in the middle of the vacuole, because characterized by the weakest interaction with biopolymers (B. Hills & Duce, 1990) and unaffected by the contrast agent until plasma and tonoplast membranes breakage (Panarese, Laghi, Pisi, Tylewicz, Dalla Rosa, & Rocculi, 2012).

Fig. 3c-d show the longest relaxation time of samples immersed in isotonic and hypertonic solutions, respectively. No differences were noticed in NoPEF samples throughout the entire process, showing that this value was independent from the immersion time in an isotonic solution or from the vacuole shrinkage which usually occurs when apple tissue is immersed into an osmotic solution (Mauro, et al., 2015). The longest relaxation time of samples treated at 250 and 400 V cm^{-1} in both isotonic and hypertonic solutions showed a high decrease of relaxation time which passed from the initial value around 2000 ms to around 1000 ms during the first 15 minutes after PEF treatments and reached values around 500 ms after 120 minutes. This parameter was therefore not only sensitive to the applied electric field strength, but also able to clearly discriminate reversible from irreversible effects. Furthermore, it is worth to notice that those results closely followed mass transfer scores. For instance, 60 pulses at 400 V cm^{-1} led to the highest water removal, 20 pulses at 250 cm^{-1} to the lowest difference in mass transfer compared to control whilst 20 pulses at 400 V cm^{-1} and 60 pulses at 250 V cm^{-1} showed intermediate values which were similar between them.

FIGURE 4

In addition to water distribution analysis, water self diffusion coefficient (D_w) was evaluated by means of pulsed magnetic field gradient spin echo sequence. This parameter gives an overview of the ability of water molecules, contained inside apple tissue, to explore the surrounding space. In order to compare raw material and samples treated at different electric field strengths, a single diffusion coefficient was calculated in both compartmentalized and non-compartmentalized samples. In the former case, this represents an average value of the diffusion coefficients in the different environments while in the latter case one population was found so that only a single coefficient model was applicable. This approximation granted the use of one universal coefficient for any of the studied cases, as suggested by Santagapita et al.

(2013). When using isotonic solution no differences were found as a consequence of any PEF treatment, even when observed along 120 minutes. In case of osmotic solution, D_w showed once more behaviour independent from PEF application, but a remarkable proportionality to the osmotic dehydration. Indeed, Fig. 4 shows the linear relationship between D_w and water content of apple tissues (R^2 = 0.92), either electrically pre-treated or not, demonstrating universal applicability of D_w for water loss estimation in case of osmotic treatments. These results are in agreement with a previous study where water self-diffusion coefficient was described as a useful non-destructive tool to monitor osmotic processes applied to kiwifruit (Santagapita, et al., 2013).

4. Conclusions

In the present work osmotic dehydration found in PEF an effective aid in removing water from apple tissue and increasing solutes concentration, due to the alteration of the membranes permeability. A description of subcellular modifications which occurred upon the use of electric fields was achieved for the first time by TD-NMR. This highlighted a continuum of consequences of PEF treatments on tissue subcellular structure, from water redistribution to membranes disruption. The measurement of T_2-weighted relaxation curves and water self diffusion coefficients provided a fast and potentially non-destructive method to control PEF and osmotic processes, respectively. In particular, water redistribution through apple cellular compartments, vacuole, cytoplasm and extracellular space was found to be highly dependent on the electric field strength and number of pulses. Mass transfer data was in good agreement with the findings from TD-NMR, promoting the use of the developed method when, as in the case of pulses electric fields, the process target is the alteration of subcellular compartmentalization. Finally, it is worth mentioning that PEF treatment produced time-dependence effects on apple tissue, suggesting that the optimization of industrial applications should take into account the time elapsed from the application of pulses electric fields.

REFERENCES

Ade-Omowaye, B., Angersbach, A., Taiwo, K., & Knorr, D. (2001). Use of pulsed electric field pre-treatment to improve dehydration characteristics of plant based foods. *Trends in Food Science & Technology, 12*(8), 285-295.

Ade-Omowaye, B., Rastogi, N., Angersbach, A., & Knorr, D. (2003). Combined effects of pulsed electric field pre-treatment and partial osmotic dehydration on air drying behaviour of red bell pepper. *Journal of Food Engineering, 60*(1), 89-98.

Ade-Omowaye, B., Talens, P., Angersbach, A., & Knorr, D. (2003). Kinetics of osmotic dehydration of red bell peppers as influenced by pulsed electric field pretreatment. *Food research international, 36*(5), 475-483.

Amami, E., Vorobiev, E., & Kechaou, N. (2006). Modelling of mass transfer during osmotic dehydration of apple tissue pre-treated by pulsed electric field. *LWT-Food Science and Technology, 39*(9), 1014-1021.

Angersbach, A., Heinz, V., & Knorr, D. (1999). Electrophysiological model of intact and processed plant tissues: cell disintegration criteria. *Biotechnology Progress, 15*(4), 753-762.

Angersbach, A., Heinz, V., & Knorr, D. (2002). Evaluation of Process-Induced Dimensional Changes in the Membrane Structure of Biological Cells Using Impedance Measurement. *Biotechnology Progress, 18*(3), 597-603.

AOAC International (2002). Official methods of analysis (OMA) of AOAC International, 17th Edition, USA. Method number: 920.15. Available at. http://www.eoma.aoac.org/

Bazhal, M., Lebovka, N., & Vorobiev, E. (2001). Pulsed electric field treatment of apple tissue during compression for juice extraction. *Journal of Food Engineering, 50*(3), 129-139.

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

Donsì, F., Ferrari, G., & Pataro, G. (2010). Applications of pulsed electric field treatments for the enhancement of mass transfer from vegetable tissue. *Food Engineering Reviews, 2*(2), 109-130.

Fincan, M., & Dejmek, P. (2002). In situ visualization of the effect of a pulsed electric field on plant tissue. *Journal of Food Engineering, 55*(3), 223-230.

Hills, B., & Duce, S. (1990). The influence of chemical and diffusive exchange on water proton transverse relaxation in plant tissues. *Magnetic resonance imaging, 8*(3), 321-331.

Hills, B., & Remigereau, B. (1997). NMR studies of changes in subcellular water compartmentation in parenchyma apple tissue during drying and freezing. *International Journal of Food Science and Technology, 32*(1), 51-61.

Holz, M., Heil, S., & Sacco, A. (2000). Temperature-dependent self-diffusion coefficients of water and six selected molecular liquids for calibration in accurate 1H NMR PFG measurements. *Physical Chemistry Chemical Physics, 2*(20), 4740-4742.

Jemai, A., & Vorobiev, E. (2002). Effect of moderate electric field pulses on the diffusion coefficient of soluble substances from apple slices. *International journal of food science & technology, 37*(1), 73-86.

Lebovka, N., Bazhal, M., & Vorobiev, E. (2002). Estimation of characteristic damage time of food materials in pulsed-electric fields. *Journal of Food Engineering, 54*(4), 337-346.

Lebovka, N., Praporscic, I., Ghnimi, S., & Vorobiev, E. (2005). Temperature enhanced electroporation under the pulsed electric field treatment of food tissue. *Journal of Food Engineering, 69*(2), 177-184.

Lebovka, N. I., Praporscic, I., & Vorobiev, E. (2004). Effect of moderate thermal and pulsed electric field treatments on textural properties of carrots, potatoes and apples. *Innovative Food Science & Emerging Technologies, 5*(1), 9-16.

Mauro, M., Dellarosa, N., Tylewicz, U., Tappi, S., Laghi, L., Rocculi, P., & Dalla Rosa, M. (2015). Calcium and ascorbic acid affect cellular structure and water mobility in apple tissue during osmotic dehydration in sucrose solutions. *Food Chemistry, 195,* 19-28.

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

Nowacka, M., Tylewicz, U., Laghi, L., Dalla Rosa, M., & Witrowa-Rajchert, D. (2014). Effect of ultrasound treatment on the water state in kiwifruit during osmotic dehydration. *Food Chemistry, 144*, 18-25.

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

Parniakov, O., Lebovka, N., Bals, O., & Vorobiev, E. (2015). Effect of electric field and osmotic pre-treatments on quality of apples after freezing–thawing. *Innovative Food Science & Emerging Technologies, 29*, 23-30.

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.

Sensoy, I., & Sastry, S. (2004). Extraction using moderate electric fields. *Journal of food science, 69*(1), FEP7-FEP13.

Soliva-Fortuny, R., Balasa, A., Knorr, D., & Martín-Belloso, O. (2009). Effects of pulsed electric fields on bioactive compounds in foods: a review. *Trends in Food Science & Technology, 20*(11), 544-556.

Stejskal, E., & Tanner, J. (1965). Spin diffusion measurements: spin echoes in the presence of a time-dependent field gradient. *The journal of chemical physics, 42*(1), 288-292.

Taiwo, K. A., Angersbach, A., & Knorr, D. (2003). Effects of pulsed electric field on quality factors and mass transfer during osmotic dehydration of apples. *Journal of food process engineering, 26*(1), 31-48.

Teissie, J., Eynard, N., Gabriel, B., & Rols, M. (1999). Electropermeabilization of cell membranes. *Advanced drug delivery reviews, 35*(1), 3-19.

Tylewicz, U., Panarese, V., Laghi, L., Rocculi, P., Nowacka, M., Placucci, G., & Dalla Rosa, M. (2011). NMR and DSC water study during osmotic dehydration of Actinidia deliciosa and Actinidia chinensis kiwifruit. *Food biophysics, 6*(2), 327-333.

Van Duynhoven, J., Voda, A., Witek, M., & Van As, H. (2010). Time-domain NMR applied to food products. *Annual reports on NMR spectroscopy, 69*, 145-197.

Vorobiev, E., Jemai, A. B., Bouzrara, H., Lebovka, N., & Bazhal, M. (2005). Pulsed electric field assisted extraction of juice from food plants. *Novel food processing technologies*, 105-130.

Vorobiev, E., & Lebovka, N. (2008). Pulsed-electric-fields-induced effects in plant tissues: fundamental aspects and perspectives of applications. In *Electrotechnologies for extraction from food plants and biomaterials* (pp. 39-81): Springer.

Wiktor, A., Iwaniuk, M., Śledź, M., Nowacka, M., Chudoba, T., & Witrowa-Rajchert, D. (2013). Drying kinetics of apple tissue treated by pulsed electric field. *Drying Technology, 31*(1), 112-119.

Wiktor, A., Śledź, M., Nowacka, M., Chudoba, T., & Witrowa-Rajchert, D. (2014). Pulsed electric field pretreatment for osmotic dehydration of apple tissue: Experimental and mathematical modeling studies. *Drying Technology, 32*(4), 408-417.

Figure 1

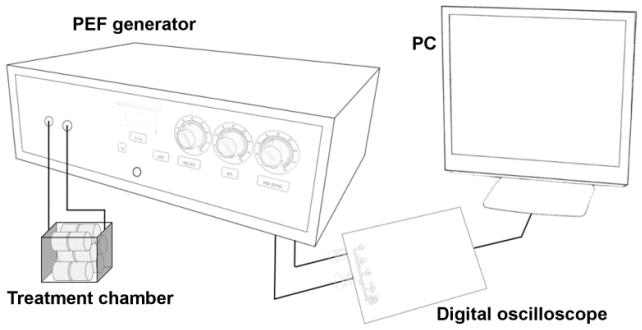

Figure2

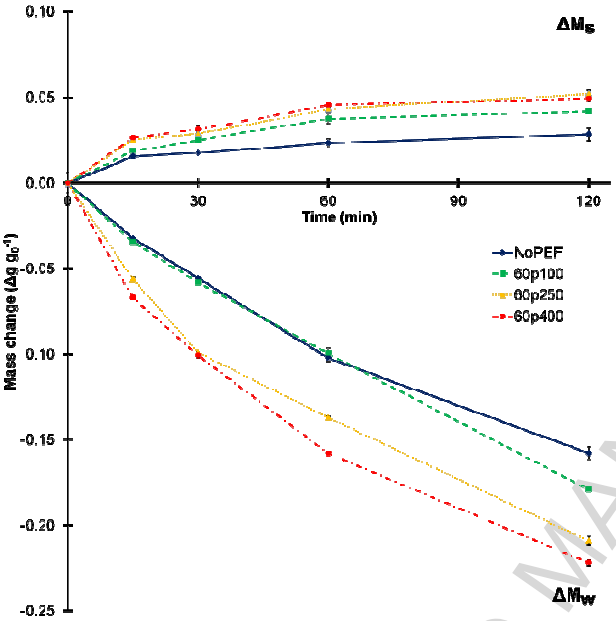

Figure 3

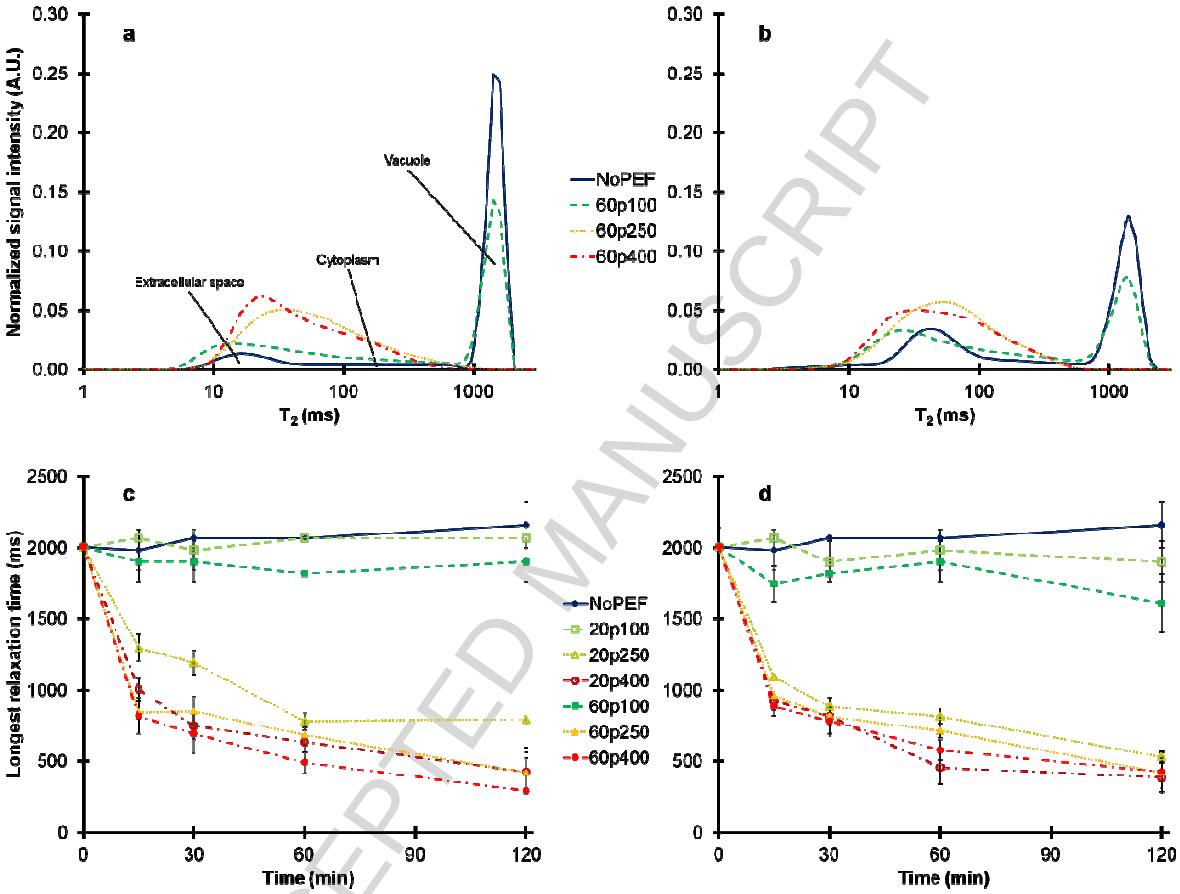

Figure 4

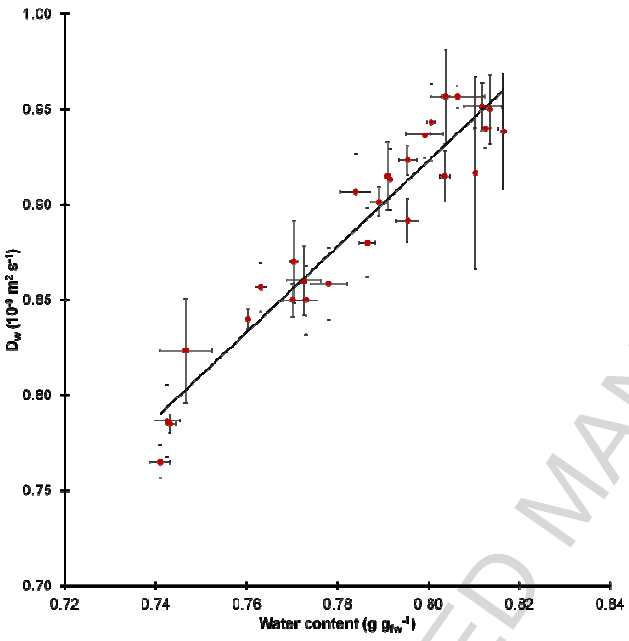

FIGURE CAPTIONS

Fig. 1. Layout of the experimental setup.

Fig. 2. Mass changes in terms of water loss (ΔM_w) and solid gain (ΔM_s) of PEF pre-treated samples with 60 pulses at different voltages throughout 120 minutes. Results are expressed as means ± standard deviation.

Fig. 3. T_2-weighted signal distribution, normalized to unitary area, of PEF pre-treated samples with 60 pulses at different voltages after 120 minutes in isotonic (a) and hypertonic (b) solutions. Longest relaxation time expressed as means ± standard deviation of samples after 120 minutes in isotonic (c) and hypertonic (d) solutions.

Fig. 4. Average ± standard deviation of water self diffusion coefficient versus water content.

Table 1

Experimental plan: sample codes and process parameters.

Sample code	Electric field (V cm^{-1})	Number of pulses (n)	Applied energy to apple (J kg^{-1})
NoPEF	0	0	0
20p100	100	20	8 ± 1
20p250	250	20	55 ± 1
20p400	400	20	135 ± 4
60p100	100	60	23 ± 1
60p250	250	60	164 ± 3
60p400	400	60	382 ± 36

Note: applied energy values are means, expressed as J per kg of fresh product, ± standard deviations (n=3).

Electric field is given as average value for the treatment chamber with electrodes spaced 3 cm.

Table 2

Effect of pulsed electric fields combined with osmotic treatment on water loss and solid gain.

Osmotic treatment	15 min	30 min	60 min	120 min
Water loss				
NoPEF	-0.032 ^{ab} ± 0.001	-0.055 ^{b} ± 0.001	-0.102 ^{b} ± 0.002	-0.158 ^{b} ± 0.004
20p100	-0.022 ^{a} ± 0.003	-0.037 ^{a} ± 0.004	-0.084 ^{a} ± 0.002	-0.144 ^{a} ± 0.002
20p250	-0.030 ^{ab} ± 0.004	-0.081 ^{c} ± 0.002	-0.129 ^{c} ± 0.003	-0.201 ^{d} ± 0.005
20p400	-0.046 ^{cd} ± 0.006	-0.093 ^{d} ± 0.001	-0.146 ^{d} ± 0.002	-0.200 ^{d} ± 0.001
60p100	-0.034 ^{bc} ± 0.001	-0.058 ^{b} ± 0.001	-0.099 ^{b} ± 0.003	-0.179 ^{c} ±0.001
60p250	-0.056 ^{de} ± 0.001	-0.099 ^{d} ± 0.001	-0.137 ^{c} ± 0.001	-0.209 ^{d} ± 0.002
60p400	-0.067 ^{e} ± 0.001	-0.101 ^{d} ± 0.002	-0.158 ^{e} ± 0.001	-0.222 ^{e} ± 0.002
Solid gain				
NoPEF	0.016 ^{a} ± 0.001	0.018 ^{c} ±0.001	0.024 ^{b} ± 0.002	0.028 ^{d} ± 0.004
20p100	0.023 ^{a} ± 0.003	0.036 ^{a} ± 0.004	0.039 ^{a} ± 0.002	0.041 ^{c} ± 0.002
20p250	0.022 ^{a} ± 0.004	0.029 ^{ab} ± 0.002	0.043 ^{a} ± 0.003	0.051 ^{abc} ± 0.005
20p400	0.024 ^{a} ± 0.006	0.031 ^{ab} ± 0.001	0.037 ^{a} ± 0.002	0.054 ^{a} ± 0.001
60p100	0.019 ^{a} ± 0.001	0.025 ^{bc} ± 0.001	0.038 ^{a} ± 0.003	0.042 ^{bc} ± 0.000
60p250	0.025 ^{a} ± 0.001	0.029 ^{ab} ± 0.001	0.043 ^{a} ± 0.001	0.052 ^{ab} ± 0.002
60p400	0.026 ^{a} ± 0.001	0.031 ^{ab} ± 0.002	0.045 ^{a} ± 0.001	0.049 ^{abc} ± 0.002

Results are means expressed as Δg per g_0(time = 0) ± standard deviations and different letters show significant differences ($p < 0.05$) between treatments for each sampling time.

Table 3

Water redistribution in sample treated at 100 V cm^{-1}.

	15 min	30 min	60 min	120 min
Extracellular space				
NoPEF	0.066 ^{b} ± 0.009	0.062 ^{a} ± 0.003	0.082 ^{c} ± 0.010	0.117 ^{b} ± 0.007
20p100	0.097 ^{a} ± 0.010	0.097 ^{a} ± 0.010	0.132 ^{b} ± 0.008	0.250 ^{a} ± 0.022
60p100	0.102 ^{a} ± 0.015	0.107 ^{a} ± 0.005	0.172 ^{a} ± 0.015	0.250 ^{a} ± 0.009
Cytoplasm				
NoPEF	0.231 ^{b} ± 0.018	0.248 ^{a} ± 0.028	0.300 ^{a} ± 0.039	0.307 ^{b} ± 0.046
20p100	0.274 ^{ab} ± 0.041	0.337 ^{a} ± 0.041	0.353 ^{a} ± 0.039	0.329 ^{ab} ± 0.027
60p100	0.338 ^{a} ± 0.048	0.373 ^{a} ± 0.073	0.424 ^{a} ± 0.068	0.493 ^{a} ± 0.107
Vacuole				
NoPEF	0.703 ^{a} ± 0.016	0.690 ^{a} ± 0.031	0.619 ^{a} ± 0.030	0.576 ^{a} ± 0.046
20p100	0.629 ^{ab} ± 0.031	0.565 ^{ab} ± 0.050	0.515 ^{ab} ± 0.047	0.422 ^{ab} ± 0.048
60p100	0.560 ^{b} ± 0.060	0.521 ^{b} ± 0.072	0.404 ^{b} ± 0.053	0.257 ^{b} ± 0.107

The intensity were scaled to have unitary values for each treatment and observation time. Results are means ± standard deviations and different letters show significant differences ($p < 0.05$) between treatments.

Highlights

- Water loss and solid gain were significantly affected by PEF pre-treatment
- TD-NMR with a contrast agent eased the observation of electroporation effects
- Fields strengths higher than 150 V cm^{-1} caused the loss of compartmentalization
- Water was redistributed from vacuole towards extracellular space at 100 V cm^{-1}
- D_w and the longest T_2 could be used to control OD and PEF effects, respectively