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Effect of pulsed electric field (PEF) pre-treatment coupled with osmotic dehydration on physico-chemical characteristics of organic strawberries

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Highlights

- Organic strawberries were used to obtain semi-dried osmodehydrated products.
- PEF prior OD positively affected the mass transfer even at the lowest intensity.
- At low PEF (100 V cm^{-1}) cell viability was partially preserved.
- Sucrose and trehalose solutions exerted a similar effects on the studied parameters.

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Effect of pulsed electric field (PEF) pre-treatment coupled with osmotic dehydration on physico-chemical characteristics of organic strawberries

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Abstract

The aim of this work was to evaluate the effect of pulsed electric field (PEF) pre-treatment on mass transfer phenomena, water distribution and some physico-chemical parameters of osmo-dehydrated organic strawberries. For PEF treatments 100 near-rectangular shaped pulses, with fixed pulse width of 100 µs and repetition time of 10 ms were used. Electric fields strength applied were 100, 200 and 400 V cm^{-1}. Afterwards, samples were subjected to OD treatments carried out in two different hypertonic solutions (40% w/w), one with sucrose and the other one with trehalose. The results shown that PEF treatment positively affected the mass transfer during OD even at the lowest electric field strength applied (100 V/cm), partially preserving the cell viability and maintaining at the same time the fresh-like characteristics of strawberries.

Keywords: fruit quality, strawberries, organic, texture, colour, non-thermal treatment

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1. Introduction

Increased consumer demand for safety, health and environmental friendly food products make the organic production one of the fastest growing market segments over the last few years. Consumers expect the quality of organic fruits to be higher or at least comparable with the conventionally produced ones, protecting at the same time the nature and reducing the environmental pollution (Barański et al., 2014).

Berries, and in particular strawberries, are very attractive for consumers, because of their unique flavour, texture and red vivid colour, both in a fresh form and in a variety of food products and snacks. They are also highly appreciated by consumers due to their high amount of ascorbic acid and antioxidants (Velickova et al., 2013; Gamboa-Santos et al., 2014). However strawberries are highly susceptible to mechanical injury and also highly perishable (Badawy et al., 2016; Kadivec et al., 2016); these characteristics could be even more pronounced in the organic fruit. Therefore, there is a need to improve the processing of these fruits in order to obtain semi-dried or intermediate moisture products with longer shelf-life. With regards to organic production practices, applied treatments and processes should be aimed at avoiding the chemical additives, while non-thermal processing are used with the aim of maintaining the nutritional and sensorial properties of food products.

Osmotic dehydration (OD) is one of the non-thermal processes used to obtain intermediate moisture products with improved stability over storage. This because, during OD a partial dewatering of plant tissue takes place, reducing both freezable water content and the water activity of the system (Tylewicz et al., 2011; Mauro et al., 2016). The application of OD process on strawberry tissue has been widely studied. Chang et al. (2014) studied the effect of power ultrasound and pulsed vacuum treatments on the dehydration kinetics and the status of water during osmotic dehydration of strawberries, showing that the highest water loss (lower freezable water content) and the highest decrease in firmness occurred using ultrasound treatment, while the highest solid gain and the highest firmness values were achieved by pulsed vacuum treatment. Castelló et al. (2010) observed that OD treatment promoted the structural collapse, however, when calcium was added to the osmotic solution a beneficial effect on the maintenance of the sample texture was observed.

Since OD treatment, especially when applied at room temperature, is a time-requiring process, other pretreatments could be used before OD in order to increase the velocity of mass transfer kinetics.

Pulsed electric filed is a process which promotes the modification of the membrane permeability by application of high voltage short time pulses (Barba et al., 2015). The application of low electric field strength creates pores in the biological membrane which affect the mass transfer in tissues. In fact, several studies of PEF-assisted OD have been carried out on different plant tissues such as apples (Dellarosa et al., 2016a; Dellarosa et al., 2016b; Amami et al., 2006), kiwifruits (Dermesonlouoglou et al., 2016; Traffano-Schiffo et al., 2016), carrots (Amami et al., 2007), potatoes (Fincan & Dejmek, 2003) etc. While the effect of PEF pre-treatment on enhancing the water loss of OD treated tissues seems to be clearly and well stated, its effect on the solid gain is ambiguous. In fact, some authors reported an increase in solid uptake, for example in mango pieces (Taiwo et al., 2002) and apples (Amani et al., 2006; Dellarosa et al., 2016a), while in PEF pre-treated kiwifruit samples the solutes uptake was lower compared to untreated ones (Traffano-Schiffo et al., 2016). The impact of high-intensity electric field pulses on the mass transfer and on some physical characteristics (leaching of cell constituents, colour and texture) of strawberry halves during osmotic dehydration (OD) has been studied (Taiwo et al., 2003). Higher water loss was obtained in samples treated with a high-intensity electric field before OD. Moreover, the application of PEF before OD minimized changes in product colour and allowed to retain product compactness.

To the best of our knowledge, this is the first work aimed to the evaluation of the effect of PEF+OD low temperature processes on the mass transfer phenomena and water redistribution of strawberry tissue. Moreover, the changes in some quality parameters of treated strawberries from organic production were evaluated.

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2. Materials and Methods

2.1. Raw material handling

Organic strawberries (*Fragaria+ananassa)* var ''Alba'' (10 ± 1 °Brix) were purchased from the local market in Cesena (Italy). The strawberries were stored at 4 ± 1 ºC at high relative humidity until use, for no longer than one week. Before processing, fruits were tempered at 25 ºC, washed, hand stemmed and cut into rectangular shape pieces of the dimension 5 x 10 x 20 mm (height x width x length).

2.2. Pulsed electric field (PEF) treatment

Two rectangular pieces (approximately 1.3 g) were placed into a rectangular treatment chamber equipped with two stainless steel electrodes (20 x 20 mm^2) with a gap between them of 30 mm and filled with 5 mL of a sodium chloride solution with the same conductivity as the strawberries (1.6 mS/cm). The PEF treatments were applied to the strawberry samples at 25°C using an in-house developed pulse generator equipment based on MOSFET technology that delivers near-rectangular shape pulses.

PEF pre-treatments were carried out by applying a train of 100 pulses at three different pulsed electric field (E) strength (100, 200 and 400 V cm^{-1}), a fixed pulse width of 100 µs and a repetition time of 10 ms (100 Hz). The procedure setting was chosen on bases of preliminary experiments.

2.3. Osmotic dehydration (OD) treatment

The OD treatment was carried out by immersing the strawberry samples in 40 % (w/w) hypertonic solutions. Two different solutions were prepared, one with sucrose and one with trehalose dissolved in distilled water. Calcium lactate (CaLac) at a concentration of 1 % (w/w) was added to both the solutions as a structuring agent. The treatment was performed at 25 ºC with continuous stirring maintaining a fruits:OD solution ratio of 1:4 (w/w) that allowed to avoid significant changes in the solution concentration during the whole treatment (data not shown).

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The samples were analyses at different treatment times: 0, 15, 30, 60 and 120 min.

Both PEF and OD procedures were repeated twice for each solution.

All obtained samples are summarised with related abbreviations as reported in table 1.

Table 1

2.2. Analytical determinations

2.4.1. Mass transfer phenomena

Mass transfer phenomena during osmotic dehydration of strawberry samples was evaluated by calculating weight reduction (WR, kg kg^{-1}), water loss (WL, kg kg^{-1}) and solutes gain (SG, kg kg^{-1}) adopting the following equations:

$$WR = \frac{m_t - m_0}{m_0} \quad (1)$$

$$WL = \frac{m_t x_{wt} - m_0 x_{w0}}{m_0} \quad (2)$$

$$SG = \frac{m_t x_{STt} - m_0 x_{ST0}}{m_0} \quad (3)$$

where:

m_0 - initial weight before osmotic treatment (kg)

m_t - weight after a time t (kg)

x_{w0}- initial water mass fraction (kg · kg^{-1})

x_{wt} - water mass fraction after a time t (kg · kg^{-1})

x_{ST0} – initial total solids (dry matter) mass fraction (kg · kg^{-1})

x_{STt} – total solids (dry matter) mass fraction after a time t (kg · kg^{-1})

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Moisture content was determined gravimetrically by drying the samples at 70°C until a constant weight was achieved (AOAC, 2002).

2.4.2. Water distribution by TD-NMR measurements

In order to measure the proton transverse relaxation time (T_2), strawberry cylinders of about 250 mg (h = 10 mm, d = 8 mm) were cut with a core borer. The samples were placed inside 10 mm outer diameter NMR tubes, in order to not exceed the active region of the radio frequency coil, and analyzed at 25 °C with the CPMG pulse sequence (Meiboom & Gill, 1958) using a 'The Minispec' spectrometer (Bruker Corporation, Germany) operating at 20 MHz. Each measurement comprised 4000 echoes over 16 scans, with an interpulse spacing of 0.3 ms and a recycle delay set at 10 s. The specified parameters, chosen to prevent sample and radio frequency coil overheat, allowed the observation of the protons with T_2 higher than a few milliseconds. According to the protocol set up by Panarese et al. (2012), the CPMG decays were analyzed with the UPEN software (Borgia et al., 1998), which inverts the CPMG signal using a quasi-continuous distribution of exponential curves, and through fittings to the sum of an increasing number of exponential curves. Furthermore, a multi exponential discrete fitting was successively applied to accurately determine T_2 and relative intensities of the water populations (Mauro et al., 2016). The experiment was conducted in triplicate at each treatment condition.

2.4.3. Cell viability test by Fluorescein diacetate (FDA) staining

The cell viability test was performed on 1 mm-thick strawberry slices, cut with a sharp scalpel, using fluorescein diacetate (FDA, Sigma-Aldrich, USA, λ_{ex} = 495 nm, λ_{em} = 518 nm), as described by Tylewicz et al. (2013) with some modifications. Strawberry slices were incubated for 5 min in a solution containing FDA (10^{-4} M) and sucrose in isotonic concentration (10 %, w/w) in the darkness at room temperature. The dye used in the experiment can passively penetrate the protoplast and then it is

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hydrolysed by cytoplasmic esterases, producing the polar product named fluorescein that only the viable cells are able to accumulate intracellularly, because it is unable to cross cellular membranes that remain intact (Mauro et al., 2016). Hence, viable cells could be easily identified by a bright fluorescence. Observations were performed under a fluorescent light in a Nikon upright microscope (Eclipse Ti-U, Nikon Co, Japan) equipped with a Nikon digital video camera (digital sight DS-Qi1Mc, Nikon Co, Japan) at a magnification of 4 ×.

2.4.4. Colour

The colour changes of fresh, PEF pre-treated and osmodehydrated samples were investigated using a spectro-photocolorimeter mod. Colorflex (Hunterlab, USA). The measurements were made using CIE $L^*a^*b^*$ scale. The instrument was calibrated with a black and white tile (L^* 93.47, a^* 0.83, b^* 1.33) before the measurements. Moreover, the hue angle (h°) parameter was calculated using the following equation:

$$h° = tan^{-1}\frac{b^*}{a^*} \quad (4)$$

where: a* (red–green) and b* (yellow–blue) are parameters of color measurement (Vega-Gálvez et al., 2012).

The analysis were conducted in twelve repetitions for randomly selected strawberry samples for each PEF pre-treatment and osmotic dehydration condition.

2.4.5. Texture analysis

Firmness (N) was evaluated by performing a penetration test on strawberry rectangular pieces using a TA-HDi500 texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5 N load cell. A stainless steel probe of 2 mm diameter was used and rate and depth of penetration were of 1 mm/s and 95 %, respectively. The analysis were performed in twelve replicates.

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2.5. Statistical Analysis

Significance of the PEF treatment and OD effects was evaluated by one-way analysis of variance (ANOVA, 95% significance level) and comparison of means by Duncan test at a 5% probability level using the software STATISTICA 6.0 (Statsoft Inc., Tulsa, UK).

3. **Results and discussion**

3.1. Mass transfer phenomena

The kinetics of water loss and solid gain during OD are shown in Figure 1 and Figure 2 for sucrose and trehalose solutions, respectively. Figure 1 shows also the effect of the different electric field strength applied on water loss and solid gain during osmotic dehydration of strawberries immersed in sucrose-based solution. Samples subjected to the PEF pre-treatment presented a significantly higher water loss compared to the untreated strawberry samples.

Figure 1

An improvement of water loss upon PEF pre-treatment has already been observed by Taiwo et al. (2003) on strawberries (1200 V cm^{-1}) and by Traffano-Schiffo et al. (2016) on kiwifruit (up to 400 V cm^{-1}). The acceleration of the kinetics of water and solids transfer is due to the effect of permeabilization of the cell membranes induced by the PEF treatment (Amani et al., 2006; Barba et al., 2015). In the present study, the application of the lowest electric field intensity (100 V cm^{-1}) resulted already sufficient to increase the water loss by 12 % after one hour of osmotic dehydration. This result is in contrast with those obtained by Dellarosa et al. (2016), who observed that the treatment with 100 V cm^{-1} did not have any effect on mass transfer of apple cylinders during the OD conducted for 60

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min. This difference could probably be explained by the different microstructure of strawberries which resulted in a different sensitivity to the electric field strength. In addition, it needs to be mentioned that, due to both the different conductivity of samples/media and the higher number of delivered pulses, the energy input applied to the strawberry samples (123 J kg^{-1}) was much higher compared to the one delivered to the apples (8 J kg^{-1}). The initial mass transfer rate in PEF treated samples was faster compared to the untreated one, proportionally to the PEF intensity. Although at the end of the osmotic treatment the samples treated at 250 and 400 V cm^{-1} did not differ significantly, in agreement with Traffano-Schiffo et al. (2016). As reported by various authors (Ade-Omowaye et al., 2003; Angersbach et al., 2002; Dellarosa et al. 2016a), PEF effects can be considered time-dependent and the formation of pores and their growth in the membrane are not immediate but continue for several minutes after the treatment. This highlights the importance of taking into account the time elapsed from the application of pulsed electric fields before any other treatment in order to optimize PEF application in a combined multi step manufacturing process.

Similarly to water loss, solid gain was favoured by the application of PEF. After 120 min of OD, the solid gain was about 4 % in the strawberry untreated tissue, while PEF pre-treated sample reached a 5–6 % gain, in agreement with the results of Dellarosa et al. (2016a).

The lower enhancement of solid gain compared to the water loss has already been observed by Ade-Omowaye et al., (2003), that attributed this result to the higher molecular size of solutes compared to water and to a selective membrane permeabilization that favour dewatering rather than solute diffusion through the tissue.

The SG and WL behaviours of strawberry samples dehydrated in the trehalose-based solution were similar (Fig.2). However, water loss in trehalose-based solution was characterized by a higher initial rate compared to the treatment in the sucrose solution but by a lower final dehydration level. At the end of the treatment, the samples treated at 200 and 400 V cm^{-1} reached the highest WL of about 50 %.

Figure 2

Interesting results were observed for solid gain. Up to 120 min, only the treatment with the lowest electric field strength caused a higher solid gain compared to the untreated sample, while the treatment at 200 and 400 V cm^{-1} reduced the trehalose uptake due to a lower initial mass transfer rate. Generally thought, samples treated at 400 V cm^{-1} showed a noticeably lower solids impregnation. Trehalose is known to exert a protective effect on cell membranes during drying or freezing (Ferrando & Spiess, 2001; Atarés et al., 2008), thanks to its ability to form hydrogen bonds with the biomolecules that allows to stabilize cells and tissues preserving viability and structures (Vicente et al. 2012). In the present study, the combination of PEF with trehalose allowed to obtain a higher dewatering effect without increasing solute uptake or even reducing it.

This could be considered a positive effect if you want to increase the stability of a perishable organic product while maintaining/considering its nutritional properties.

3.2. Water redistribution upon treatments

Osmotic dehydration itself, generally, promotes important changes in cellular structure of different plan tissues, that can affect the water mobility and its distribution through different parts of the cellular tissue (Tylewicz et al., 2011; Panarese et al., 2012; Mauro et al., 2016). TD-NMR permitted to separately observe two main water populations located in vacuoles and cytoplasm plus extracellular spaces of strawberry tissue that corresponded to the relaxation time (T_2) of 1139.82 ± 129.56 and 251.24 ± 23.51, respectively. During OD treatment it was possible to observe the decrease of the signal intensity related to the water protons located in the vacuole throughout 120 min. As a consequence, the shrinkage of vacuole led to the increase of the intensity of the water protons belonging to the cytoplasm and extracellular space, as shown in the way of example for the sucrose treated samples in figure 3a.

Figure 3

Results are in agreement with those reported by Cheng et al. (2014), who studied the effect of water-osmotic solute exchange on the strawberry cell compartments (vacuole, cytoplasm plus intercellular space, and cell wall) subjected to the ultrasound and vacuum assisted OD treatment in sucrose solution. The authors also observed that, upon OD treatments, the relative space occupied by the vacuole decreased while the one occupied by the cytoplasm and intercellular space increased. In other fruits such as kiwifruit (Tylewicz et al., 2011; Panarese et al., 2012) and apples (Mauro et al., 2016) similar behaviour on water distribution was observed, confirming the migration of water from the inner compartments toward the external ones.

Figure 3b shows the effect on water distribution due to the application of PEF on the strawberry tissue before immersion in the hypertonic solution. The electroporation induced by the treatment led to a loss of compartmentalization that is highlighted by the merging of the two proton populations into a single one. This effect was more pronounced when applied E was increased from 100 to 400 V cm^{-1}.

Dellarosa et al. (2016) studied the water distribution in apple tissue subjected to PEF treatments at similar voltages and determined a no-reversibility threshold at around 150 V cm^{-1} with 60 train pulses. In the present study even the lower voltage applied (100 V cm^{-1}) seemed to promote a collapse of the cellular structures although less markedly compared to the higher voltages. As mentioned above, this discrepancy could be explained by the higher energy input applied in the present experiment and the different sensitivity of strawberry tissue to the field strength in comparison with apples.

Figure 3c illustrates mean T_2 values of the water populations throughout 120 min of the osmotic treatment. As expected, this value decreased during OD due to the water removal and the different water-solutes-biopolymers interaction. Indeed, the water that is leaving the tissue during OD is characterized by high mobility, hence with long T_2. Therefore, a marked decrease of T_2 values, from

755 ± 60 ms to 478 ± 89 ms, for untreated strawberries was observed. Interestingly, each applied electric field strength also showed values spanning in the range 390-500 ms, immediately after PEF treatment. Such results might not be attributed to the different water content, but to the dissimilar water-solutes-biopolymers induced by the loss of compartmentalization within the strawberry tissue. In addition, similarly to control trends, T_2 values continued to decline during the whole duration of the osmotic dehydration process, so when water was also removed. These results, in accordance with mass transfer data, demonstrated that OD efficiency could be highly influenced by PEF pre-treatments which eased the diffusion of inner water by markedly affecting the permeability of membranes.

The samples dehydrated in trehalose-based solution (data not shown) followed a similar trend as the samples dehydrated in sucrose. Probably the marked effect of PEF contributed to hide the effect of different solutes used for dehydration.

3.3. Cell viability test by Fluorescein diacetate (FDA) staining

Figure 4 presents images of strawberry tissue after the PEF treatment followed by staining with FDA in order to investigate the possible loss of cell viability.

Indeed, the creation of pores in the cell membrane, through the phenomenon of electroporation, which is a function of temperature, intensity of the applied electric field, number of pulses, pulse shape, type of tissue etc. (Buckow et al., 2013), may lead to irreversible damages causing loss of cell viability.

In order to determine the threshold of irreversible electroporation, Dellarosa et al. (2016 b) measured the metabolic heat production and the respiration rate of apple cylinders subjected to 100, 250 and 400 V cm^{-1}. The authors found that the medium and the high applied voltages promoted a drastic loss of cell viability that was attributed to the irreversible damages of the membranes. On the other hand, the tissue treated with 100 V/cm showed metabolic indexes comparable to the fresh tissue indicating that the electroporation was only reversible and did not cause loss of cell viability. In the present experiment, although cell viability was not completely lost, strawberry samples treated with an intensity of the electric field strength of 100 V cm^{-1}, showed residual cell viability, also if much lower than the fresh sample intensity, as shown in figure 4. The increase of the electric field strength induced a greater structural damage, as found in samples treated at 200 and 400 V cm^{-1} where there was a complete loss of cell viability. Consequently, cell viability was maintained even after 120 min of osmotic treatment of untreated samples (data not shown). The preservation of cell viability was observed also by Mauro et al. (2016) after 120 min in 40 % of sucrose solution. In the Mauro's study when 30% sucrose + 3 % of calcium lactate was used the cell viability was also preserved, while increasing quantity of calcium lactate up to 4% in 40% of sucrose compromised the cell viability. However, in the present study, only 1 % of calcium lactate was used, therefore this parameter was not affected by OD process, but just by PEF pre-treatment. Moreover, the PEF treated samples at 100 V cm^{-1} partially preserved their viability also after OD process (data not shown), while samples treated with higher E were not further investigated, due to the viability loss following PEF treatment.

Therefore, with the aim of increasing the shelf-life of an organic product, characterized by quality parameters as close as possible to the fresh one, the lowest electric field strength applied in the tested range seems to be the suitable.

Figure 4

3.4. Colour

Table 2 shows the L* and hue angle (h°) values of untreated and PEF treated strawberry tissues subjected to osmotic dehydration for 120 min in both solutions. L* parameter of untreated samples did not change during the whole OD treatment. Similar results were obtained by Nuñez-Mancilla et al. (2013) who did not notice any variation of the L* parameter in strawberry samples subjected to the OD process, while this parameter was influenced significantly by the application of high hydrostatic pressure.

The luminosity of the samples resulted to be affected by the electric field intensity. In fact, this parameter increased significantly after the application of PEF at the intensity of 100 V cm^{-1}, while decreased due to the application of PEF at highest field intensity. Also Wiktor et al. (2015) observed that the colour measurement showed unchanged or lower L* value of PEF treated samples at E=1.85 kV cm^{-1} and E=3 or E=5 kV cm^{-1}, respectively, in comparison with the untreated apple tissue. The darkening of the PEF treated samples at 400 V cm^{-1} could be related to the higher release of enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) and their substrates after the electroporation of the strawberry cells membrane. In fact, Chisani et al. (2007) observed that the browning of the strawberry fruit during the storage was related to both oxidase activities. However, after 120 min of OD treatment the PEF treated samples increased their L* values, which was significantly higher in comparison to untreated ones.

Table 2

Since the colour of strawberries is the mixture of red and yellow, the hue angle (h°) was also calculated and its values are reported in table 2, respectively for strawberries treated in sucrose and trehalose solution. In general OD treatment promoted a decrease of this parameter. The application of PEF promoted a further decrease of hue angle in comparison with untreated samples, which was proportional to the electric field strength applied, at least in samples dehydrated in sucrose solution. Similar results were observed by Osorio et al. (2007). The reduction of h° colorimetric parameter could be due to both solubilisation of pigments in the osmotic solution and degradation of anthocyanin induced by PEF-treatment (Fathi et al., 2011; Odriozola-Serrano et al., 2008). In samples dehydrated

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in trehalose non significant differences were observed among PEF-treated samples, if not for the samples treated by 100 V cm^{-1} at 30 min after OD that showed a significantly lower h° value compared to the others. Wiktor et al. (2015) observed that the effect of PEF treatment strongly depends on the raw material properties and the treatment conditions. In fact, the authors noticed the different behaviour of carrot and apple tissue subjected to electric field strength at different intensities. In both cases browning of the tissue was observed, however in carrots it was more pronounced when the low voltage treatment was applied, while in apple with high voltage.

3.5. Texture

It is well known that OD induces plasmolysis, shrinkage of the vacuole compartment, changes in size and structure of the cell walls of outer pericarp and dissolution of the middle lamella, which could be translated in decreasing of the firmness of the plant tissue (Chiralt & Talens, 2005; Panarese et al. 2012). The changes of firmness of untreated and PEF treated strawberry tissue subjected to OD treatment up to 120 min in sucrose-based solution is shown in Figure 5. OD of untreated samples promoted a decrease of strawberry firmness, already 15 min after the treatment, and increased slightly during the OD treatment. In the present experiment, PEF pre-treatment drastically reduced the hardness of strawberry samples; further, the PEF treated samples remained below the untreated ones during the whole OD process and the effect was proportional to the electric field strength applied. Also Taiwo et al. (2003) observed the decrease in firmness of strawberries halves treated with PEF (1200 V cm^{-1}; 350 μs) and then osmodehydrated for 4 hours in binary (sucrose, NaCl) solution. The reduction of firmness of PEF treated samples could be due to the alteration of the membrane permeability due to the pores creation and the rupture of internal structure, which promotes the softening of the tissue (Fincan & Dejmek, 2002; Wiktor et al., 2016).

Figure 5

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Figure 6

The slight increase of the texture observed after longer OD times could be probably due to the penetration of Ca^{2+} into the strawberry tissue. The structural role of calcium ions in the cell wall is due to their interaction with pectic acid polymers to form cross-bridges that reinforce the cell adhesion, thereby reducing cell separation, which is one of the major causes of plant tissue softening (Van Buggenhout et al., 2008; Mauro et al., 2016). This increase has not been observed in the samples treated at 400 V cm^{-1}, probably because the tissue was already completely disintegrated after the PEF treatment, and did not permit the incorporation of calcium ions in the cell walls.

Similar results were observed in strawberries samples dehydrated in trehalose-based solution (Fig. 6). However, considering that the firmness of the material (0.8 ± 0.1 N) used for the experiment was almost half compared with the value relative to the raw material used in the experiment with sucrose (1.35 ± 0.2 N), the decrease of firmness following the OD process was less marked. In fact, the firmness of samples dehydrated in trehalose decreased only by 36 % in comparison to 57 % of decrease observed in sucrose dehydrated samples already 15 min after the treatment. This behaviour could probably be due to the protective effect of trehalose on the tissue structure, as reported by Phoon et al. (2008). The intensity of the applied electric field strength seems to be not so relevant in comparison to samples dehydrated with sucrose. Shayanfar et al. (2013) observed texture softening and loss of turgor in frozen/thawed potatoes after the PEF treatment. However, when CaCl2 and trehalose were added to the liquid medium used in PEF treatment, the samples maintained their firmness when compared to solely PEF treated samples.

4.Conclusions

PEF treatment prior to osmotic dehydration was found to positively affect the mass transfer, in term of water loss from the strawberry tissue. The application of the lowest electric field intensity (100 V cm^{-1}) resulted already sufficient to increase the water loss by 12 % and 6%, after one hour of osmotic dehydration, respectively for strawberries dehydrated in sucrose and trehalose solution, partially preserving the cell viability and maintaining at the same time the fresh-like characteristics of fruits. Concerning the solid gain results, while the solid gain was favoured by the application of all the PEF intensities in samples dehydrated in sucrose solution, the treatment at 200 and 400 V cm^{-1} reduced the trehalose uptake due to a lower initial mass transfer rate.

In most of the cases, the PEF effect on different strawberry characteristics investigated was proportional to the electric field strength applied.

TD-NMR results showed that the diffusion of inner water was eased by PEF application because of a marked effect on membranes permeability.

Although similar effects on the investigated parameters were observed by using sucrose or trehalose solutions, the combination of PEF with trehalose allowed to obtain a higher dewatering effect without increasing solute uptake or even reducing it.

Definitely, the application of the lower field intensity and the use of trehalose for the dehydration process, seem to be the optimal combination for obtaining a semi-dried strawberry product with quality characteristics similar to the fresh one, that is a fundamental requirement for an organic production.

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Figure Captions

Figure 1. Solid gain and water loss of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in sucrose-based solution.

The same letter on the same column means no significant difference between the samples by the Duncan test ($p < 0.05$).

Figure 2. Solid gain and water loss of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in trehalose-based solution.

The same letter on the same column means no significant difference between the samples by the Duncan test ($p < 0.05$).

Figure 3. T_2 -weighted signal distribution, normalized to unitary area, of OD samples with sucrose (a) and sample immediately after PEF pre-treatments (b). Mean transverse relaxation time (T_2) values ± standard deviation PEF pre-treated and control strawberries during 120 min from immersion into the sucrose solution (c).

Figure 4. Microscopy images of fresh strawberry tissue and after the PEF treatment followed by staining with FDA.

Figure 5. Firmness (N) of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in sucrose-based solution.

The same letter on the same column means no significant difference between the samples by the Duncan test ($p < 0.05$).

Figure 6. Firmness (N) of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in trehalose-based solution.

The same letter on the same column means no significant difference between the samples by the Duncan test ($p < 0.05$).

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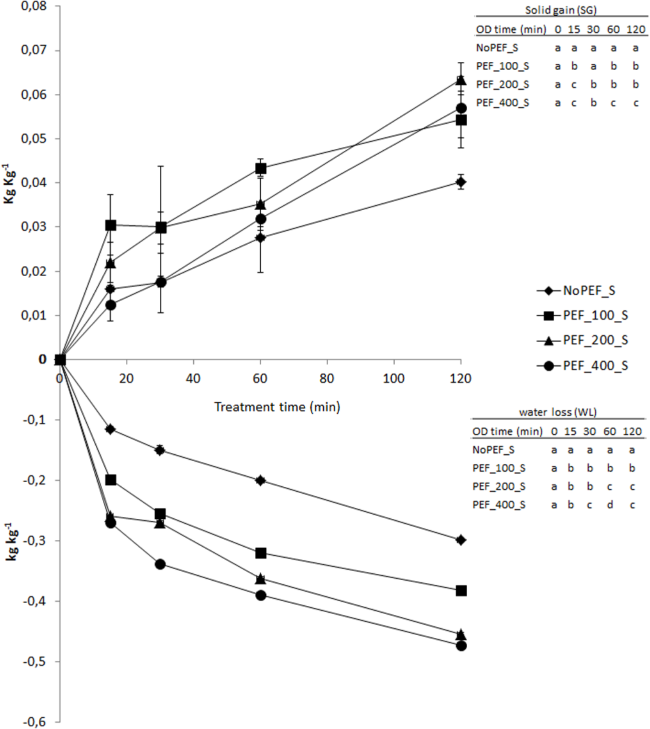
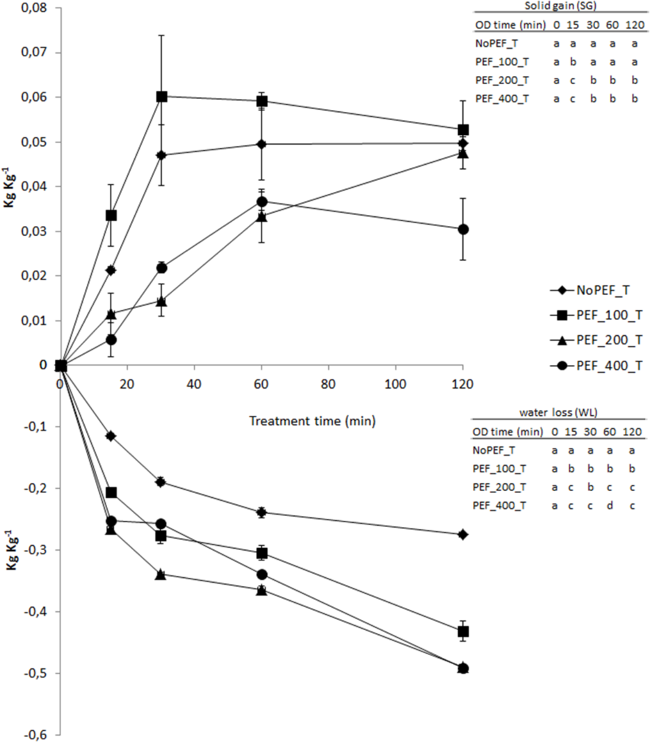
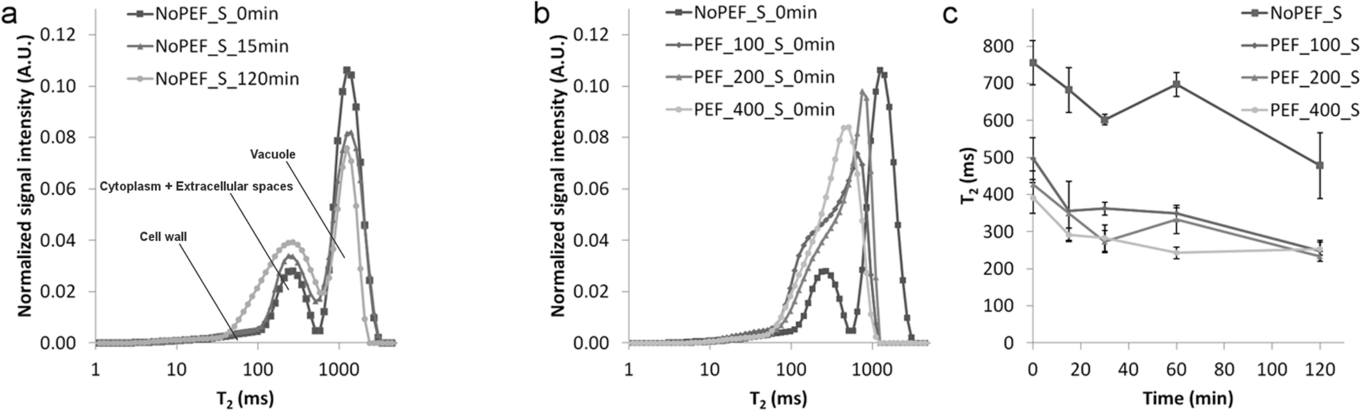


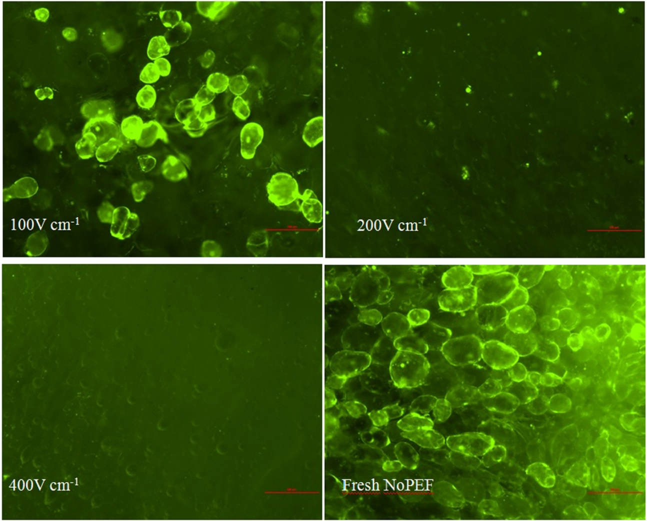
Solid gain (SG)
OD time (min) 0 15 30 60 120
NoPEF_S a a a a a
PEF_100_S a b a b b
PEF_200_S a c b b b
PEF_400_S a c b c c
Kg Kg-1
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
NoPEF_S
PEF_100_S
PEF_200_S
PEF_400_S
0 20 40 60 80 100 120
Treatment time (min)
water loss (WL)
OD time (min) 0 15 30 60 120
NoPEF_S a a a a a
PEF_100_S a b b b b
PEF_200_S a b b c c
PEF_400_S a b c d c
kg kg-1
-0,1
-0,2
-0,3
-0,4
-0,5
-0,6

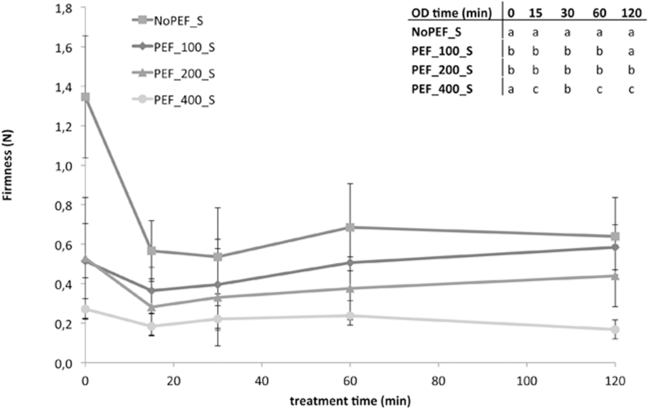


Solid gain (SG)
OD time (min) 0 15 30 60 120
NoPEF_T a a a a a
PEF_100_T a b a a a
PEF_200_T a c b b b
PEF_400_T a c b b b
water loss (WL)
OD time (min) 0 15 30 60 120
NoPEF_T a a a a a
PEF_100_T a b b b b
PEF_200_T a c b c c
PEF_400_T a c c d c
NoPEF_T
PEF_100_T
PEF_200_T
PEF_400_T
Kg Kg-1
Kg Kg-1
Treatment time (min)
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
-0,1
-0,2
-0,3
-0,4
-0,5
-0,6
0
20
40
60
80
100
120



a
NoPEF_S_0min
NoPEF_S_15min
NoPEF_S_120min
Vacuole
Cytoplasm + Extracellular spaces
Cell wall
Normalized signal intensity (A.U.)
T2 (ms)
b
NoPEF_S_0min
PEF_100_S_0min
PEF_200_S_0min
PEF_400_S_0min
Normalized signal intensity (A.U.)
T2 (ms)
c
NoPEF_S
PEF_100_S
PEF_200_S
PEF_400_S
T2 (ms)
Time (min)


100V cm-1
200V cm-1
400V cm-1
Fresh NoPEF


NoPEF_S
PEF_100_S
PEF_200_S
PEF_400_S
OD time (min) | 0 | 15 | 30 | 60 | 120
NoPEF_S | a | a | a | a | a
PEF_100_S | b | b | b | b | a
PEF_200_S | b | b | b | b | b
PEF_400_S | a | c | b | c | c
Firmness (N)
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120
treatment time (min)

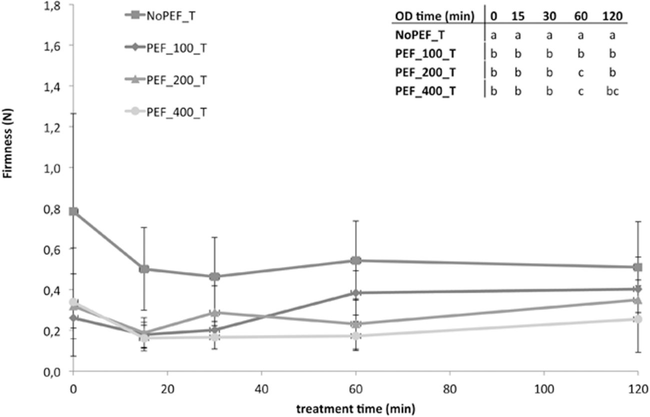

NoPEF_T
PEF_100_T
PEF_200_T
PEF_400_T
OD time (min) 0 15 30 60 120
NoPEF_T a a a a a
PEF_100_T b b b b b
PEF_200_T b b b c b
PEF_400_T b b b c bc
Firmness (N)
treatment time (min)
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120

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Table 1. Codification of analysed samples

Sample code	Electric field ($V\ cm^{-1}$)	Type of solution
NoPEF_S	0	Sucrose
PEF_100_S	100	Sucrose
PEF_200_S	200	Sucrose
PEF_400_S	400	Sucrose
NoPEF_T	0	Trehalose
PEF_100_T	100	Trehalose
PEF_200_T	200	Trehalose
PEF_400_T	400	Trehalose

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Table 2. Colour parameters (L* - Lightness, h° - hue angle) of untreated and PEF pre-treated strawberry samples, as a function of the osmotic dehydration time in both sucrose and trehalose solutions.

OD Time	0 min	15 min	30 min	60 min	120 min
			L*		
NoPEF_S	35 ± 4 b	32 ± 6 b	40 ± 6 a	38 ± 3 bc	37 ± 4 de
PEF_100_S	42 ± 4 a	38 ± 5 ab	38 ± 3 ab	42 ± 3 a	45 ± 5 a
PEF_200_S	35 ± 1 b	35 ± 2 b	34 ± 2 bc	39 ± 2 ab	42 ± 2 ab
PEF_400_S	26 ± 2 c	42 ± 2 a	34 ± 2 bc	35 ± 2 cd	41 ± 2 abc
NoPEF_T	35 ± 4 b	37 ± 6 ab	36 ± 5 abc	37 ± 5 bc	34 ± 5 e
PEF_100_T	41 ± 4 a	35 ± 6 ab	33 ± 2 c	35 ± 3 cd	35 ± 4 ce
PEF_200_T	28 ± 3 c	30 ± 1 c	34 ± 2 bc	33 ± 2 d	39 ± 3 cd
PEF_400_T	27 ± 2 c	37 ± 4 ab	33 ± 3 c	35 ± 3 cd	38 ± 2 cde
			h°		
NoPEF_S	40 ± 2 a	36 ± 4 a	36 ± 2 a	35 ± 1 a	35 ± 2 a
PEF_100_S	35 ± 2 c	29.9 ± 0.9 b	29 ± 2 cd	29 ± 2 b	29 ± 2 c
PEF_200_S	38 ± 2 ab	29 ± 1 b	31 ± 2 bc	28 ± 1 b	25 ± 3 de
PEF_400_S	35 ± 4 bc	24 ± 1 c	27 ± 3 de	24 ± 1 c	23 ± 2 e
NoPEF_T	40 ± 1 a	37 ± 2 a	38 ± 1 a	33 ± 1 a	32.1 ± 0.7 b
PEF_100_T	35 ± 2 bc	30 ± 2 b	24 ± 2 e	24 ± 5 bc	26 ± 2 d
PEF_200_T	34 ± 3 d	28 ± 1 b	27 ± 1 d	25.5 ± 0.8 c	23 ± 2 e
PEF_400_T	36 ± 2 bc	28 ± 2 b	32 ± 3 b	28 ± 1 b	24 ± 2 e

The same letter on the same column means no significant difference by the Duncan test ($p < 0.05$).

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