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Abstract

This research aims at evaluating the effect of pulsed electric field (PEF) pre-treatment before osmotic dehydration (OD) on physiological changes in organic strawberries and kiwifruits, in terms of metabolic heat production measured by isothermal calorimeter and of tissue damage evaluated by fluorescence microscopy, texture analysis and electrolytes leakage. Fruits pre-treated at two electric field strengths (100 and 200 V/cm) using 100 near-rectangular shaped pulses (pulse width: 10 μs, repetition time: 10 ms) were subjected to OD in hypertonic solutions (40% w/w) of sucrose or trehalose, both with addition of 1 % of calcium lactate. Results showed that OD alone allowed to retain the functionality of the membranes causing only a decrease in the endogenous heat production. The application of low electric field strength (100 V/cm) generally preserved the cell viability, which was drastically reduced following OD treatment. On the contrary, the application of 200 V/cm caused tissue damage and loss of cell vitality, probably due to irreversible electroporation.

Industrial application

PEF could be an interesting pre-treatment for reducing time and energy necessary for the osmotic dehydration of fruits. However, it is important to understand the implication of the treatment on the tissue metabolism and structure in order to control the effect on the quality of the final product. This study provides some useful information that could be exploited for the industrial production of intermediate moisture fruit products.

Keywords: PEF; OD; metabolic response; texture; organic fruits

1. Introduction

Since the mid-1990s the market for organic foods has been expanding rapidly and, among these products, organic fruit and vegetables have been growing the fastest. The worldwide total area under organic fruit production by 2015 has been recorded as 288 K hectares and 375 K hectares for temperate (including strawberry) and subtropical/tropical (including kiwifruit) fruits, respectively (Willer & Lernoud, 2016). This could be attributed to the increased consumer demand for safe, healthy and environmentally friendly food products, with high quality characteristics.

When the organic strawberries and kiwifruits are intended for processing in semi-dried products with longer shelf-life, but with fresh-like characteristics, the applied treatments should be chemical additives free, while the retention of nutritional and sensorial properties could be achieved by application of non-thermal processing (Tylewicz et al., 2017).

Osmotic dehydration, which consists in partial dewatering of the fruits by the action of osmotic pressure differences between the products and solutions, is often coupled with other non-thermal technologies such as ultrasound and pulsed electric fields (PEF) in order to accelerate the mass transfer and make the process faster (Dermesonlouoglou, Zachariou, Andreou, & Taoukis, 2016; Nowacka, Tylewicz, et al., 2018; Nowacka, Tylewicz, Romani, Dalla Rosa, & Witrowa-Rajchert, 2017; Traffano-Schiffo et al., 2017; Tylewicz et al., 2017).

PEF is a non-thermal technology, which leads to electroporation of the cell membrane by applying an external electric field to the cellular tissue. The electric field could range from 100-600 V/cm (Tylewicz et al., 2017; Phoon et al., 2008) to 20–80 kV/cm, depending on the desired effect to be obtained (Barba, Galanakis, Esteve, Frigola, & Vorobiev, 2015). The PEF application could promote the reversible or irreversible permeabilization, depending on both the intensity of the electric field strength applied and the characteristics of the raw materials. Reversible electroporation, which means that cell membranes are able to recover their structure and functionality after removing the electrical field, is used to assure the survival of the electrically stimulated cells. The irreversible permeabilization instead, will cause permanent membrane damage and consequently the cell death (Donsì, Ferrari, & Pataro, 2010).

Considering fruit to be a biologically active tissue, it is of extreme importance to evaluate the effect of different technologies on the tissue metabolic response and cell integrity and vitality. The metabolic response of the fruit tissue has been widely studied by application of isothermal calorimetry (Dellarosa et al., 2016; Dymek et al., 2016; Nowacka, Fijalkowska, et al., 2018; Panarese, Tylewicz, Santagapita, Rocculi, & Dalla Rosa, 2012; Panarese et al., 2014; Tappi et al., 2017; Yusof, Wadsö, Rasmusson, & Gómez Galindo, 2017). This technique is based on the measurements of heat production by the plant tissue, due to different physical, chemical and biological reactions (Rocculi

et al., 2012). Osmotic dehydration promoted the reduction of metabolic heat in kiwifruits (Nowacka, Tappi, et al., 2018; Panarese et al., 2012), and apples (Tappi et al., 2017), as a consequence of a partial loss of vitality. PEF technology has been also reported to reduce the metabolic heat production in apples (Dellarosa et al., 2016); however, when PEF was coupled with vacuum impregnation using trehalose solution an increase in metabolic heat production was observed in spinach leaves (Dymek et al., 2016). The decrease in metabolic heat production of different plant tissues could be due to the loss of cell viability as a consequence of different treatments (Mauro et al., 2016; Nowacka, Fijalkowska, et al., 2018; Tylewicz et al., 2017).

To follow the changes in cell structure integrity and membrane permeability, the measurement of electrolyte leakage by changes in conductivity and texture is often used. Electrolyte leakage is used to assess the degree of permeability and integrity of the membranes, while texture measurements reflect the loss of turgor pressure, softening of the cell walls or loss of the cellular tissue integrity (Ersus & Barrett, 2010; Faridnia, Burritt, Bremer, & Oey, 2015; Lebovka, Praporscic, & Vorobiev, 2004).

The aim of this work was to evaluate the effect of pulsed electric field (PEF) pre-treatment before osmotic dehydration (OD) on physiological changes in organic strawberry and kiwifruit tissues. The effect on metabolism was examined assessing endogenous heat production by isothermal calorimetry, cell viability by fluorescence microscopy, cell membrane integrity by electrolytes leakage and changes in mechanical properties.

2. Materials and Methods

2.1. Raw material handling

Strawberries (*Fragaria+ananassa)* var. *Alba* (9.8 ± 0.5 °Brix, 91 ± 0.7 % of water content) and kiwifruits (*Actinidia Deliciosa*) var. *Hayward* (12 ± 1 °Brix, 83 ± 2 % of water content) from organic farming were purchased from the local market in Cesena (Italy). The fruits were stored at 4 ± 1 ºC and high relative humidity for no longer than one week. The strawberries were washed, hand stemmed and cut into half along the central axis of the fruit. The kiwifruits were washed, peeled and cut into slices of 10 mm, which were further divided into four triangular pieces.

2.2. Pulsed electric field (PEF) treatment

Approximately 35 g of samples, corresponding to six strawberry halves and to eight-ten triangular kiwifruit pieces, were placed, separately for each fruit species, into a parallel plate treatment chamber equipped with two stainless-steel electrodes with a gap between them of 4.7 cm and filled with a

sodium chloride solution with the conductivity of 1.6 mS/cm (measured by EC-Meter basic 30+, Crison). This conductivity value was chosen as the average conductivity for strawberry and kiwifruit samples. The PEF treatments were applied by using a lab-scale pulse generator S-P7500 60A 8kV (Alintel srl., Bologna). Treatment conditions were selected on the basis of previous experiments (Tylewicz et al., 2017; Traffano-Schiffo et al., 2016, 2017) that allowed to discriminate between reversible and irreversible electroporation for both fruit tissues. Two different electric field strengths were selected (100 and 200 V/cm) and 1,000 rectangular pulses of fixed 10 µs width at the frequency of 100 Hz were applied (**Fig. 1**). The total treatment time was of 10 s. Moreover, a specific energy input was calculated, as suggested by Raso et al. (2016), showing the values of 0.96 and 1.92 kJ/kg respectively for samples treated at 100 and 200 V/cm.

2.3. Osmotic dehydration (OD) treatment

The OD treatment was carried out by immersing the strawberry and kiwifruit samples, separately, in 40 % (w/w) sucrose or trehalose solutions. Sucrose was selected as the most commonly used sugar for OD, while trehalose was selected on account of its ability to protect cell membrane from physical stresses (Crowe et al., 2001). To both solutions, 1 % (w/w) of calcium lactate (CaLac) was added as a structuring agent (Tylewicz et al., 2017). The treatment was performed at 25 ºC with continuous stirring maintaining a fruits: OD solution ratio of 1:4 (w/w) in order to avoid changes in the solution concentration during the 120 min of treatment.

All obtained samples with related abbreviations are reported in **Table 1**.

2.4. Analytical determinations

2.4.1. Metabolic activity by isothermal calorimeter (TAM)

The metabolic activity of samples was evaluated on the basis of heat production measured by isothermal calorimetry. For each sample, six cylinders (ø 5 mm) with a total weight of about 3 g were obtained from the central part of kiwifruit slices and of strawberry halves and were placed in 20 mL glass ampoule, sealed with a teflon coated rubber seals and an aluminium crimp cap. For each sample, two replicates for three independent treatments were analysed (in total six replicates for each sample). A TAM air isothermal calorimeter (TA Instruments, New Castel, USA) with a precision of ±10 µW was used to measure the heat production. Water was chosen as reference material; its quantity was calculated according to Panarese, Laghi, et al. (2012). The analysis was carried out at 10 °C for 20 h and baseline (30 min) was recorded before and after each measurement. Specific thermal powers ($mW \cdot g^{-1}$) were calculated according to Gómez Galindo, Wadsö, Vicente, & Dejmek (2008). The average metabolic heat production was calculated by integrating the metabolic heat profiles. The first

4 h of analysis was excluded in order to prevent the influence of the initial disturbance due to sample loading and conditioning (Dellarosa et al., 2016), hence values reported refer to 16 h at 10 °C. In order to verify the effect of dehydration on metabolic heat production, water content was determined gravimetrically by drying the samples at 70 °C until a constant weight was achieved (AOAC, 2002).

2.4.2. Cell viability

The cell viability test was performed using fluorescein diacetate dye (FDA, Sigma-Aldrich, USA, kex = 495 nm, kem = 518 nm) as reported by Tylewicz et al. (2017). For each sample, 1-2 mm thick slices were incubated for 5 min in a 10^{-4} M FDA prepared in isotonic sucrose solution and then rinsed in distilled water. Then the images were examined 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 10. Viable cells could be easily identified by a bright fluorescence. For each sample a total of 8-10 different images were collected.

2.4.3. Electrolytes leakage

Electrolytes leakage was evaluated by changes in the conductivity of the solution. To perform the analysis two pieces of strawberries (or kiwifruit) for each sample (about 10g), were placed in solution with distilled water in a proportion of 1: 4 and kept in constant agitation at room temperature (25 ± 1 °C). The electrolyte content was determined with the measurement of the electrical conductivity at room temperature with a Conductivity meter (EC-Meter basic 30+, Crison), immediately (T0), after three hours of stirring (T3h) and after ten minutes of boiling (TC), to ensure the complete cell disintegration and removal of all electrolyte substances from the sample immersed in water.

2.4.4 Texture analysis and texture disintegration index

Firmness (N) of strawberry halves and kiwifruit slices was evaluated at room temperature (~ 20 °C) performing a penetration test using a TA-HDi500 Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell. The test was completed using a stainless-steel probe of 8 mm diameter and penetrating the samples for 90% of depth. Analysis were performed in 24 replicates and results expressed as means of maximum force values.

Mechanical properties changes can be used as an indirect measure of PEF-induced cell disruption, as extensively mentioned in literature studies (Fincan & Dejmek, 2003; Wiktor et al., 2018). In order to characterise the extent of tissue damage, the texture disintegration index (TDI) was calculated as follows:

$$Z_t = (F - F_d)/(F_i - F_d)$$

where F is the maximum penetration force measured, and subscripts i and d refers to the values of intact and completely damaged tissue, respectively (Lebovka & Vorobiev, 2017). This equation gives a range of Z_t from 0 for intact tissue to 1 for complete disrupted tissue. Complete damaged tissue was obtained by subjecting samples to a freezing-thawing cycle.

2.4.5. Statistical Analysis

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

3. Results

3.1. Endogenous heat production

Fresh fruit tissues produce heat and CO_2 by consuming O_2 as a direct result of respiratory activity. However, heat production can be altered by various types of tissue damage, as reported in various studies (Wadsö et al., 2004; Rocculi et al., 2012; Tappi et al., 2017). This parameter also represents an indirect index of cell viability, useful to understand if the applied treatments have determined the loss of the tissues viability and the consequences associated with it. In the present study, the metabolism of the examined samples was characterized through the quantity of endogenous heat produced by the tissues after the different applied treatments.

As an example, **Fig. 2** shows calorimetric signals obtained by strawberries samples after the different applied treatments.

Considering only the first 20 hours, the production of heat due to microbial growth on the sample can be negligible, therefore the recorded metabolic heat can be attributed exclusively to the endogenous metabolism of the tissue, the sum of the basal and the one due to the "wounding response" or the reaction of the tissues to damage due to the cut and the treatments used (Wadsö, Gomez, Sjöholm, & Rocculi, 2004).

Table 2 reports the total specific heat production of kiwifruit samples subjected to the different treatments compared to the fresh one. Fresh samples showed a heat production of 2.22 J/g, that is remarkably lower compared to the one measured by Panarese, Tylewicz, Santagapita, Rocculi, & Dalla Rosa (2012). However, measurements were carried out at a higher temperature (20°C) compared to the present study, explaining in part the difference in the results. Moreover, the physiological state of the tissue can have great influence on the gross metabolism. OD with both sucrose and trehalose promoted only a slight reduction of the heat production, that did not

significantly differ from the fresh tissue, despite the significant reduction in water content of around 12 and 8% for sucrose and trehalose respectively. The effect of PEF, as expected, depended on the applied voltage. 100 V/cm did not alter the metabolic heat production of the tissues, while 200 V/cm promoted a strong reduction of it. For both treatments, no reduction of water content was observed. The combination of PEF with OD promoted a decrease of the metabolic heat production. However, due to the high variability of the data, average values were not significantly different compared to samples treated with only OD or PEF. In the samples dehydrated after the 100 V/cm treatment, water content was not significantly further reduced compared to the only application of OD, while the higher electric field strength allowed to increase dehydration of 22-23% compared to the fresh sample. The near absence of metabolic heat was instead observed following the combination of PEF treatments at 200 V/cm combined with OD using trehalose.

Table 3 reports the total specific heat production of strawberries samples subjected to the different treatments compared to the fresh one. The specific heat production of fresh strawberries was 5.29 J/g. As observed for kiwifruit, despite the significant water loss due to OD of around 13 and 11% for sucrose and trehalose respectively, this value was only slightly decreased upon OD with both sugars. After the application of the lowest PEF voltage, no significant difference was found, while the application of 200 V/cm led to a strong reduction of heat production of the tissue, without any change in water content, similarly to kiwifruit.

When OD and PEF were combined, in the case of the lowest voltage, a further reduction of this parameter was observed; while in the 200 V/cm treated samples, heat produced was even lower compared to the 100 V/cm treatment, with no significant differences between the use sugars. with regards to the water content, the application of 100 V/cm in combination with OD resulted in an increase of dehydration only when trehalose was used (around 17%), while the higher treatment allowed to increase water loss (around 18%) with both sugars.

3.2. Cell viability

Cell viability was assessed using the fluorescence dye fluorescein diacetate (FDA), which is able to actively penetrate through the cell membrane, where it is hydrolyzed to a fluorescent compound. This polar compound is accumulated intracellularly in the viable cells and is no longer able to cross the intact membrane. Therefore, if the membrane functionality is preserved, the cell will be characterized by fluorescent green coloration (Saruyama et al., 2013). **Fig. 3** shows the representative photos of kiwifruit samples stained with FDA subjected to the different treatments compared to the fresh one. It can be observed that the samples treated with OD alone did not compromise cell viability. The effect of PEF, as expected, depended on the applied voltage: 100 V/cm did not alter the cell viability,

while 200 V/cm promoted the complete loss of fluorescence in the considered tissue. When the lower PEF treatment and OD with both sugars, were combined, the viability was not completely compromised but was only partially retained. As expected, when 200 V/cm and OD were combined no viable cells were observed.

Fig. 4 shows the representative photos of strawberries samples stained with FDA subjected to the different treatments compared to the fresh one. The cell viability of strawberry samples was similar to the fresh one, when lower PEF voltage and OD were used singularly, while the application of 200 V/cm field led to the loss of cell viability, as observed in kiwifruits. When OD and PEF were combined, in the case of the lowest voltage, a better cell viability preservation was observed for samples treated with trehalose compared to sucrose, while in the 200V/cm treated samples, cell viability was completely compromised.

3.3. Electrolyte leakage

Table 2 reports the values of electrolyte leakage of kiwifruit samples subjected to the different treatments compared to the fresh one.

Unfortunately, the results obtained for the kiwifruit were not satisfactory. Fresh samples already showed a very high electrolyte leakage of about 52 %, and the PEF treatment decreased or retained these values respectively for the 100V/cm and the 200V/cm treated samples.

In general, it was not possible to observe a real trend in the behaviour of the electrolytes losses. Samples treated with the combination of PEF and OD showed, instead, an opposite behaviour; in fact the samples treated with trehalose presented a higher EL at the end of the process in comparison to the samples treated with sucrose. However, the values of EL in 200 V/cm treated samples were significantly higher than those of samples treated at 100 V/cm, even if both were significantly lower in comparison to the samples treated with only OD. The high inhomogeneity in ripening degree of the samples used could have determined alterations in the permeability of cell membranes (Antunes & Sfakiotakis, 2008).

The loss of electrolytes in the strawberry samples is shown in **Table 3.** The fresh sample presented the lowest value of EL. The PEF treatement alone seemed to slighlty increased the EL but differences were not significant. The OD treatment with both sugars increased the EL by about 15 %. In combined treatments, a further reduction in the ability to retain the solutes by the samples and the consequent increase of released electrolytes was observed, which was proportional to the intensity of the PEF treatment applied but only in the case of sucrose.

3.4. Texture

Firmness values of kiwifruit and strawberry samples subjected to the different treatments compared to the fresh one are reported in table 2 and 3, respectively.

In kiwifruit, after OD with sucrose a slight decrease was observed, while values were unchanged when trehalose was used. The application of PEF promoted a strong reduction of tissue firmness, proportional to the applied voltage. When the two treatments were combined, a further reduction occurred after the 100 V/cm PEF treatment using both sugars, while values were very low and unchanged after the 200 V/cm treatment.

For strawberries, a similar trend was observed. However, the effect of the 100 V/cm treatment seemed to affect the tissues less than kiwifruit. Firmness values were not lower compared to the fresh sample, however, combining OD with the 100 V/cm PEF treatment resulted in a significant reduction of firmness.

Firmness values have been used to calculate the TDI, as an index of tissue disruption after PEF treatments. Values are reported in table 2 and 3, for kiwifruit and strawberries, respectively. In both fruits, TDI was proportional to the voltage applied, although they were generally lower in strawberries. For the 200 V/cm treatment, TDI reached 0.934 and 0.818 in kiwifruit and strawberries respectively. Changes in structural properties depended on both the applied voltage and the type of sugar used for OD treatment. In fact, kiwifruits better retained firmness at lower electric field (100 V/cm) when combined with trehalose, while strawberries showed better results when OD treatment with sucrose solution was used for both electric fields applied.

4. Discussion

Since the physiological response of a tissue to a treatment is very complex, in this study various parameters have been assessed in order to understand the effect of OD, PEF and their combination on two different fruit tissues.

The effect of OD on fruit tissue metabolism has been studied by different authors. Castelló, Fito, & Chiralt (2010), Moraga, Moraga, Fito, & Martínez-Navarrete (2009) and Torres, Castelló, Escriche, & Chiralt (2008) found that osmotically dehydrated fruits were characterized by a lower respiration rate, but a higher respiratory quotient indicating the onset of anaerobic metabolism, as a consequence of the removal of oxygen from the tissues. Mauro et al. (2016) observed that the use of sucrose solution at different concentration (20%, 30% and 40%) did not alter the viability of apple cells after 2 h. Similar results were observed by Nowacka et al. (2018) for kiwifruit and by Tylewicz et al. (2017) for strawberry samples. In the first study the kiwifruit samples were subjected to the OD treatment in sucrose at 61.5 %, while in the second one the strawberry samples were treated in

305 solutions of sucrose and trehalose both at 40 %. The cell viability was maintained in both fruits after
306 the sole OD treatment.
307 However, a reduction of metabolic heat production upon osmotic dehydration has been observed for
308 kiwifruit and apple tissues (Panarese et al., 2012; Tappi et al., 2017). Moreover, the addition of
309 calcium to the osmotic solution was shown to further decrease the tissue metabolic activity that was
310 attributed to a decrease of respiration rate the causes of which are still not completely understood
311 (Lester, 1996; Luna-Guzmán et al., 1999; Castelló et al., 2010; Tappi et al., 2017).
312 Some authors (Blum and Tuberosa, 2018; Mavroudis et al., 2004) reported that the cell survival
313 depends on the water status and content of the products, showing a progressive loss of the cell survival
314 with the increase of the dehydration rate.
315 In the present study, for both fruits, OD allowed to maintain cell viability and did not seem to alter in
316 a significant way the metabolism of the tissues, despite the decrease of water content in OD treated
317 samples (table 2 and 3). Mavroudis et al. (2004) suggests that death in the outer layer of apples occurs
318 when the osmotic medium concentration is 50% or above, which is higher than the one used in the
319 present research. However, the duration of the process and the structural properties of the considered
320 tissue also have an influence on the survival rate. Panarese et al. (2012) observed that the
321 physiological response to OD is influenced also by the ripening degree.
322 Electrolyte leakage generally increased after OD in both sugar solutions, however these differences
323 were significantly different only in kiwifruit samples treated with sucrose This may indicate that,
324 although cell viability is fully maintained, a partial damage of cell membranes occurred, more
325 pronounced in kiwifruit compared to strawberries. In general, when fruits are subjected to the osmotic
326 dehydration several phenomena could take place, like plasmolysis, shrinkage of the vacuole, changes
327 in the structure of the cell walls among others, which could cause the softness of the tissue (Panarese
328 et al., 2012). However, while in kiwifruit samples dehydrated in sucrose, this was also reflected in a
329 slight loss of firmness, in samples dehydrated using trehalose textural parameters were unaffected.
330 Concerning the strawberry tissue, the trend was similar as for the kiwifruit samples, however, no
331 statistically significant differences were found in texture after OD treatment. Also Tylewicz et al.
332 (2017) observed a less marked changes in the texture of strawberry samples dehydrated in trehalose
333 in comparison to those in sucrose solution, probably due to the protective effect of trehalose on the
334 cellular structure (Velickova et al., 2013).
335 The study of Tylewicz et al. (2017) also showed that the PEF treatment itself caused the cell death
336 only when the field strength of 200 V/cm or higher were used, while lower intensities (100 V/cm)
337 allowed to preserve the tissue viability, indicating that the process was reversible and that cell
338 membranes were able to retain their structure and functionality after the treatment. Ersus & Barrett

339 (2010) studied the effect of PEF at different intensities on cellular integrity of onion cells by staining
340 them with neutral red dye, which is able to color the intact vacuoles. They observed that at the electric
341 field strength applied (167 V/cm), regardless the pulse number used (10 or 100), no cell rupture
342 appeared. They suggested that probably the applied electric field was lower than the critical electric
343 field strength, which is necessary to rupture the cell membranes.
344 Fincan & Dymek (2002) developed a method to visualize single permeabilized cells in onion tissue
345 upon PEF treatment showing how their distribuion in the tissue is not homogeneous and that a time
346 scale of internal transport and mixing exists because of the heterogenicity of the permeabilized tissue.
347 However, the effect of PEF on vegetable tissue metabolism is less known. Dellarosa et al. (2016)
348 characterized the effect of PEF treatments on the metabolism of apple tissues; results showed that
349 while 100 V/cm field strength allowed to maintain cell viability, it determined an increase in the
350 production of heat compared to the fresh sample. A similar effect was also observed for strawberries
351 in the present study. According to the authors, this effect may have been caused by a tissue response
352 to the stress generated by the electroporation of membranes; the increased heat production reflected
353 the energy expended by the tissue to compensate for reversible changes in the membrane. According
354 to Gomez-Galindo (2016), the transient permabilization of the membrane and the struggle of the cells
355 to recover normal functionality promote changes on cell metabolism and tissue properties.
356 According to Gómez Galindo, Wadsö, Vicente, & Dejmek (2008), the formation of pores and their
357 resealing, caused by reversible electroporation, the induced physiological response in the tissue
358 involves the oxygen consuming pathway and may last up to several hours after the treatment. On the
359 other side, no effect was observed in kiwifruit.
360 In the study of Dellarosa et al. (2016), operating at process field strength of 250 V/cm and 400 V/cm,
361 promoted damages to the apple cells that caused their death bringing the metabolic heat production
362 very close to zero. In the present study, cell metabolism was evaluated by calorimetric measurements
363 and microscopic observations. It has to be noted that the evaluation of cell viability by FDA analysis
364 is limited to a restricted area of the whole bulk, and can therefore only give partial information that
365 should be considered complementary to the calorimetric results. Indeed, although a complete loss of
366 viability was observed by fluorescent microscopy in sample treated at 200 V/cm, a residual, even if
367 drastically reduced, metabolic heat production was observed. These results indicate that the field
368 strength applied lead to a strong reduction of cell viability in the tissue, probably due to irreversible
369 electroporation.
370 This hypothesis was confirmed by the EL in strawberries and by the texture measurements. Ion
371 leakage in strawberries increased proportionally to the EF applied. Faridnia, Burritt, Bremer, & Oey
372 (2015) investigated the ion leakage from potato tissue following PEF treatment at different intensities

and duration. They observed a higher ion leakage when electric field streght of 1.1 kV/cm was used, while the application of 0.4 kV/cm did not cause any changes in this parameter, demonstrating that higher field strengths caused greater cell disruption.

Similarly, a loss of firmness proportional to the applied electrical field was observed. The TDI index in samples treated at 200 V/cm clearly indicates an extensive cell structures breakdown, more pronounced in kiwifruit in which it was very close to the tissue subjected to freezing-thawing.

The combination of PEF and OD showed some interesting results. Metabolic heat production was reduced for both fruit after reversible electroporation (100 V/cm treatment) and the viability of cell appeared partially preserved for both tissues, in agreement with Tylewicz et al. (2017). While for kiwifruit, as stated previously, EL measurements were not clear, in strawberries, an increase of this parameter indicated a higher membrane disuption proportional to the applied field strength. Indeed, although the 200 V/cm itself seemed to have caused irreversible electroporation, the ion leakage was further increased when samples were subjected to osmosis. Similarly, texture disintegration index increased significantly when PEF and OD were combined, indicating a more pronounced disruption of membrane.

However, comparing the effect of the 2 considered sugars, contrasting results were found. Specifically, during OD trehalose seemed to be able to further reduce metabolic heat production and to better maintain samples structure with respect to sucrose. Trehalose is a non-reducing disaccharide that has shown the ability of reducing damages on biological systems during freezing and thawing (European Patent Application, 1999) and in general in dry conditions (Crowe et al., 2001). This effect has been observed on both animal (Doygan et al., 2017) and vegetable (Phoon et al., 2008) tissues. For this reason, OD with trehalose has been used as a pre-treatment before drying improving characteristics of the rehydrated products (Aktas, Yamamoto, & Fujii, 2004). Nevertheless, ion leakage seemed to increase when using trehalose, while texture showed different results but without a clear trend in relation to both the applied filed strength and the type of fruit tissue. Therefore, the effect of the sugar used should be better clarified.

5. Conclusions

The application of PEF prior to OD is used with the main aim of increasing mass transfer rate. Results showed that the reversible electroporation obtain after the 100 V/cm treatment, effectively increased water loss only in strawberries dehydrated with trehalose, while the irreversible electroporation was effective for all samples.

405 The application of OD alone to organic strawberries and kiwifruits allowed to preserve the
406 functionality of the cell membranes causing only a slight decrease in the endogenous heat production
407 and a higher electrolyte leakage.
408 The application of the lower strength (100 V/cm) PEF generally did not alter the metabolic and
409 structural indexes, although it seemed to promote a physiological reaction in strawberry tissue
410 evidenced by an increase of metabolic heat production. The combination with OD led to a further
411 decrease in metabolic heat production and an increase of textural breakdown. On the contrary, the
412 application of 200 V/cm PEF caused total loss of cell vitality and tissue breakdown, probably due to
413 the irreversibility of the electroporation. For a possible industrial application, it would be important
414 to evaluate the effect of the increased textural breakdown on the final products quality, also in relation
415 to the intended use (e.g. minimally processed product, further drying, freezing ecc)
416 Further clarifications are needed on the effect of the substitution of sucrose with trehalose.
417
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420
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554 **Figure captions**

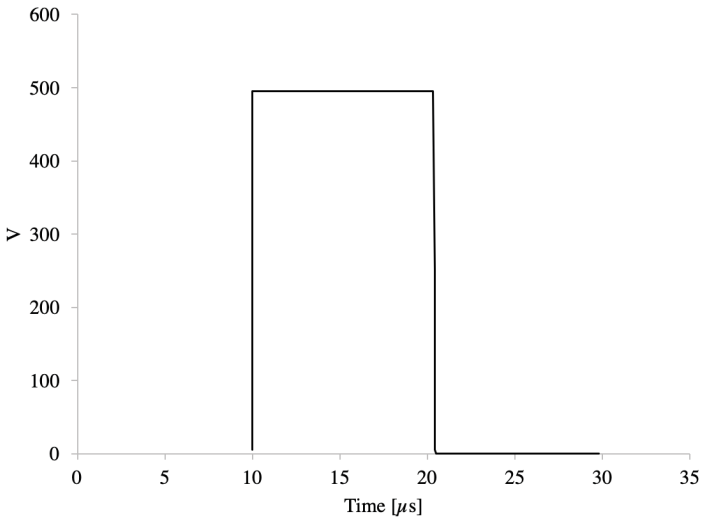
555 **Fig. 1** Representative pulses applied at two different electric field strengths (100 V/cm and 200 V/cm)

556 **Fig. 2** Example of calorimetric signals obtained from strawberries as fresh (F), after OD with sucrose
557 (OD-S), after PEF treatment at 100 (PEF100) and 200 (PEF200) V/cm, and after the combined
558 treatments (PEF100 OD-S; PEF200 OD-S),

559 **Fig. 3** Representative photos of kiwifruit samples stained with FDA subjected to the different
560 treatments compared to the fresh one.

561 **Fig. 4** Representative photos of strawberries samples stained with FDA subjected to the different
562 treatments compared to the fresh one.

563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V
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10
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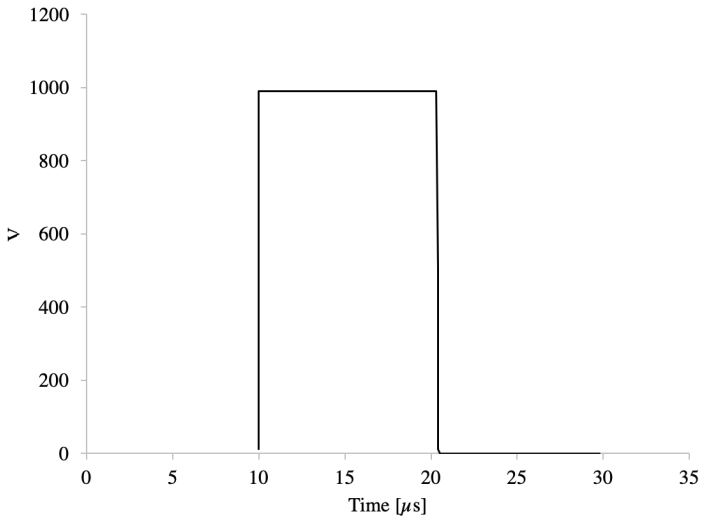
Time [μs]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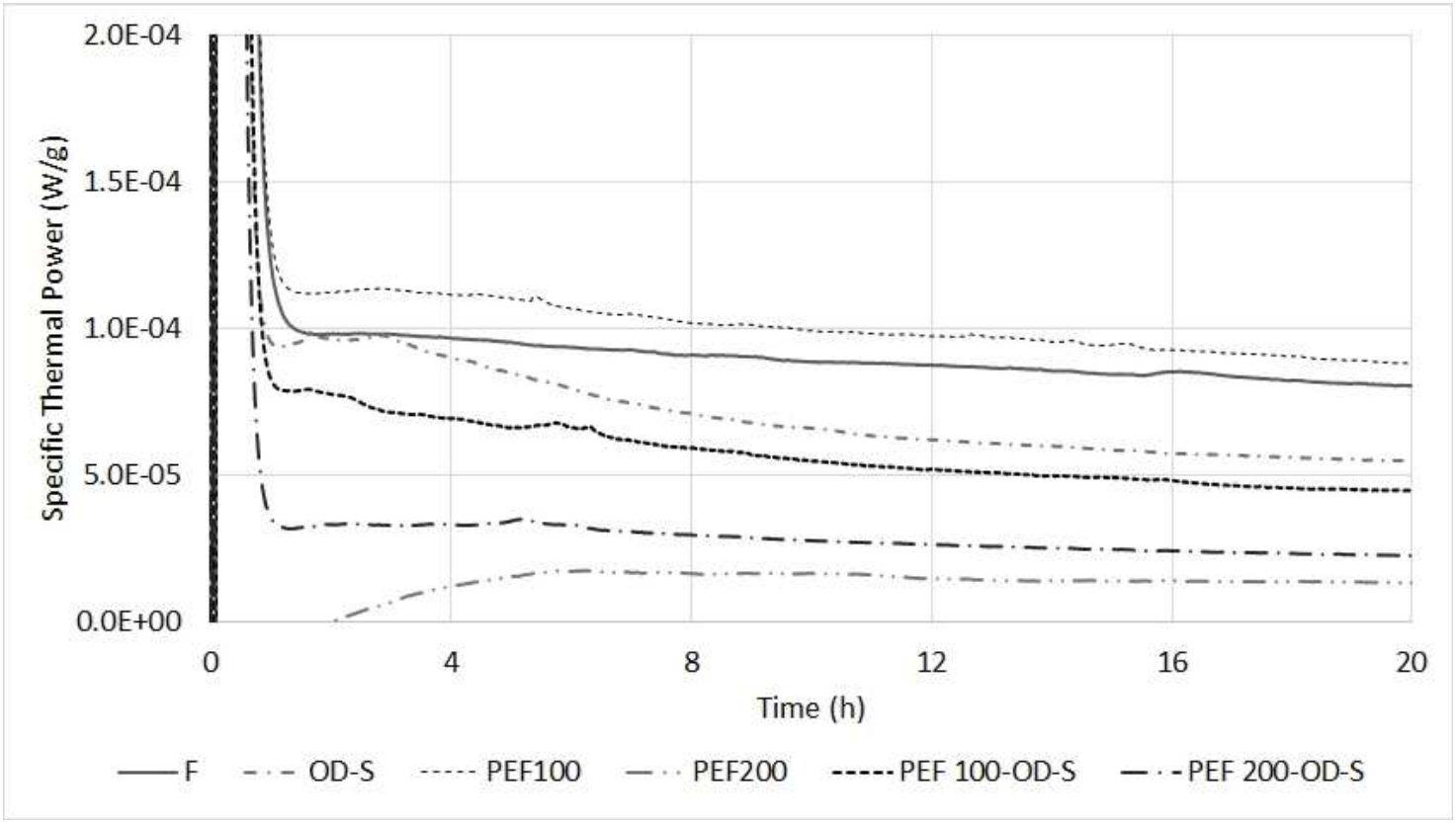
Time [μs]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Specific Thermal Power (W/g)
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Time (h)
F
OD-S
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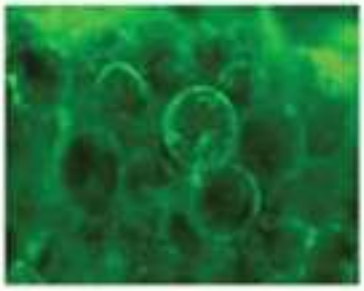
PEF 100-OD-S
PEF 200-OD-S



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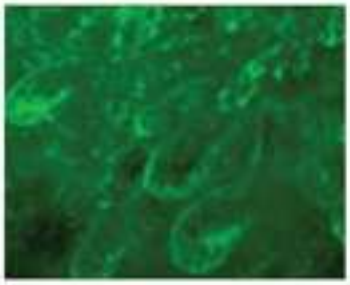

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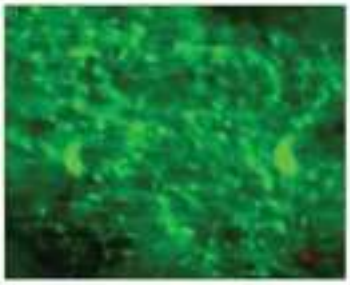

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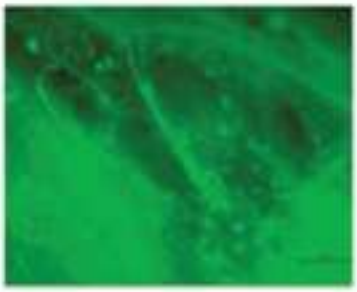

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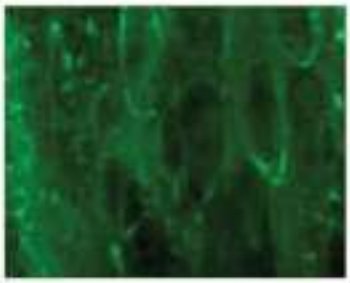

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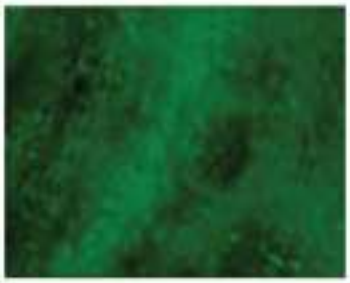

PEF_100_T

PEF_200

PEF_200_S

PEF_200_T

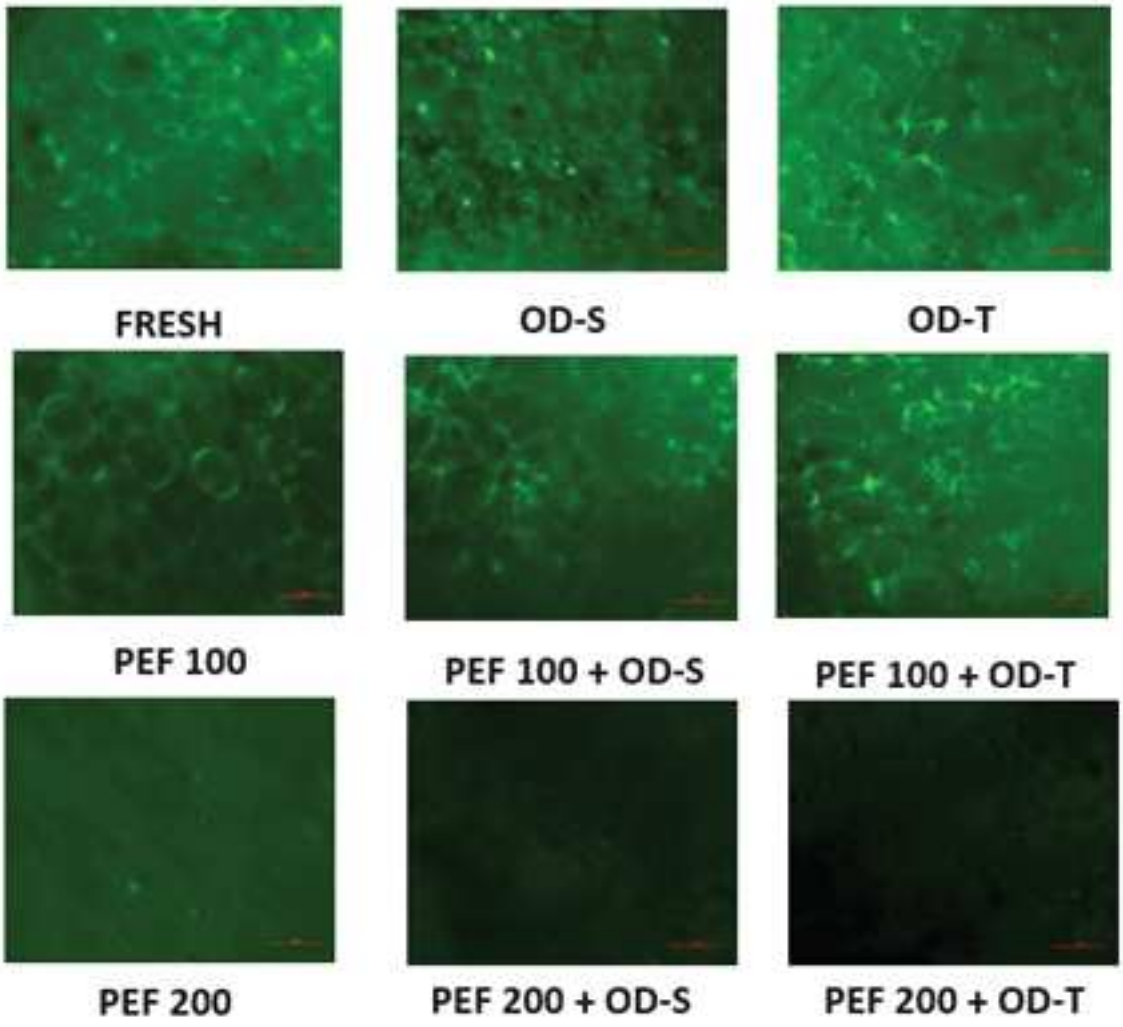
FRESH
OD-S
OD-T
PEF 100
PEF 100 + OD-S
PEF 100 + OD-T
PEF 200
PEF 200 + OD-S
PEF 200 + OD-T

Table 1. Abbreviations of analysed samples

Sample code	Electric field ($V\ cm^{-1}$)	Type of solution
FRESH	-	-
PEF_100	100	-
PEF_200	200	-
OD_S	-	Sucrose
OD_T	-	Trehalose
PEF_100_S	100	Sucrose
PEF_100_T	100	Trehalose
PEF_200_S	200	Sucrose
PEF_200_T	200	Trehalose

Table 2. Metabolic heat production, water content, electrolyte leakage (EL), firmness and Texture disintegration index (TDI) of kiwifruit samples subjected to the different treatments compared to the fresh one.

Sample	Heat (J/g)	Water content (%)	EL (%)	Firmness (N)	TDI
Fresh	$2.22^{a} \pm 0.45$	$82.8^{a} \pm 1.8$	$52.52^{bc} \pm 0.41$	$3.10^{a} \pm 0.49$	-
OD-S	$1.59^{ab} \pm 0.18$	$78.5^{c} \pm 0.5$	$68.61^{a} \pm 1.84$	$2.15^{b} \pm 0.43$	-
OD-T	$1.51^{ab} \pm 1.12$	$79.4^{bc} \pm 0.7$	$59.05^{b} \pm 0.91$	$3.11^{a} \pm 0.45$	-
PEF 100	$1.83^{ab} \pm 0.69$	$84.4^{a} \pm 0.7$	$44.78^{de} \pm 1.15$	$1.83^{b} \pm 0.52$	$0.487^{d} \pm 0.121$
PEF 200	$0.96^{bc} \pm 0.07$	$81.8^{ab} \pm 0.4$	$51.82^{bcd} \pm 3.32$	$0.77^{d} \pm 0.12$	$0.934^{a} \pm 0.041$
PEF100+OD-S	$0.88^{bc} \pm 0.01$	$77.7^{cd} \pm 0.2$	$43.02^{e} \pm 0.52$	$0.98^{d} \pm 0.24$	$0.842^{b} \pm 0.052$
PEF100+OD-T	$1.04^{bc} \pm 0.77$	$77.4^{cd} \pm 0.2$	$51.94^{bcd} \pm 3.43$	$1.42^{c} \pm 0.41$	$0.661^{c} \pm 0.053$
PEF200+OD-S	$0.81^{bc} \pm 0.15$	$75.8^{d} \pm 0.3$	$50.38^{cd} \pm 0.91$	$0.96^{d} \pm 0.14$	$0.853^{b} \pm 0.030$
PEF200+OD-T	$0.07^{c} \pm 0.19$	$74.9^{d} \pm 0.2$	$55.45^{bc} \pm 0.65$	$0.69^{d} \pm 0.14$	$0.968^{a} \pm 0.022$

Values in a column bearing different letters are significantly different at P level of 0.05.

Table 3. Metabolic heat production, water content, electrolyte leakage (EL), firmness and Texture disintegration index (TDI) of strawberries samples subjected to the different treatments compared to the fresh one.

Sample	Heat (J/g)	Water content (%)	EL (%)	Firmness (N)	TDI
Fresh	$5.29^{ab} \pm 0.55$	$90.9^{a} \pm 0.7$	$31.27^{d} \pm 0.24$	$4.25^{ab} \pm 1.14$	-
OD-S	$5.67^{a} \pm 1.16$	$88.5^{bc} \pm 0.7$	$38.73^{bcd} \pm 5,74$	$3.96^{abc} \pm 1.03$	-
OD-T	$4.69^{ab} \pm 0.47$	$88.2^{bc} \pm 1.1$	$34.89^{cd} \pm 0.59$	$4.47^{a} \pm 1.07$	-
PEF 100	$4.24^{ab} \pm 0.64$	$90.2^{a} \pm 0.5$	$35.81^{cd} \pm 0.95$	$3.38^{abc} \pm 1.11$	$0.339^{e} \pm 0.143$
PEF 200	$0.99^{c} \pm 0.12$	$91.2^{a} \pm 0.3$	$35.00^{cd} \pm 2.89$	$2.13^{ef} \pm 0.43$	$0.818^{b} \pm 0.011$
PEF100+OD-S	$1.72^{c} \pm 0.11$	$87.7^{cd} \pm 0.8$	$35.90^{cd} \pm 2.54$	$2.98^{cde} \pm 1.45$	$0.532^{d} \pm 0.065$
PEF100+OD-T	$0.83^{c} \pm 0.01$	$86.4^{d} \pm 0.3$	$50.17^{abc} \pm 3.01$	$2.68^{def} \pm 0.72$	$0.628^{c} \pm 0.041$
PEF200+OD-S	$3.92^{b} \pm 0.43$	$87.0^{d} \pm 0.7$	$52.31^{ab} \pm 7.07$	$3.09^{cd} \pm 1.36$	$0.560^{d} \pm 0.121$
PEF200+OD-T	$2.17^{c} \pm 0.74$	$86.2^{d} \pm 0.8$	$61.71^{a} \pm 6.29$	$1.95^{f} \pm 0.48$	$0.973^{a} \pm 0.023$

Values in a column bearing different letters are significantly different at P level of 0.05.