Antioxidant and antimicrobial properties of organic fruits subjected to PEF-assisted osmotic dehydration

Highlights

PEF assisted osmotic dehydration of organic strawberry and kiwifruit were studied.

Antioxidant and antimicrobial properties were influenced by PEF-pre-treatment.

Good maintenance of colour following the combined treatment.

Combined treatment (PEF+OD) decreased the antioxidant activity and capacity.

Antioxidant and antimicrobial properties of organic fruits subjected to PEF-assisted osmotic dehydration

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Abstract

The effect of PEF pre-treatment prior to osmotic dehydration on mass transfer parameters, colour, antioxidant and antimicrobial properties in organic strawberries and kiwifruits was evaluated. An increase of water loss in both fruits upon the application of combined processes was noticed (up to 21.6 %), while a decrease of solid gain was observed only in kiwifruit samples dehydrated in sucrose (about 45 %). In general, the combined treatments were beneficial for colour maintenance in both fruit tissues. The antioxidant capacity (DPPH) and activity (ORAC) increased after PEF treatment, however, all the combined treatments reduced significantly these values (of about 20 and 28 % for strawberry and of about 56 and 35 % for kiwifruit, respectively for DPPH and ORAC methods). In general, PEF treatment alone was also effective with regard to an increase in the antimicrobial activity of the samples against the tested microorganisms (*B. subtilis, E.coli, S. cerevisiae*).

Keywords: Pulsed electric field, osmotic dehydration, antioxidant capacity, antimicrobial activity, organic fruit

Industrial relevance

PEF pre-treatment coupled with osmotic dehydration could be applied at industrial level to obtain semi-dried fruit products. Moreover, both processes could be used as pre-treatments for drying process, in order to develop healthy and microbiologically stable fruit snacks. In fact, in the present work we observed that PEF pre-treatment alone promoted higher antioxidant and antimicrobial activity. The combined process decreased both parameters, suggesting that an accurate study is necessary to evaluate the benefits of these processes in terms of bioactive compounds retention and time and energy consumption in further drying process. The results of the present study could be used as a starting point for the industries to design novel products with intermediate moisture content intended for further processing.

1. Introduction

There is an increasing awareness of the importance of a healthy diet among consumers nowadays. Moreover, consumers give also huge attention to the environment pollution, therefore the organic fruit production has been expanding rapidly in recent years, reaching about 69.8 million hectares in 2017 (Willer et al., 2019).

Berries and other fruits are rich sources of polyphenols, vitamins and fibre, which can have high beneficial effects on health due to their potential to reduce risk of cardiovascular, cognitive, cancer diseases, etc. (Szajdek & Borowska, 2008). However, their availability is affected by seasonal and climatic conditions and their shelf-life is generally limited to a few days. The shelf-life could be even more reduced in organic fruits. During the processing of organic berries, the focus should be put on the application of mild/non-thermal green technologies for processing and preservation of food products while maintaining high nutritional quality and safety (Tylewicz et al., 2017).

In particular, mild technologies like osmotic dehydration can be used alone or in combination with other non-thermal treatments in order to obtain intermediate moisture content fruits. Pulsed electric field (PEF) treatment could be applied before osmotic dehydration to improve mass transfer phenomena, saving time and energy required for this process (Dermesonlouoglou et al., 2016; Tylewicz et al., 2017; Traffano-Schiffo et al., 2016, 2017a,b; Yu et al., 2017).

PEF treatment leads to electroporation of the cell membrane, as a consequence of an external electric field to the product placed between two electrodes. This energy is usually applied in form of short pulses (Barba et al. 2015). Electroporation of the cell membranes, apart of affecting mass transport phenomena, could have an impact on colour, structure and the nutritional composition of treated samples.

Colour and texture are important parameters since they determine the preferences of the consumers when choosing the food product. PEF treatment can have a dual impact on colour. From one side it can cause alteration of the colour due to enzyme activities (e.g. peroxidase and polyphenol oxidase) caused by the pore formation in the cell membrane, and great leakage of pigment into the treatment solution (Wiktor et al., 2015) or degradation of anthocyanins (Fathi et al., 2011). On the other hand, in some cases, the inactivation of certain enzymes by PEF could promote colour retention (Wiktor et al., 2015).

Concerning bioactive compounds, some studies have shown that PEF treatment may cause faster leaching of vitamin C into the osmotic solution (Ade-Omowaye et al., 2001). However, Yu et al. (2018) observed that blueberry samples treated with PEF showed lower loss of total polyphenols (66 %) in comparison to the control (79%) samples, after osmotic dehydration. It was explained that PEF pre-treatment caused inactivation of PPO, which significantly increased the retention of anthocyanins,

predominantly phenolic acids and flavonols, total phenolics, and antioxidant activity in dehydrated blueberries. Phenolic compounds in berries and fruits in general are well known for their antimicrobial properties, and therefore PEF pre-treatment of berries maintaining phenolics may increase also their antimicrobial potential.

The aim of this work was to evaluate the effect of pulsed electric field (PEF) pre-treatment before osmotic dehydration (OD) on antioxidant and antimicrobial properties in strawberry and kiwifruit tissues. Moreover, the mass transfer parameters and colour of the samples were evaluated.

2. Materials and Methods

2.1. Raw material handling

Organic strawberries (*Fragaria+ananassa)* var. *Alba* and kiwifruit (*Actinidia Deliciosa*) var. *Hayward* were purchased from the local market in Cesena (Italy). The fruits were stored at 4 ± 1 ºC at 95 % of relative humidity for 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, afterwards, each slice was cut into four triangular pieces.

2.2. Pulsed electric field (PEF) pre-treatment

Six strawberry halves and eight to ten triangular kiwifruit pieces (approximately 35 g) were placed into a rectangular treatment chamber (5 x 5 x 5 cm) equipped with two stainless steel electrodes (5 x 5 cm) with a gap between them of 5 cm, and filled with sodium chloride solution with a conductivity of 1.6 mS/cm (measured by EC-Meter basic 30+, Crison). This conductivity was chosen as the average conductivity for strawberry and kiwifruit samples. PEF pre-treatments were carried out by applying 1000 rectangular pulses at two different electric field strengths (100 and 200 V/cm), a fixed pulse width of 10 μs and a frequency of 100 Hz. The PEF parameters have been selected on the basis of our previous experiments, giving the possibility to obtain reversible (100 V/cm) and irreversible (200 V/cm) electroporation (Tylewicz et al., 2017; 2019). The PEF treatments were applied using a pulse generator S-P7500 60A 8kV (Alintel srl., Bologna). The total treatment time was set to 10 s. The specific energy input was calculated, and it was 0.96 and 1.92 kJ/kg for samples treated at 100 and 200 V/cm respectively. The effectiveness of PEF treatment has been study in the previous work (Tylewicz et al., 2019), by measuring the disintegration index (Zt) on the basis of the texture data, showing Zt of about 34 and 82 % respectively for samples treated by PEF at 100 V/cm and PEF at 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 (refined commercial sugar, Eridania Italia S.p.A, Italy) or trehalose (EXACTA + OPTECH Labcenter S.p.A., Italy) solutions. Sucrose was used as a most common agent for the osmotic dehydration, while trehalose was chosen in order to evaluate the possibility to replace sucrose in the dehydration process. Trehalose is a naturally occurring dietary disaccharide, with almost half the sweetness of sucrose, and has a low cariogenic potential when compared to sucrose and moderate glycaemic index with low insulinemic response (Galmarini et al., 2009). Moreover, trehalose is able also to preserve lipid bilayers of the cell membrane from physical stresses during the dehydration and therefore can protect biomolecules (Crowe et al., 2001). To both solutions 1 % (w/w) of calcium lactate (CaLac) (calcium-l-lactate 5-hydrate powder, PURACAL® PP Food, Corbion PURAC, Netherlands) was added as structuring agent (Tylewicz et al., 2017). The treatment was performed at 25 ºC during 120 min (according to Tylewicz et al., 2011, 2017) with continuous stirring, maintaining a fruit:OD solution ratio of 1:4 (w/w) in order to avoid concentration changes in the solution during the treatment. The osmotic treatment was performed twice. All obtained samples with related abbreviations are shown in table 1.

Table 1

2.4. Analytical determinations

2.4.1. Mass transfer phenomena and water activity (a_w)

Mass transfer parameters (weight reduction - WR, water loss - WL and solutes gain - SG) following the osmotic dehydration treatment were calculated adopting the following equations:

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

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

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

where:

m_0 - weight before osmotic treatment (kg)

m_t - weight after osmotic treatment (kg)

x_w - water mass fraction ($kg \cdot kg^{-1}$)

x_{ST} - total solids mass fraction ($kg \cdot kg^{-1}$)

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

Water activity (a_w) was measured in the water activity meter AquaLab Series 3TE (Decagon Devices, Inc., USA) at room temperature. Both moisture content and aw analyses were conducted in triplicate.

2.4.2. Colour

The colour parameters of fresh, PEF pre-treated and osmo dehydrated samples were investigated using a spectro-photocolorimeter mod. Colorflex (Hunterlab, USA). The CIE $L^*a^*b^*$ scale was used. 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 and total colour change (ΔE) were calculated using the following equation:

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

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

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (5)$$

where:

ΔL*, Δa*, Δb* are the differences of mean L*, a* and b* parameters, respectively, between fresh fruits and osmodehydrated untreated and US treated samples (Radojčin et al., 2015).

The colour analysis was performed in ten repetition.

2.4.3. Preparation of extracts from fruit samples

The treated and untreated samples were freeze-dried using a freeze-dryer model Lio2000 (CinquePascal S.r.l., Milano, Italy). The initial sample temperature was −35 °C, and the pressure during the primary drying vacuum phase was 25.12 Pa.

The treated and untreated samples were extracted from freeze-dried powders using acidified methanol (purity of 99.8 %, Merck) according to the methods described by Howard et al. (2003) and Barnes et al. (2009), with some adaptions. Freeze-dried samples (0.200 g ± 0.015 g) were mixed by vortexing for 15 s with 5 ml of methanol [MeOH:H_2O (70:30 mix) plus 1 % trifluoracetic acid] in glass tubes with screw caps. The samples were sonicated for 15 min at 20 °C, 37KHz (S15 Elma Sonicator, Elma Schmdbauwer GmbH), and posteriorly incubated for 30 min in water bath (60 °C, 100 rpm). After incubation, the samples were mixed by vortexing for 15 s, placed in ice for 30 min and centrifuged at 5000 × g for 10 min. The supernatant fluid was collected, and re-extraction was carried out by adding 5 ml of acidified methanol to the pellet, followed by vortexing, centrifugation, and finally pooling the

supernatants from each extraction. The supernatants (10 ml) were centrifuged at 5000 × *g* for 15 min and stored at -20 °C until analysis. All analyses were made in triplicate.

2.4.4. Evaluation of antioxidant properties by DPPH and ORAC

DPPH assay

The free radical scavenging capacity of the extracts was measured, using the DPPH assay (2,2´-diphenyl-1-1-picrylhydrazyl) based on the methods of Brand-Williams et al. (1995) and Reque et al. (2014), with some modifications. Briefly, 100 µl of sample extracts and control (Trolox) were diluted with methanol and combined with 100 µl DPPH solution (250 µM) in a 96-well microplate. After incubation in the dark for 30 min at 20 °C, the absorbance was measured at 517 nm (Savini et al., 2017) in a Safire^2 plate reader (TECAN, Magellan software), using methanol as blank. The absorbance of the blank was used as 100%, and the concentration required to scavenge 50% of DPPH free radicals (EC50), was calculated using the SPSS® Statistics ver. 22 software and expressed as micromoles of Trolox equivalent per gram of dry weight powder (µmol Trolox/g DW).

ORAC assay

The Oxygen Radical Absorbance Capacity (ORAC) assay was used to evaluate the antioxidant activity according to the methods of Prior et al. (2003) and Dávalos et al. (2004). The ORAC assay is based on the scavenging of peroxyl radicals, generated by AAPH (2,2´- azobis (2 amidinopropane hydrochloride) which prevent the degradation and consequently the loss of fluorescein in the sample. A trolox standard curve was used for quantification.

The reaction was carried out in 75nM phosphate buffer (pH 7.4) and the final reaction mixture was 200µl. Twenty µl of the sample and 120µl of fluorescein (1.17mM) were pipetted into a black 96 well microplate. After pre incubation for 15 min at 37°C, 60µl of AAPH solution (40mM) was added quickly using a multichannel pipet. The microplate was immediately placed into a Safire^2 plate reader (TECAN, Magellan software). The fluorescence was recorded each 0.5 min for 120 min. The microplate was automatically shaken prior each blank reading and a trolox standard was prepared for each assay. The area under the fluorescence decay curve was calculated and the antioxidant capacity was expressed as µmol Trolox equivalent/g DW.

2.4.5. Antimicrobial activity

Microbial strains *Bacillus cereus* VTT E-83178 (ATCC 21228), *Escherichia coli* E-94564^T (DSM 30083) and *Saccharomyces cerevisiae* VTT C-00370 were used in antimicrobial activity analysis of the organic strawberry and kiwifruit samples combined in Table 1. Each sample used in antimicrobial

205 analysis was pooled of two parallel samples, except only one sample representing PEF_100 OD_S of
206 strawberry.
207 *B. subtilis* and *E.coli* were cultivated aerobically in or on Nutrient medium (Difco), and the yeast *S.*
208 *cerevisiae* in or on YM medium (Difco). The antimicrobial activity was evaluated using liquid culture
209 method (Nohynek et al., 2006), with modification of using reduced culture volumes of 1.0 ml
210 (Puupponen-Pimiä et al. (2016). Samples of 5 mg were aseptically measured in round bottom
211 Eppendorf tubes of 2 ml serving for cultures of 1 ml. Microbial culture with no fruit samples was
212 included as positive controls of each strain, and the potential antimicrobial effect of low pH was
213 observed with control microbial cultures of pH 4.7. Chloramphenicol (50 µg ml^{-1}) and hygromycin
214 (25 µg ml^{-1}) were used as negative growth controls for bacterial and yeast cultures, respectively. The
215 liquid microbial cultures were incubated for 24 h as follows: *E. coli* at 37 °C with agitation (150 rpm),
216 *B. subtilis* at 30 °C (150 rpm) and *S. cerevisiae* at 30 °C (100 rpm). During this incubation period
217 four samples at time points of 0, 3, 6 and 24 h were taken from each culture for microbial colony
218 counts (cfu ml^{-1}). The growth curves of each cultures were generated based on their colony counts,
219 and antimicrobial activity was evaluated by comparison of control growth curves to the ones with
220 strawberry and kiwifruit samples.
221 Low pH often causes antibacterial activity, and therefore the pH values of the strawberry and kiwifruit
222 extracts (5 mg ml^{-1}) were measured in Nutrient broth. In addition, the control sample with pH 4.7 was
223 included in the antimicrobial activity test for each microbial strain for evaluation of the effect of the
224 strong acidity.
225
226 2.5. Statistical Analysis
227 One-way analysis of variance (ANOVA) and *Tukey's* Honestly Significant Difference (HSD) test
228 were applied to compare the mass balance parameters, colour and antioxidant capacity/activity of the
229 samples. Statistical Significance was defined as $p < 0.05$. Statistical analyses were performed using
230 the IBM® SPSS® Statistics ver. 24 software.
231
232 3. Results and discussion
233 3.1. Mass transfer parameters
234 Osmotic dehydration causes an intense water loss from the plant tissue and a simultaneous enrichment
235 in solids from the osmotic solution. This is due to the permeability of the cell membrane and to the
236 difference in the chemical potential of water between the product and the osmotic medium (Chiralt
237 & Talens, 2005; Tylewicz et al., 2011a, Tylewicz et al.2011b; Dermesonlouoglou et al., 2018a). PEF
238 treatment causes the electroporation of the plant tissue membranes, facilitating the removal of water

from the product (Dellarosa et al., 2016; Dermesonlouoglou et al., 2016, 2018b). Since these two processes involve a complex mass transfer, it is of fundamental importance to characterize the parameters involved in the mass exchange. Moreover, water activity is also a crucial parameter for OD treatments, since it gives important information on product stability. In table 2 and 3 mass transfer parameters in terms of water loss, solid gain and weight reduction and water activity values following the osmotic treatment of strawberries and kiwifruits are reported, respectively.

As it can be seen in table 2, the strawberry samples treated with PEF_100 and PEF_200 showed a slight weight loss, probably due to the loss of water and native soluble solids during PEF pre-treatment, however, the values did not exceed 1% and therefore these changes could be considered insignificant. In samples treated only with OD (OD_S and OD_T) it was possible to notice an effective loss of moisture compared to the fresh sample and this loss was significantly higher in the dehydrated sample with the sucrose solution. Also, Tylewicz et al. (2017) observed a higher final dewatering rate in strawberry samples treated in sucrose than in trehalose solution at the same concentration. The combination of PEF pre-treatment with osmotic dehydration showed a further water loss in the samples, reaching values of about 16-19%. The intensity of the electric field applied influenced significantly this parameter; in fact, the water loss was higher in the samples pre-treated with PEF at 200 V/cm in both osmotic solutions in comparison to samples treated with PEF at 100V/cm. The obtained data are in agreement with those obtained by Tylewicz et al. (2017), showing an increase in water loss in the strawberry samples proportional to the intensity of the electric field strength applied. However, in the present study the final values of the dewatering by the application of PEF at 200 V/cm were much lower (18-19 %) against 44-50% obtained in the study of Tylewicz et al (2017), probably due to the lower agitation rate used in the present experiment.

Concerning the solid gain data, the samples dehydrated in trehalose presented a slightly higher increase than those treated in sucrose, even though the differences were not significant. This was in contrast to the study of Ferrando and Spiess, (2001), who stated that trehalose should have a much lower capacity for inclusion in cells in comparison to sucrose. Tylewicz et al. (2017) observed that PEF treatment at three different intensities (100, 200 and 400 V/cm) increased the solid gain in strawberries when sucrose was used, while when trehalose was used this increase was only observed at the lowest PEF intensities.

Table 2

Osmotic dehydration of kiwifruit alone promoted slightly lower water loss (Table 3) in comparison to the strawberry samples, probably because of the differences in the tissue structure and the different

preparation of the sample. Kiwifruit outer pericarp is composed by large cells dispersed in a matrix of smaller cells (Hallett et al., 1992). Strawberry instead has heterogeneous structure, with dense, external, epidermal layer (Suutarinen et al., 1988; Velickova et al., 2018), giving a better protection of the tissue structure to the PEF pre-treatment. Moreover, in case of kiwifruit, the peeling and cutting operations and the shape obtained caused a higher exposure of the pulp surface to the treatments used with respect to the strawberry. Significantly higher water loss was observed when kiwifruit was dehydrated in sucrose solution rather than in trehalose. However, following pre-treatment with PEF, osmotic dehydration with trehalose resulted in greater dehydration than sucrose, thus negating its protective effect on cell membranes (Ferrando and Spiess, 2001), probably because the cell membrane integrity was already compromised due to pre-treatment with PEF.

The solid gain of kiwifruit treated with sucrose decreased upon the application of PEF at both intensities. This result is in agreement with the study of Traffano-Schiffo et al. (2016), which showed the reduction of the overall amount of electrolytes, causing the alteration of the functionality of Na^+ pump, responsible for the sucrose transport into the cells, showing a lower solute gain in PEF treated samples. This was not observed for the trehalose treated samples, which seemed to be non-influenced by the PEF treatment. When hypertonic solution, containing glycerol and trehalose among others, was used for the dehydration of kiwifruit a higher solid gain was observed in the samples pre-treated with PEF independently than the electric field strength applied (Dermesonlouoglou et al., 2016), suggesting that the solution composition could have an important role on the inclusion of the compounds in the kiwifruit structure. Moreover, the structural alteration and physicochemical reactions which occurs during PEF treatment could influence the solid penetration during osmotic dehydration process (Ade-Omowaye et al., 2001).

Table 3

In addition, table 2 and 3 report the data of water activity measurements, respectively for strawberry and kiwifruit samples. It has been seen that in general water activity of fresh kiwifruit samples was much lower in comparison to the strawberry ones, showing the values of 0.986 ± 0.001 and 0.995 ± 0.002 for kiwifruit and strawberry, respectively. The values of a_w decreased after OD treatment, which was more pronounced in the combined treatments, showing the final a_w values in the range of 0.972-0.977 for kiwifruit and 0.980-0.985 for strawberry. In general, the reduction in the water activity in our study could be consider slight, however, according to Gianotti et al. (2001) it can help to reduce a possible growth of microorganism, due to an increase of viscosity of kiwifruit liquid phase. Also, Dermesonlouoglou et al. (2016) observed that combined treatment with PEF and OD substantially lowered water activity of kiwifruit samples and that it was proportional to the electric field strength applied, indicating a product of increased stability without compromising sensory

quality suitable for further processing. Similarly, for strawberry samples, Dermesonlouoglou et al. (2017) observed the reduction of water activity and consequently a lower microbial grown during osmotic dehydration, thanks to the limited availability of the intercellular spaces for the microorganism penetration.

3.2. Colour

The results of colour measurement for both strawberry and kiwifruit samples are reported in Table 4. Concerning the strawberry samples, PEF application promoted a significant decrease in all colour parameters, which was higher in samples treated with higher intensity (200 V/cm). These changes were pronounced in a less vivid red colour, tending to grey.

The osmotic treatment, as expected, promoted a change in strawberry colour, causing a reduction of all investigated parameters in comparison to the fresh samples. In general, samples dehydrated with trehalose presented higher values of a*, b* and chroma, suggesting more saturated red colour. The combined treatments showed a similar tendency, in fact samples dehydrated with trehalose pre-treated either by 100 or 200 V/cm showed a better maintenance of colour in terms of redness/yellowness and saturation, even if the luminosity was significantly lower in these samples. Moreover, when combined treated samples were compared with those just PEF pre-treated it was observed that the samples treated at 200 V/cm and dehydrated in trehalose presented unchanged L* and a* parameters and enhanced b*, hue angle and chroma values. Similar results were observed by Tylewicz et al. (2017) in strawberry samples osmo-dehydrated with sucrose and trehalose. In general, the reduction of colour parameters following PEF and OD process could be due to the leakage of the pigments into the solution used for osmotic dehydration, as well as the degradation of some compounds, such as anthocyanins following the PEF treatment (Fathi et al., 2011; Odriozola-Serrano et al., 2008). However, the combined treatment with PEF at 200 V/cm and OD with trehalose better preserved the colour of the samples. This could be due to the protective role of trehalose on the cell and vacuole membrane of the biomolecules, such as anthocyanins, which are localized inside vacuoles in the plant cells where these pigments are protected (Oliveira et al., 2019).

Table 4

Concerning the colour of kiwifruit samples (Table 4), PEF treatment alone did not promote significant changes in colour in most of the investigated parameters, contrary to Dermesonlouoglou et al. (2016) study, who showed the significant differences in kiwifruit colour immediately after the PEF pre-treatment. However, when kiwifruit samples were subjected to the OD alone with trehalose an increase of L* was observed. This is probably due to the leaching of compounds, such as

chlorophylls, responsible for the color, to the osmotic solution, even though this could be also related to incorporations of solids from the solution into the material (Rząca et al., 2009). However, the L* values remained unchanged in samples dehydrated in sucrose, in contrary to the results obtained by Nowacka et al. (2017), who observed a decrease of L* value in kiwifruit dehydrated for 120 min in sucrose. The combined treatment at high PEF intensity (200 V/cm) in both solutions promoted an increase of hue angle, highest in PEF_200+OD_T sample. The increase of the hue angle parameter indicates samples with low green component and darker. Also, Dermesonlouoglou et al. (2016) observed that the combined PEF and OD treatment promoted changes in colour parameters, mainly an increase in a* values and a decrease in the b* ones.

Figure 1

Figure 1 shows the total colour difference of untreated and differently treated samples. There are different studies indicating the threshold of delta E, corresponding to the visible changes, which depends on the initial colour of the products. For example, for blood oranges, it has been stated that the ΔE values higher than 2 indicates the differences visible to the naked eye (Choi, Kim, and Lee, 2002), while for products with more intense colouring, like blueberries, even the delta E values of 6-7 were subtle to the eye (Stojanovic & Silva, 2007).

PEF treatment at low intensity (PEF_100) promoted the lowest changes in total colour difference of strawberries, however, when combined treatment was used, the delta E values were in the same range as those in samples treated with 200 V/cm alone or in combination with OD. Concerning the kiwifruit samples, lower differences between different samples were observed, actually, they were significantly higher only in samples treated with combined treatment of PEF 100 with OD sucrose and PEF 200 with OD trehalose. OD in both solutions presented ΔE values of 4-5, which are in agreement with those found by Nowacka et al. (2017) in 120 min OD treated kiwifruit samples. In general, in our study the differences in colour were more marked in strawberry samples in comparison to those of kiwifruit, probably due to the different extractability of chlorophyll in comparison to the anthocyanins. It has been seen that most solvents used to extract anthocyanins are polar solvents (e.g, water), possessing electrical conductivity, and can let electricity pass thorough to sample cells. On the other hand, electric field cannot pass through a non-polar solvent, usually used for the extraction of chlorophylls, since it is an electrical resistance possessing low or negligible conductivity (Yuhas, 1995). Therefore, the differences observed in our study are probably due to the easier extraction and availability of anthocyanins during PEF treatment than for chlorophylls (Ngamwonglumlert et al., 2017).

3.3.Antioxidant capacity/activity

The effect of PEF and OD treatments on the antioxidative potential of strawberry and kiwifruit tissues was evaluated using DPPH and ORAC assays.

According to Apak et al. (2013), the concepts of antioxidant "capacity" and "activity" are different. The former refers to the measurement of the thermodynamic efficiency in reactions between an oxidant with an antioxidant, while the second term deals with the kinetics of a reaction between an antioxidant and the radical it reduces. The DPPH method is an electron transfer based assay, which measures the antioxidant capacity and ORAC is a hydrogen atom transfer based assay, which determines the antioxidant activity of a solution.

The application of PEF at 200 V/cm increased the antioxidant capacity and the antioxidant activity of strawberry extracts by 13.6% and 11.5%, respectively, while for kiwifruit samples a lower electric field strength was more beneficial, in fact PEF at 100 V/cm was responsible for increasing the antioxidant capacity and activity of kiwifruit extracts by 7% and 15.6%, respectively, even though in most of the cases these differences were non statistically different (Table 5). These findings are in agreement with those reported by Tylewicz et al. (2017), who demonstrated that lower electric fields are suitable to preserve quality parameters in fruits. The biological difference between strawberry and kiwifruit tissues, and consequently different sensitiveness to electric field strengths, might explain these findings. Table 5 also shows that, for strawberries, the application of OD with sucrose solution instead of trehalose (hypertonic solution) was more beneficial to retain antioxidants than for the same treatments with kiwifruit. The antioxidant activities of kiwifruit extracts were similar between OD treatments with sucrose and trehalose based solutions, while the antioxidant capacity was higher in samples treated with OD and trehalose (Table 5). All the combination of PEF as a pre-treatment of OD significantly reduced the antioxidant capacity of the extracts of strawberry and kiwifruit (Table 5). The combination of the treatments possibly leads to losses of antioxidant compounds in strawberry and kiwifruit due to the higher diffusion rate of solutes from the fruit tissues - as a result of changes at the membrane cells - which underwent simultaneously electropermeabilization and osmotic flux of water to the external hypertonic solution. The antioxidant capacity and activity were higher in Strawberry (Fragaria+ananassa) variety Alba in comparison with Kiwifruit (Actinidia deliciosa) variety Hayward (Table 5). This finding is in agreement with earlier studies (Perez-Burill et al., 2018; Asami et al., 2003), which show lower content of polyphenols (antioxidant compounds) in kiwifruit than in strawberries. Even though kiwifruit has high content of ascorbic acid (a well-known antioxidant compound) this vitamin is more sensitive to heating, light and oxygen exposure in comparison with polyphenols, which may explain the lower antioxidant capacity and activity in kiwifruit. Moreover, these differences could

also be due to higher exposure of the kiwifruit pulp surface to the treatments used compared with the strawberry. Moreover, some authors showed a protective role of sucrose in the retention of bioactive compounds in strawberries, however it has been proven just when a low concentration of sucrose (20 %) was used (Nikkhah, et al., 2007). Instead, trehalose is associated with the protection of cell membranes since this disaccharide, differently from sucrose, is able to interact with higher stability with phospholipids on the membrane. Considering that some bioactive compounds such as anthocyanins are stored in membrane vacuoles in the cell, the application of trehalose may protect these compounds inside the cells during processing where they are more stable (Oliveira et al., 2019).

Table 5

3.4.Antimicrobial activity

The antimicrobial activity of strawberry and kiwifruit extracts (5 mg ml^{-1}) on *E. coli*, *B. subtilis* and *S. cerevisiae* was evaluated in liquid cultures during 24 h incubation.

In general, *B. subtilis* was the most sensitive of the tested microbes against studied extracts (Figure 2). Fresh and PEF_200 treated strawberry samples showed strong bactericidal effect on *B. subtilis*, and the other strawberry samples inhibited totally the growth of the culture. The only exception among strawberry extracts was the *B. subtilis* culture with PEF_100+OD_T, which was growing over the positive microbial growth control. The strongest antimicrobial effects in kiwifruit extracts were observed after PEF_100, PEF_200 and OD-S treatments. Growth of *B. subtilis* was retarded in culture with fresh kiwifruit, whereas the other treated kiwifruit extracts enhanced the growth of *B. subtilis* cultures over the control culture. The pH 4.7 of the culture as the control had the strongest antimicrobial effect on the *B. subtilis*, and chloramphenicol 50 µg ml^{-1} as negative growth control was also very bactericidal.

Figure 2

The growth of *E. coli* was retarded in cultures with fresh and PEF_200 -treated strawberry, as well as with PEF_200 -treated kiwifruit (Figure 3). The colony counts in these samples (after 24 h incubation), were 5 x 10^7 cfu ml^{-1}, compared to 5 x 10^9 cfu ml^{-1} in the control culture. PEF_100 and OD -treated kiwifruit samples were almost as effective, followed by fresh fruit and all the combinations of PEF+OD -treatments with mild antimicrobial effect. Similar to *B. subtilis*, the pH control of 4.7 and chloramphenicol (50 µg ml^{-1}) had strong bactericidal effect on *E. coli*.

Figure 3

The *Saccharomyces cerevisiae* strain VTT C-00370 used in this study originated from the dairy industry (as contaminant), and was very resistant against most of the tested samples (data not shown). Only PEF_100 pre-treated strawberry caused minor inhibition on the growth of this yeast culture, which was also very tolerant to the pH in the control (pH 4.7).

In general, PEF - and OD -treatments increased the antimicrobial activity over the activity caused by the fresh berries only with PEF-200 and OD-S -treated kiwifruit against Bacillus subtilis. The pH was measured of each extract as 5 mg ml-1 in NB, and the values varied from pH 4.7 of the fresh strawberry to pH 5.6 of PEF+OD_T -treated kiwifruit (Table 2). Many bacterial species are sensitive to low pH, especially below pH 5, whereas yeasts often tolerate acidic environments (Piper et al., 2001). This pH effect was observed also in this study and complicates the evaluation of the antimicrobial properties of the extracts.

PEF and OD-S treatments increased antimicrobial activity especially of kiwifruit against *Bacillus subtilis*, which, however, had lower antioxidant activity compared to the strawberry (Table 5). Chemical analysis of phenolic contents in organic and conventionally cultivated berries, fruits and vegetables have shown increased levels of specific phenolic compounds in organic berries and fruits, such as ellagic acid and kaempferol (Häkkinen and Törrönen, 2000; Asami et al., 2003; Veberic et al., 2005). PEF and OD_S -treatments may have modified phenolic composition of kiwifruit favouring these strong antimicrobial compounds (ellagic acid and kaempferol), which would be interesting to evaluate by analysing phenolic profiles of the strawberry and kiwifruit samples.

Conclusions

The obtained results showed the differences in the behaviour of strawberry and kiwifruit samples upon the application of PEF pre-treatment prior to osmotic dehydration. Moreover, the composition of the hypertonic solution used for osmotic dehydration had a significant influence on some of the parameters evaluated. Concerning the mass transfer phenomena, the combined process increased the dehydration rate of both fruits, proportionally to the electric field strength applied. Solid gain was unchanged in strawberries, while a decrease of this parameter has been observed in kiwifruit treated with sucrose.

The combination of PEF at 200 V/cm and OD with trehalose solution was best for the preservation of colour of strawberry samples, while for kiwifruit lower intensities of PEF and dehydration in both solutions were more beneficial for colour maintenance.

The antioxidant capacity and activity were higher in strawberry in comparison with kiwifruit samples and these parameters increased significantly following the application of PEF_200 in

strawberry (13.6% and 11.5%, respectively for antioxidant capacity and activity) and PEF_100 in kiwifruit (7% and 15.6%, respectively for antioxidant capacity and activity). However, in both samples, even if there was an increase after PEF treatment, all the combinations of PEF and OD reduced the initial values of these parameters, without any statistical differences between samples treated in combined treatments.

Concerning the antimicrobial activity of the extracts against the tested microorganisms (*B. subtilis, E.coli, S. cerevisiae*), a general increase was observed. In addition, OD_S -treated kiwifruit extract showed strong antimicrobial activity against *B. subtilis*, while PEF_100 treated strawberry extract was the only one that caused moderate antimicrobial effect against *S. cerevisiae*, which resulted the most resistant among all tested microbes.

Acknowledgements: Financial support for this project is provided by funding bodies within the FP7 ERA-Net CORE Organic Plus, and with cofounds from the European Commission (No 618107).

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FIGURE CAPTIONS

Figure 1. Total colour difference (ΔE) of untreated and PEF pre-treated strawberry (grey) and kiwifruit (black) samples before and after osmotic dehydration in sucrose and trehalose solutions.

Figure 2. Antimicrobial activity of the strawberry (A) and kiwifruit (B) extracts against *Bacillus subtilis*. The tested samples of 5 mg ml^{-1} were as follows: fresh (before treatments), PEF -treated at 100 V/cm 200 V/cm, osmotic dehydration (OD) -treated using sucrose (OD_S) or trehalose (OD_T), as well as the combinations of the treatments. The antimicrobial activities of berry samples were evaluated against non-treated bacterial culture (Growth control), and Chloramphenicol was included as negative control.

Figure 3. Antimicrobial activity of the strawberry (A) and kiwifruit (B) extracts against *Escherichia coli*. The tested samples of 5 mg ml^{-1} were as follows: fresh (before treatments), PEF -treated at 100 V/cm 200 V/cm, osmotic dehydration (OD) -treated using sucrose (OD_S) or trehalose (OD_T), as well as the combinations of the treatments. The antimicrobial activities of berry samples were evaluated against non-treated bacterial culture (Growth control), and Chloramphenicol was included as negative control.

Figure 1

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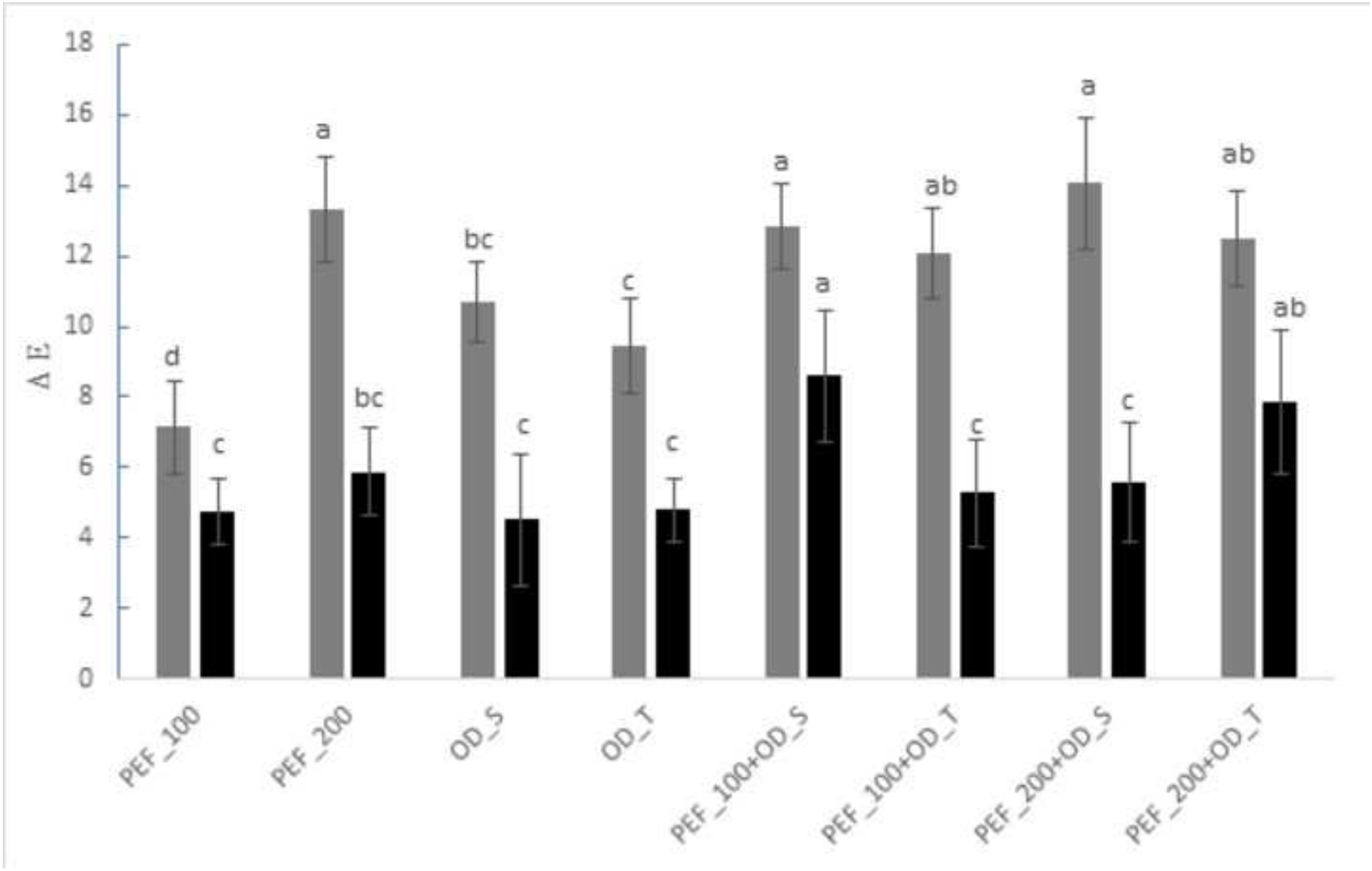

Figure 2

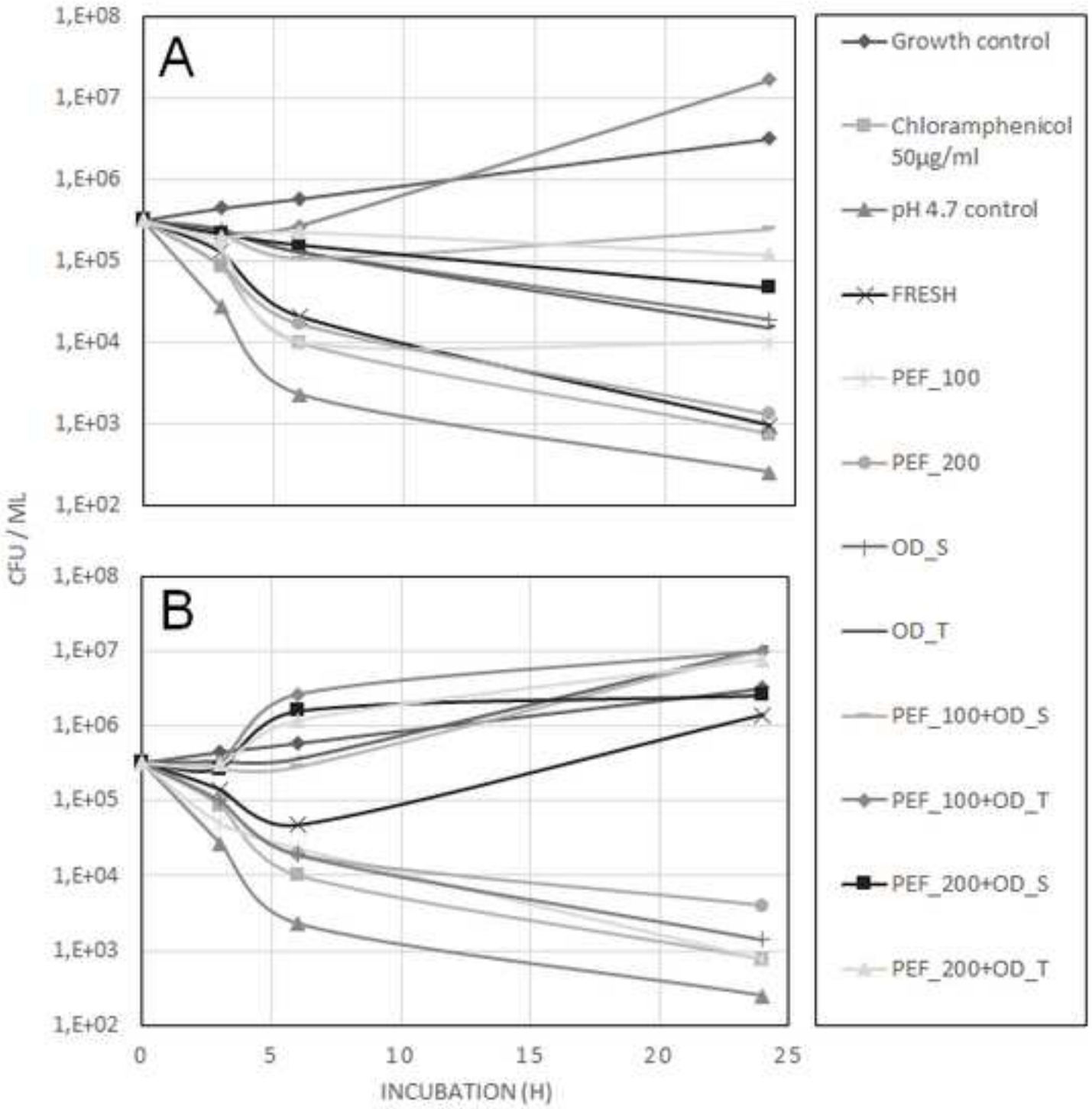

Figure 3

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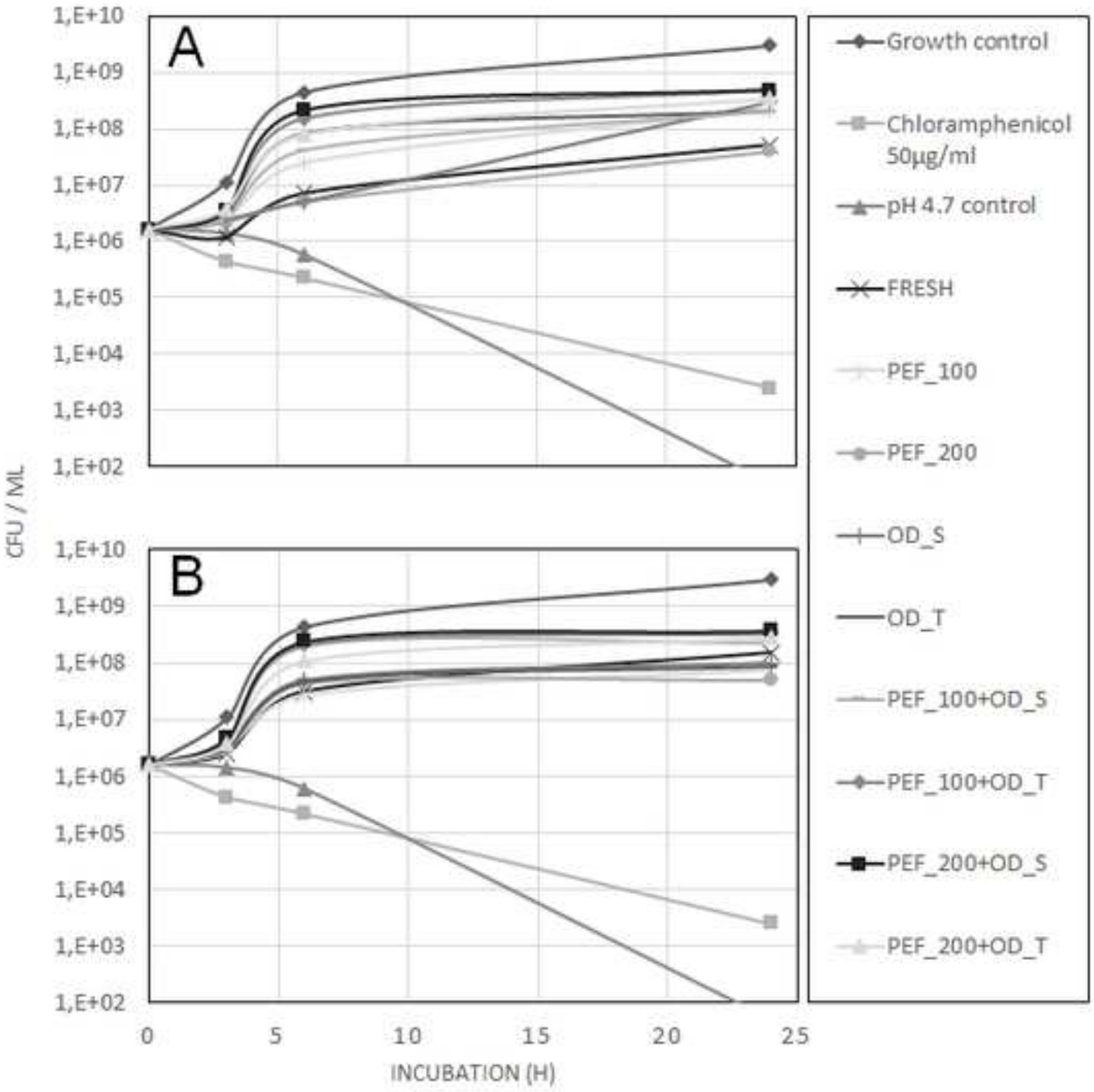

Table 1
Abbreviations of analysed samples.

Sample code	Electric field strength (V/cm)	Type of solution
FRESH	–	–
PEF_100	100	–
PEF_200	200	–
OD_S	–	Sucrose
OD_T	–	Trehalose
PEF_100 + OD_S	100	Sucrose
PEF_100 + OD_T	100	Trehalose
PEF_200 + OD_S	200	Sucrose
PEF_200 + OD_T	200	Trehalose

Table 2
Mass balance parameters and water activity for fresh and treated strawberry samples following 120 min of osmotic dehydration. Values with the same letter within a column at superscript, for each fruit type, were not significantly different at $P < 0.05$, based on Tukey's test.

Samples	Water loss (%)	Solute gain (%)	Weight reduction (%)	Water activity (a_w)
FRESH	–	–	–	0.995 ± 0.002^{a}
PEF_100	0.6 ± 0.1^{a}	–	0.6 ± 0.1^{a}	0.997 ± 0.002^{a}
PEF_200	0.8 ± 0.2^{a}	–	0.8 ± 0.2^{a}	0.996 ± 0.003^{a}
OD_S	13.2 ± 0.2^{c}	1.4 ± 0.2^{a}	11.8 ± 0.2^{c}	0.987 ± 0.001^{c}
OD_T	11.0 ± 0.9^{b}	1.6 ± 1.1^{a}	9.4 ± 0.3^{b}	0.989 ± 0.002^{b}
PEF_100 + OD_S	16.0 ± 0.7^{d}	1.4 ± 0.7^{a}	14.6 ± 0.4^{d}	0.985 ± 0.001^{d}
PEF_100 + OD_T	17.3 ± 1.1^{de}	2.5 ± 1.1^{a}	14.8 ± 0.2^{d}	0.985 ± 0.002^{d}
PEF_200 + OD_S	19.1 ± 0.6^{e}	1.7 ± 0.6^{a}	17.3 ± 0.5^{f}	0.980 ± 0.002^{f}
PEF_200 + OD_T	18.4 ± 0.7^{e}	2.5 ± 0.7^{a}	15.9 ± 0.3^{e}	0.983 ± 0.002^{e}

Table 3
Mass balance parameters and water activity for fresh and treated kiwifruit samples following 120 min of osmotic dehydration. Values with the same letter within a column at superscript, for each fruit type, were not significantly different at $p < .05$, based on Tukey's test.

Samples	Water loss (%)	Solute gain (%)	Weight reduction (%)	Water activity (a_w)
FRESH	–	–	–	0.986 ± 0.001^{a}
PEF_100	0.5 ± 0.1^{a}	–	0.5 ± 0.1^{a}	0.987 ± 0.003^{a}
PEF_200	0.7 ± 0.1^{a}	–	0.7 ± 0.1^{a}	0.987 ± 0.001^{a}
OD_S	11.9 ± 0.4^{c}	4.5 ± 0.5^{a}	7.4 ± 0.3^{c}	0.979 ± 0.002^{c}
OD_T	7.4 ± 0.9^{b}	2.1 ± 0.6^{b}	5.3 ± 0.2^{b}	0.982 ± 0.002^{b}
PEF_100 + OD_S	14.7 ± 2.0^{d}	2.3 ± 0.7^{b}	12.4 ± 0.3^{d}	0.977 ± 0.001^{c}
PEF_100 + OD_T	17.7 ± 0.4^{de}	3.7 ± 0.4^{ab}	14.0 ± 0.4^{de}	0.975 ± 0.001^{d}
PEF_200 + OD_S	18.4 ± 1.1^{e}	2.6 ± 0.6^{b}	15.8 ± 0.3^{e}	0.975 ± 0.001^{d}
PEF_200 + OD_T	21.6 ± 0.4^{f}	3.2 ± 0.4^{b}	18.4 ± 0.3^{f}	0.972 ± 0.002^{e}

Table 4
Colour parameters (L* - luminosity, a* - green/red index, b* -blue/yellow index, h°-hue angle, C-Chroma) of untreated and PEF pre-treated strawberry and kiwifruit samples before and after osmotic dehydration in sucrose and trehalose solutions. Values with the same letter within a column at superscript, for each fruit type, were not significantly different at $p < 0.05$, based on Tukey's test.

Sample	L*	a*	b*	h°	C
Strawberry (*Fragaria + ananassa)* var. *Alba*					
FRESH	42.9 ± 2.0^{a}	33.2 ± 1.7^{a}	24.4 ± 1.6^{a}	36.4 ± 0.9^{a}	41.2 ± 2.3^{a}
PEF_100	40.2 ± 1.6^{b}	28.9 ± 2.2^{bcd}	19.7 ± 0.9^{b}	34.5 ± 0.6^{b}	35.1 ± 1.6^{bc}
PEF_200	33.9 ± 1.7^{ef}	28.9 ± 1.3^{bcd}	15.5 ± 1.7^{d}	28.4 ± 1.5^{e}	32.7 ± 1.4^{cde}
OD_S	39.4 ± 0.9^{bc}	26.7 ± 1.0^{de}	17.5 ± 1.1^{c}	33.3 ± 1.9^{bc}	31.6 ± 0.9^{def}
OD_T	35.2 ± 1.1^{de}	30.9 ± 1.6^{b}	19.1 ± 1.2^{bc}	31.8 ± 1.6^{c}	35.9 ± 1.4^{b}
PEF_100 + OD_S	39.3 ± 1.6^{bc}	25.8 ± 1.7^{e}	14.8 ± 1.6^{d}	29.5 ± 1.4^{de}	29.9 ± 2.2^{f}
PEF_100 + OD_T	33.9 ± 1.9^{ef}	29.3 ± 1.3^{bc}	18.0 ± 1.1^{bc}	27.4 ± 2.8^{cd}	30.6 ± 1.6^{bcd}
PEF_200 + OD_S	37.0 ± 1.2^{cd}	27.1 ± 1.7^{cde}	14.3 ± 1.9^{d}	31.1 ± 1.4^{e}	34.2 ± 1.8^{ef}
PEF_200 + OD_T	32.5 ± 2.2^{f}	30.2 ± 1.6^{b}	19.7 ± 1.0^{b}	33.2 ± 1.2^{bc}	35.8 ± 1.2^{b}
Kiwifruit (*Actinidia deliciosa*) var. *Hayward*					
FRESH	36.4 ± 2.7^{b}	-5.5 ± 0.5^{f}	22.8 ± 1.8^{a}	76.4 ± 0.7^{def}	23.5 ± 1.3^{a}
PEF_100	36.1 ± 1.9^{b}	-5.1 ± 0.3^{def}	18.9 ± 1.4^{bcd}	75.5 ± 1.3^{f}	19.6 ± 1.5^{bcd}
PEF_200	31.9 ± 0.9^{c}	-5.1 ± 0.7^{ef}	20.4 ± 1.9^{abc}	75.8 ± 1.2^{ef}	21.5 ± 1.4^{abc}
OD_S	35.8 ± 1.8^{b}	-3.9 ± 0.5^{bc}	20.7 ± 1.9^{abc}	79.6 ± 1.6^{b}	21.1 ± 1.9^{abc}
OD_T	39.6 ± 1.6^{a}	-4.8 ± 0.8^{de}	21.4 ± 2.5^{ab}	77.4 ± 0.9^{cd}	21.7 ± 2.5^{ab}
PEF_100 + OD_S	37.9 ± 2.1^{ab}	-3.4 ± 0.2^{ab}	15.2 ± 1.6^{e}	77.1 ± 0.5^{cde}	15.6 ± 1.7^{e}
PEF_100 + OD_T	37.1 ± 1.7^{ab}	-4.2 ± 0.6^{bcd}	18.6 ± 2.8^{cd}	77.1 ± 1.0^{cde}	18.8 ± 2.1^{cd}
PEF_200 + OD_S	32.6 ± 1.6^{c}	-4.4 ± 0.7^{cde}	19.5 ± 1.2^{bcd}	78.0 ± 0.9^{bc}	19.9 ± 1.9^{bc}
PEF_200 + OD_T	32.3 ± 1.3^{c}	-2.6 ± 0.5^{a}	16.9 ± 1.6^{de}	81.4 ± 1.3^{a}	17.1 ± 1.7^{de}

Table 5
Antioxidant capacity (DPPH) and activity (ORAC) of extracts from treated and untreated strawberry and kiwifruit samples. Values with the same letter within a column at superscript, for each fruit type, were not significantly different at $p < .05$, based on Tukey's test.

Sample	DPPH	ORAC
Strawberry (*Fragaria + ananassa*) var. *Alba*		
Untreated	344.0 ± 19.3^{b}	998.3 ± 47.4^{ab}
PEF_100	367.5 ± 19.4^{ab}	933.3 ± 33.9^{bc}
PEF_200	398.1 ± 15.9^{a}	1128.1 ± 58.1^{a}
OD_S	368.6 ± 12.9^{ab}	803.3 ± 47.8^{cd}
OD_T	336.5 ± 26.1^{b}	735.0 ± 23.5^{d}
PEF_100 + OD_S	270.6 ± 3.8^{c}	703.3 ± 51.3^{d}
PEF_200 + OD_S	291.7 ± 14.0^{c}	708.2 ± 30.8^{d}
PEF_100 + OD_T	266.6 ± 28.4^{c}	708.6 ± 53.4^{d}
PEF_200 + OD_T	272.6 ± 7.3^{c}	738.7 ± 38.7^{d}
Kiwifruit(*Actinidia deliciosa*) var. *Hayward*		
Untreated	56.1 ± 3.3^{ab}	72.9 ± 5.3^{b}
PEF_100	60.1 ± 5.0^{a}	86.4 ± 5.7^{a}
PEF_200	56.9 ± 4.7^{ab}	59.5 ± 4.2^{c}
OD_S	49.5 ± 3.4^{b}	52.1 ± 3.7^{c}
OD_T	55.1 ± 5.4^{ab}	58.4 ± 4.1^{c}
PEF_100 + OD_S	28.4 ± 3.2^{b}	53.9 ± 4.5^{c}
PEF_200 + OD_S	25.7 ± 3.0^{c}	52.8 ± 3.6^{c}
PEF_100 + OD_T	20.7 ± 3.6^{c}	28.8 ± 2.3^{d}
PEF_200 + OD_T	24.0 ± 4.8^{c}	53.3 ± 3.4^{c}

Values represent the mean ± SD ($n = 3$) and are expressed as micromole Trolox equivalents per gram of dried fruit powder (μmol Trolox equivalent/g dw).

Table 6
The pH values of strawberry and kiwifruit pre-treated with PEF followed by osmotic dehydration in hypertonic solutions with sucrose (OD_S) or trehalose (OD_T).

Treatment	pH (Strawberry)	pH (Kiwifruit)
FRESH	4.7	5.0
PEF_100	5.0	5.2
PEF_200	4.9	5.1
OD_S	5.1	5.1
OD_T	5.1	5.1
PEF_100 + OD_S	5.0	5.3
PEF_200 + OD_S	5.2	5.4
PEF_100 + OD_T	5.3	5.6
PEF_200 + OD_T	5.2	5.5

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

X All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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Urszula Tylewicz: Investigation, Writing-Original Draft, Writing-Review-editing; **Gabriel Oliveira:** Investigation, Writing-Original Draft, Writing-Review-editing; **Marie Alminger:** Project administration, Supervision; **Liisa Nohynek:** Investigation, Writing-Original Draft, Writing-Review-editing; **Marco Dalla Rosa**: Supervision; **Santina Romani**: Conceptualization, Writing-Review-editing.