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TITOLO TESI

Application of Pulsed Electric Fields for the Modulation of Chemico-Physical Properties of Different Foods

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Abstract

Pulsed Electric Field (PEF) technology is one of the most appealing emerging non-thermal technology thanks to its lower energy consumption and short treatment times. It consists of an electric treatment of short duration (from several ns to several ms) with electric field strengths from 0.1 to 80 kV cm^{-1} that result in an increase of cell membrane permeability, phenomenon termed as electroporation.

In this PhD thesis, PEF technology was investigated with the aim of improving mass transfer in plant- and animal-based food matrices by using it alone or in combination with conventional food processes. Different electroporation assessment methodologies were investigated for the optimization of PEF processing parameters. In this respect, the degree of membrane permeabilization in plant and animal foods was explored employing electrical impedance spectroscopy, current-voltage measurements and magnetic resonance imaging as electroporation assessment methods.

The research findings provided useful insights and calls for critical choice of electroporation assessment methods for the selection of adequate PEF treatment conditions. It was outlined that the effect of electroporation is highly dependent on the food matrix characteristics, and on secondary phenomena occurring in the cell structure undergoing PEF treatment, such as the water re-distribution in the tissue due to the exchange of fluids between intra- and extra-cellular environments.

This study also confirmed the great potential of combining PEF technology with conventional food processes, with the main purpose of improving the quality of the food material and accelerating the kinetics of mass transfers, in both plant and animal tissues. Consistent reduction of acrylamide formation in deep-fat fried potato crisps was achieved by monitoring key PEF process parameters and subsequent manufacturing steps. Kiwifruit snacks showed a significant reduction in drying kinetics when pre-treated with PEF, while their quality was well maintained. Finally, the research results showed that PEF pre-treatments can shorten the brine process as well as the rehydration kinetics of fish muscles.

Contents

List of Papers

This PhD thesis is based on the work contained in the following Papers, which are referred to in the text by their Roman numerals. The Papers are attached at the end of the thesis.

I **Genovese, J.**, Tappi, S., Luo, W., Tylewicz, U., Marzocchi, S., Marziali, S., Romani, S., Ragni, L. and Rocculi, P. (2019). Important factors to consider for acrylamide mitigation in potato crisps using pulsed electric fields. I*nnovative Food Science & Emerging Technologies*, 55, pp.18-26.

II Tylewicz, U., Tappi, S., **Genovese, J.**, Mozzon, M. and Rocculi, P. (2019). Metabolic response of organic strawberries and kiwifruit subjected to PEF assisted-osmotic dehydration. *Innovative Food Science & Emerging Technologies*, 56, p.102190.

III Schouten, M.A., **Genovese, J.**, Tappi, S., Di Francesco, A., Baraldi, E., Cortese, M., Caprioli, G., Angeloni, S., Vittori, S., Rocculi, P. and Romani, S. (2020). Effect of innovative pre-treatments on the mitigation of acrylamide formation in potato chips. *Innovative Food Science & Emerging Technologies*, 64, p.102397.

IV Cropotova, J., Tappi, S., **Genovese, J.**, Rocculi, P., Dalla Rosa, M. and Rustad, T. (2021). The combined effect of pulsed electric field treatment and brine salting on changes in the oxidative stability of lipids and proteins and color characteristics of sea bass (*Dicentrarchus labrax*). *Heliyon*, 7(1), p.e05947.

V Cropotova, J., Tappi, S., **Genovese, J.**, Rocculi, P., Laghi, L., Dalla Rosa, M. and Rustad, T. (2021). Study of the influence of pulsed electric field pre-treatment on quality parameters of sea bass during brine salting. *Innovative Food Science & Emerging Technologies*, 70, p.102706.

VI Tylewicz, U., Mannozzi, C., Castagnini, J.M., **Genovese, J.**, Romani, S., Rocculi, P., Dalla Rosa, M. (2021). Application of PEF- and OD- assisted drying for kiwifruit waste valorization. *Innovative Food Science & Emerging Technologies* (under review).

VII **Genovese, J.**, Tylewicz, U., Mannozzi, C., Castagnini, J.M., Romani, S., Rocculi, P., Dalla Rosa, M. (2021). Kiwifruit waste valorization through innovative snack development. *Acta Horticulturae, X International Symposium on Kiwifruit* 1332, pp. 407-414. 2021.

VIII **Genovese, J.**, Tappi, S., Tylewicz, U., D'Elia, F., de Aguiar Saldanha Pinheiro, A.C., Rocculi, P. (2021). Dry-salted cod (*Gadus morhua*) rehydration assisted by pulsed electric fields: modelling of mass transfer kinetics. *Journal of the Science of Food and Agriculture* (under review).

IX **Genovese, J.**, Kranjc, M., Serša, I., Petracci, M., Rocculi, P., Miklavčič, D., Mahnič-Kalamiza, S. (2021). PEF-treated plant and animal tissues: insights by approaching with different electroporation assessment methods. *Innovative Food Science & Emerging Technologies*, 74, 102872.

1 Introduction and Objectives

Among the emerging non-thermal technologies, Pulsed Electric Field (PEF) technology represents one of the most appealing due to its reduced heating effects and short treatment times. Moreover, thanks to the reduced energy consumptions (generally between 1 and 15 kJ kg^{-1} of product), PEF technology is gaining industrial attractiveness. Nevertheless, due to the unique characteristics and properties of the biological tissues processed, a case-by-case PEF treatment optimization is often required. Therefore, a deeper knowledge on the modifications occurring after electroporation and their relation with the complexity of the food material tested are of high value to open new ideas for electroporation-based treatments in the food industry. Lab- and pilot-scale tests are needed for the optimization of new electroporation-based treatments for food production. Moreover, an appropriate selection of electroporation assessment methods, able to quantify the effects of PEF treatment in highly inhomogeneous matrices, is crucial for the optimization of the treatment itself.

In this direction, the present PhD project aimed at studying the application and optimization of PEF technology for the improvement of mass transfer phenomena in biological tissues of interest for food, both of plant and animal origin.
This PhD thesis is based on the Papers published within the research project, and it specifically reports on:

- The comprehension of the electroporation phenomenon due to the application of an external electric field to plant and animal tissues, and the evaluation of physical and structural modifications. Moreover, various electroporation assessment methodologies have been investigated for the optimization of PEF processing parameters.
- The combination of PEF pre-treatment with conventional food processes for the improvement of food quality and/or to accelerate the kinetic of mass transfers. In particular, the optimization of PEF-treatment in real food production has been clustered in the following potential industrial applications:
  - enhance the release of Maillard reaction substrates from the potato tissue and mitigate the concentration of toxicants (i.e. acrylamide) in fried potato crisps.
  - accelerate the dehydration and drying kinetics for the production of fruit snacks and for the preservation of their physical and nutritional quality features.

- accelerate the kinetics of curing and rehydration of fish muscles, and examine the effects of the application of high-voltage pulses on the fish protein and lipid oxidation stability.

Chapter II aims at giving a brief overview of the up to date knowledge on the electroporation phenomenon and its exploitation in plant- and animal-based food matrices. The main findings of this research project are elucidated in Chapter III and IV, with the main focus on the electroporation assessment methods investigated and the combination of PEF treatment with conventional food processes, respectively. The connections between the research findings and the related relevant published paper are marked in each section. The materials and methods used are described in detail in the papers appended at the end of the thesis.

2 Pulsed Electric Fields (PEF)

2.1 Overview

Pulsed electric field (PEF) technology is based on the so-called electroporation phenomenon. When a biological tissue is exposed to an externally applied electric field of sufficient strength, cell membrane permeability increase will occur. This phenomenon is explained by the creation of aqueous pathways in the lipid domain of the cell membrane [1]. PEF technology consists of an electric treatment of short time (from several ns to several ms) with electric field strengths from 0.1 to 80 kV cm^{-1} [2]. High electric fields (> 20 kV cm^{-1}) are generally used to inactivate alterative and pathogenic microorganisms, and quality related enzymes [3]; while lower electric field strengths (0.5 – 1 kV cm^{-1}) are used alone or in combination with other food processes to increase mass transfers [4].

The effectiveness of PEF treatments is strictly related to both, the process parameters, also definable as independent variables, and the characteristics of the biological material (dependent variables), which change during the high-voltage treatment. The main operating parameters include [5]:

- The voltage across the electrodes (U_0). For parallel plate electrode configuration with constant interelectrode gap, the electric field (E) in V cm^{-1} can be estimated as $E = U_0/d$, where d is the distance between the electrodes (in cm).
- The pulse characteristics, including pulse shape, pulse duration (τ) and polarity.
- The total number of successive pulses (n).
- The pulse frequency (f) reported in Hz. Some devices allow the delivery of different sequences of pulses at variable frequency.

Experimental works have revealed that electroporation occurs when a threshold value of potential difference across the membrane is exceeded (i.e. transmembrane potential that depends on size and shape of cells, as well as the structure and properties of the tissue [6]). Depending on the PEF treatment conditions described above and on the total exposure time, the electroporation process can be reversible with cell membrane recovering its integrity, or it can irreversibly damage the cell membrane. The reversibility of the process has given many advantages in biomedical applications for *in vivo* delivery of drugs, plasmids, antibodies, etc.[7]–[9]; while most of the food applications rely on the irreversibility of the process [10].

It has been widely demonstrated that pulsed electric field can be used in food processes as a pre-treatment method in order to enhance the subsequent process kinetics or to modify the quality of the final product. It can be used in combination with the most energy-consuming processes, such as freezing [11], drying [12], extraction [13], which are based on heat and/or mass transfers. Due to electrically induced disintegration of the cell membrane (and some other organelles), the progress of drying, osmotic dehydration, or freezing can be enhanced, and thus the specific energy consumption of aforementioned processes can be reduced. The energy consumption is an important factor for the estimation of PEF industrial attractiveness. PEF treatment requires a moderate energy consumption, generally between 1 and 15 kJ kg^{-1} of product [14].

Electroporation mechanisms have been widely investigated in model systems and static operation conditions; however, how dynamic processes and the complex geometry of matrices could influence the outcome is not clear and need further explanations.

2.2 PEF in Plant-Based Food Processes

In the last decades, most of the published food-related PEF applications focused on the treatment of biological tissues of plant origin, either for their preservation or for the enhancement of mass transfer phenomena [15]. Plant materials constitute an important category of foodstuff, and PEF treatment of fruit and vegetables meet a variety of applications. Specific characteristics of the plant matrices, such as cell size and shape, electrical resistivity and structure, to name some, contribute, together with processing parameters (as described in Section 2.1), to the effectiveness of PEF treatment. Plant tissues are organized in cells, of different sizes and shapes, and having specific functions. A plant cell can be simply described as composed by protoplasm surrounded by the plasma membrane and the cell wall, and embedded in an extracellular media. Adjacent cells are joined together by a layer of polymeric substances (i.e. the *middle lamella*), and junctions between middle lamellae are filled with air [16]. The cell membranes constitute a physical barrier to the diffusion processes, thus their permeabilization due to PEF treatment can influence mass transfers. Many researchers have already demonstrated that the electroporation of plant-cell membrane promotes the selective release of different intracellular components [14], [17], [18]. The application of PEF for the disruption of food plant tissues has been proposed as an alternative to the conventional food processing, thanks to the low-energy consumption requirements and the non-thermal characteristic of such technology. Many studies have reported on the increased efficiency of food processes implying mass transports if the plant material undergoes to a high-voltage pre-treatment. For instance, the improved extraction yields in PEF-assisted juice and valuable compounds extractions have been deeply studied for different plant-based materials, such as grapes, olives, sugar beets, etc. [19]–[21]. Several studies have also demonstrated the significant impact of PEF pre-treatment in accelerating the drying rate, successfully achieving time

reductions from 5 to 60 % [22], [23].

Within the present PhD project, different papers report on the application of PEF treatment, alone or in combination with other subsequent processing steps, to plant-based food materials. **Paper I** and **III** focused on the increasing the release of Maillard reaction substrates in PEF-treated potato tubers for the mitigation of toxicants concentration (i.e. acrylamide). The enhancement of drying kinetics (hot air drying or osmotic dehydration) of kiwifruits and strawberries pre-treated by PEF is described in **Papers II**, **VI** and **VII**.

2.3 PEF in Animal-Based Food Processes

The potential application of PEF technology for meat and fish processing has been mentioned for the first time about 20 years ago [24], and although there is a general interest, the literature appears to be still very scarce in the field. Compared to plant cells, skeletal muscle cells present a higher degree of structure complexity and a greater variability among raw materials. Moreover, the *post-mortem* maturation phase (i.e. conversion of muscle to meat) causes significant tissue structural modification, that should be taken into consideration. Skeletal muscles are predominantly comprised of muscle fibres each surrounded by a layer of connective tissue (i.e. endomysium). The connective tissue also compartmentalizes muscles in fibre bundles (i.e. perimysium), and constitute also the external envelope of muscle (i.e. epimysium). Almost the entire intracellular volume is occupied by myofibrils [25]. The effectiveness of PEF treatment on tissues of animal origin depends on PEF processing parameters, but also on the physicochemical characteristics of the meat and the *pre-* and *post-mortem* conditions. Researchers have reported that the application of PEF treatments to meat and fish products could affect the structure and modify the functional attributes such as tenderness [26], water-holding capacity [27] and color [28]. Therefore, PEF treatment of animal-based food products could influence the maturation rate and increase the mass transfers, accelerating the removal of water during drying [29] or accelerating the marination / curing processes [30].

Within the scope of this PhD project, **Papers IV**, **V** and **VIII** report on the application of PEF technology to animal tissues for the enhancement of curing processes (**Paper IV** and **V**) and to increase the rehydration rate (**Paper V**) in fish products.

3 Electroporation Assessment Methods

The application of PEF treatment in food processing is currently under intensive research and development, thus an appropriate choice of methods assessing changes occurring in the biological matrices due to electroporation becomes crucial. Although a considerable number of scientific papers have accumulated in the field, nor or very few studies report detailed information on the detection and quantification of the effect of electroporation in complex and highly inhomogeneous multicellular systems, such as real food systems. In fact, due to the high complexity of the biological tissues processed (of plant and animal origin), generally a case-by-case PEF treatment optimization protocol is required [31]–[33]. This chapter presents the electroporation assessment methods used in this research and the main findings also related to the different characteristics of the biological tissue analysed (plant and skeletal muscle tissues of interest for food).

3.1 Electrical Impedance Spectroscopy

The electrical impedance spectroscopy (EIS) is a powerful technique for the characterization of biological materials and the investigation of their electrical properties. Most food matrices are composed of cells, and by the simplest electrical model of an individual cell, the cell is represented as an insulating membrane, while the intra- and extra-cellular media are modelled as ionic solutions (electrolytes). Electrolytes behave as a resistive (ohmic) load up to hundreds of MHz, while, in contrast, membranes exhibit high resistance but also considerable capacitance. Typically, passive electric properties (conductivity, σ, and permittivity, ε) are strongly dependent on the frequency [34]. Looking at one single cell or a suspension of cells between electrodes, several dispersions related to different polarization mechanisms can be identified. At low frequencies (f = 1 – 10^4 Hz), the α-dispersion is related to the lateral movement of ions along the insulating membrane; the β-dispersion (f = 10^4 – 10^8 Hz) reflects the polarization of the cell membrane; the rotation of molecules having a permanent dipole (water, proteins) cause the γ-dispersion ($f > 10^9$ Hz) [35], [36]. Therefore, cells with their membranes will influence tissue impedance in a frequency range up to several MHz. Since the cell membrane acts as a capacitance, low-frequency currents cannot pass via the intracellular route, thus the magnitude of impedance will be higher across the low frequency range. High-frequency currents are, on the contrary, free to flow through the cell membrane, i.e. the membrane is 'invisible' to high-frequency

fields.

Typically, for most plant and animal tissues, the transition between low- and high-frequency behaviour arises at the spectra band starting from about 50 Hz and ending at about 10 MHz [4], [37]. Being that the electrical impedance is very sensitive to the permeability of cell membranes, measurement of changes in the complex impedance have been suggested as a method to estimate the degree of tissue damage due to PEF treatment [38]. The utility of impedance measurement has already been demonstrated for various biological systems [39]–[41].

3.1.1 New Findings

In **Paper IX** measurements of changes in electrical properties of different plant and animal tissues exposed to various amplitudes of applied voltage [U] are reported. The electrical impedance of the biological tissues of plant and animal origin (i.e. potatoes, apples and chicken breasts) was measured before the application of high-voltage pulses and at the end of treatment (immediately, i.e. between 3-5 seconds after pulsation). For more details in the methodology used please refer to Paper IX.

In Figures 3.1 and 3.2 is shown the ratio between the absolute impedance value after applying PEF treatment ($|Z_2|$) and before the treatment ($|Z_1|$). In the plant tissues studied (potato and apple), as a result of β-dispersion, it has been observed the frequency dependence of the impedance (Fig. 3.1, above). Moreover, at sampling frequency of 5 kHz, a consistent decrease of the normalized impedance following PEF treatment was attained, and the non-linear trend suggested the thresholding nature of the phenomenon (Fig. 3.1, below). Threshold values were found to be lower in potatoes than in apples ($U \sim 150$ V; 250 V cm^{-1} for potatoes , $U \sim 300$ V; 500 V cm^{-1} for apples), and these results were not expected. For instance, since the critical transmembrane potential is reached with external electric fields proportionally decreasing with the cell radius [42], and since the potato cell size is smaller than that of the apple (i.e. potato ≈ 35 μm, apple ≈ 130 μm) a higher electroporation threshold for potato was awaited. The obtained results could be justified taking into account that the measured electrical properties of plant tissues could be affected, not only by the permeabilization of cell membrane due to electroporation, but also by the physico-chemical characteristic of the tissue, such as the cell packing density, the distribution of moisture, the initial cell turgor pressure, the spatial distribution of air.

Measurements of the electrical properties of animal tissues were performed in chicken broiler *Pectoralis major*. Chicken breast was selected as a reference skeletal muscle, as it is one of the most homogeneous muscle (i.e. constituted almost entirely of fast-twitch glycolytic fibre type [43]). As it is shown in Figure 3.2, the decrease of the absolute impedance was much smaller compared to plant tissues. In this case, the measurements were also performed considering the possible anisotropy of the skeletal muscle at different level of PEF (i.e. impedance varies with current passing along or across the muscle fibres). For this purpose, a 4-needle electrode configuration system was employed (more details in Paper IX). The electrical impedance spectroscopy

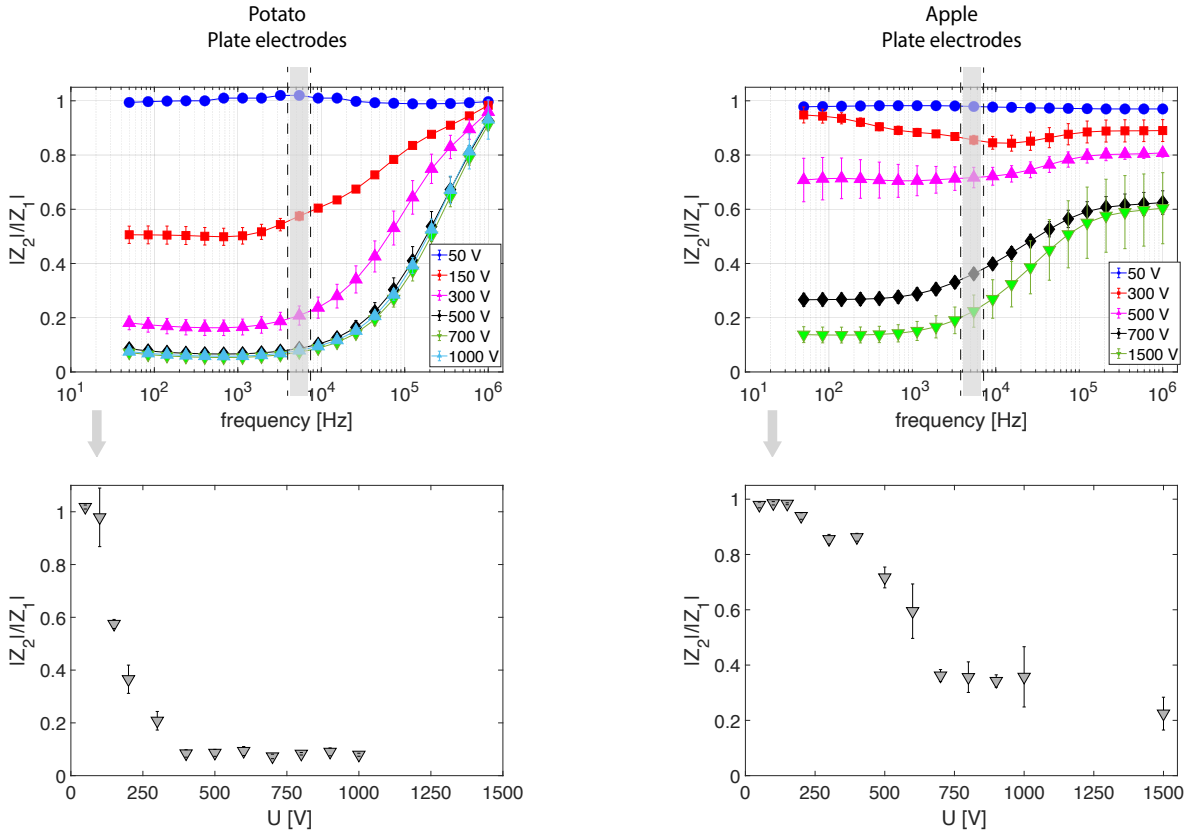


Figure 3.1: Plant tissues normalized absolute impedance versus frequency at different applied voltage [U] amplitudes measured with plate-electrodes configuration (not all the applied voltages are shown for clarity of presentation) (above). Values at 5 kHz are marked with gray color background. Normalized absolute impedance at sampling frequency of 5 kHz versus the applied voltage [U] (below). Results are expressed as means ± standard deviations (error bars) of $n = 4$ (adapted from Figures 1 and 2 in Paper IX).

did not show any skeletal muscle tissue anisotropy, as the variations of the normalized absolute impedance did not present any statistical differences ($p > 0.05$) between the parallel and the perpendicular orientations of the muscle fibres (Fig. 3.2, right).

This result was expected as, in the present study, the analyses of chicken electrical properties were performed ca. 48 h *post-mortem*, a long enough ageing period to cause significant structural modification of the meat. In fact, in the *post-mortem* maturation phase (i.e. conversion of muscle to meat), the proteolytic stage activation leads to fragmentation of myofibrils and degradation of the cytoskeleton, that in turn lead to a decrease of the electrical anisotropy of meat [44].

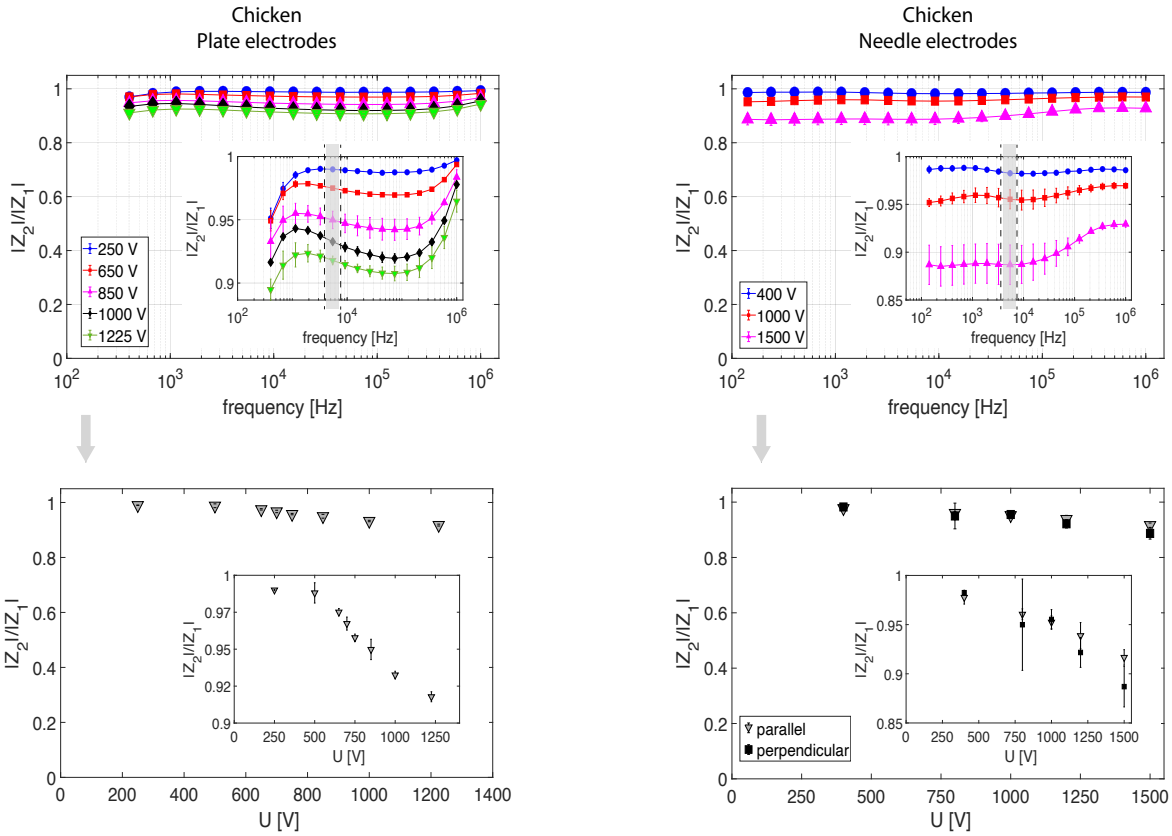


Figure 3.2: Animal tissues normalized absolute impedance versus frequency at different applied voltage [U] amplitudes measured with plate-electrodes, and 4-needle-electrodes configuration (not all the applied voltages are shown for clarity of presentation) (above). Values at 5 kHz are marked with gray color background. Normalized absolute impedance at sampling frequency of 5 kHz versus the applied voltage [U] measured with plate-electrodes, and 4-needle-electrodes configuration (below). Measurements with 4-needle electrodes were performed applying high-voltage pulses parallelly or perpendicularly to the muscle fibre orientation. Results are expressed as means ± standard deviations (error bars) of $n = 4$ (adapted from Figure 3 in Paper IX).

3.2 Voltage-Current Signals Analysis

Although not frequently used, another approach for the evaluation of tissue electrical properties involves the analysis of the voltage and current waveforms recorded during the application of the electric pulses. Electric current signals can be used to detect changes of the dielectric properties of the cell membrane in real-time and, thus, can be used to assess the electroporation process. A discrete number of research papers reported the analysis of the current signals during the application of the high-voltage pulse, and it has been demonstrated that the dynamics of current can be used as a key characterization feature of tissue electroporation [40], [45], [46]. A typical current-time course, during PEF application to a tissue, consist of a rapid initial current increase followed by an exponential decrease which corresponds to membrane charging. When electroporation occurs, the current shows further increase before reaching a constant level at the end of the pulse (a schematic presentation in Fig. 3.3 A). This further increase is consistent with the cell membrane permeabilization. In fact, when cell membrane electroporation / permeabilization increases, the conductivity increases, so the total current increases at constant voltage applied.

From voltage and current recordings it is possible to define also the conductivity during the pulse, if the cell constant of the experimental set up is know. Dynamic conductivity measurements can be used to study the cell membrane conductivity changes induced by electroporation [37].

3.2.1 New Findings

Raw plants and skeletal muscle of food interest were subjected to various amplitudes of applied voltage (U) and changes in the electrical properties due to electroporation were evaluated analysing the current waveforms recorded during the application of PEF treatment. Detailed methodology is described in **Paper IX**. Figure 3.3 A depicts the electric current difference between the value of I_{fin} (current reached at the end of the pulse, i.e. 100 μs after initial current increase) and I_{init} (current value at 10 μs after initial current increase) normalized to the current value at the end of the pulse (ΔI_{norm}) for all the specimens investigated. In plant tissues (potato and apple), it can be observed that the maximum increase of current during the pulse has been observed after applying a certain 'critical' U value, definable as electroporation threshold. In particular, in potato tissue, the permeabilization level was attained at applied voltage amplitudes of about 100 V (i.e. approx. 166 V cm^{-1} of the voltage-to-distance ratio), while in apple tissue the maximum increase of current during the pulse has been observed at permeabilizing applied voltage intensities of about 150 V (i.e. approx. 250 V cm^{-1} of the voltage-to-distance ratio) (Fig. 3.3 B). On the contrary, the animal tissue investigated (i.e. chicken breast) did not show identical behaviour in terms of the current-time course, displaying a constant level of current during the pulse at all the applied voltage amplitudes measured.

In addition, measured voltage and current waveforms, when a treatment chamber with parallel-plate electrode configuration was used, were analysed in terms of con-

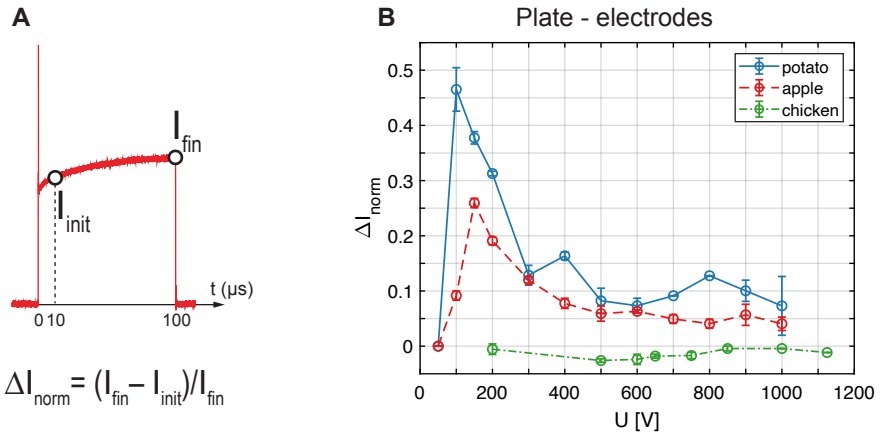


Figure 3.3: Normalized electric current difference (ΔI_{norm}) between the final (I_{fin}) and initial (I_{init}) current values of the pulse portion with current exponentially increasing during the pulse (A). The difference was normalized to the current value at the end of the pulse (I_{fin}). An example of ΔI_{norm} obtained in potato, apple and chicken tissue exposed to electric pulses applied using plate electrodes (B). U – applied voltage. (adapted from Figure 4 in Paper IX)

ductivity at 100^{th} μs of the pulse (i.e. at the end of the first pulse). The conductivity was calculated as:

$$\sigma = (I/U)(d/S)$$

where (I/U) is the ratio between current and voltage measured at 100^{th} μs of the pulse, d is the distance between the electrodes, and S the contact surface between the electrode and the sample.
Figure 3.4 displays the conductivity dependence on applied voltage amplitudes (U) for plant and skeletal muscle studied. Raw data were modelled following a sigmoid curve (potato R^2 = 0.90; apple R^2 = 0.83; chicken R^2 = 0.08). While in potato specimen the conductivity slowly increases at U between 50 and 500 V, reaching a plateau at U $\geq$ 600 V; in apple σ shows an abruptly increase between 100 and 200 V, and saturates at higher voltage amplitude applied. Conversely, the conductivity of the chicken sample did not display any increase with increased voltage amplitude applied. A simple interpretation of the observed conductivity trends would be that potato tissue is, from the water distribution perspective, much more homogeneous, and the conductivity increases almost linearly with the delivered electroporation pulses as more and more cells are electroporated since this renders a proportionally greater amount of extracellular liquid available for ionic transport (and thus current conduction). In the apple tissue, due to large amount of air in the tissue (i.e. higher porosity than potato) and disassociated domains of tissue caused by pockets of air, higher field strength is necessary to cause a sufficient release of liquid. In fact, once a sufficiently

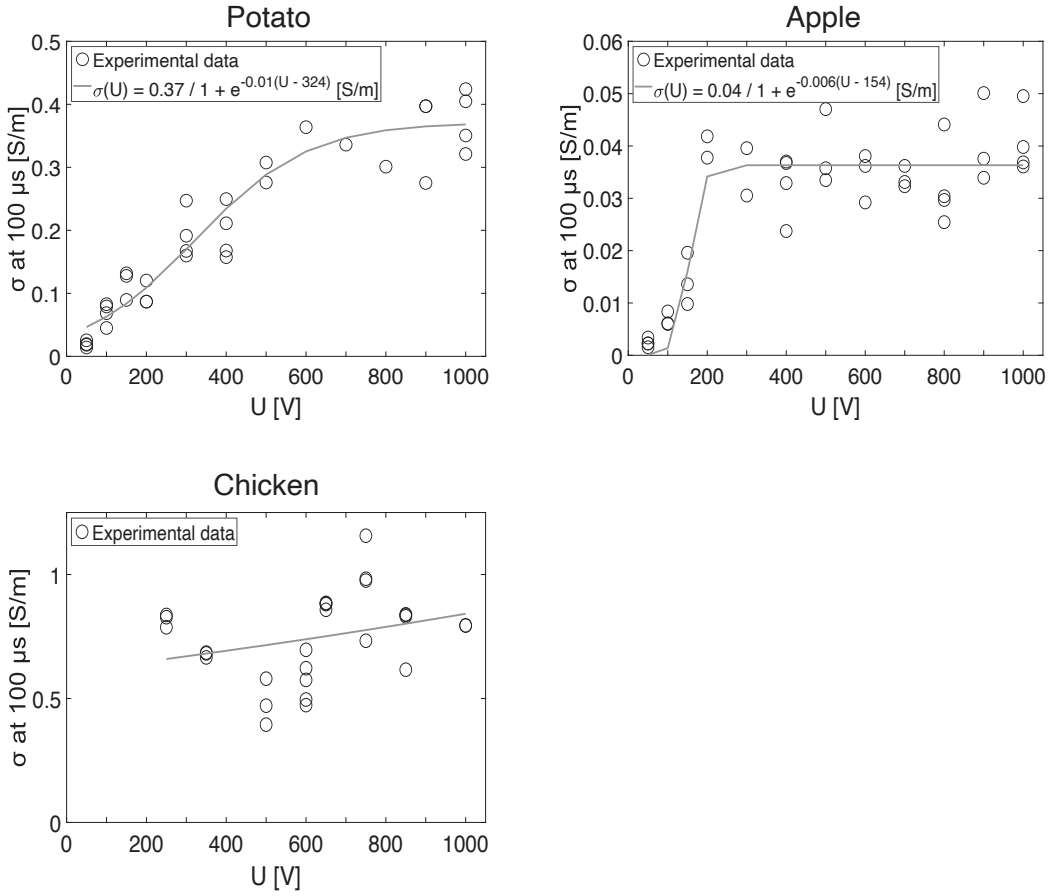


Figure 3.4: Conductivity values at 100^{th} μs of the first pulse recorded versus the amplitude of applied voltage [U]. Dashed lines represent the sigmoid function used to model the experimental data (adapted from Figure 5 in Paper IX).

conductive pathway in tissue is formed by the critical amount of released liquid, a step-like increase of conductivity is observed. When chicken breast was analysed negligible changes of current/conductivity were observed (Figures 3.3 and 3.4).

Some of the consequences of membrane permeabilization are related to structure modifications and intracellular fluid leaking outside the cell [47]. These consequences could play an important role in affecting the measured electrical properties of plant cells, characterised by high gradient of osmotic pressure between the intra- and extracellular fluids. On the other hand, *post-mortem* skeletal muscles undergoes to a series of physical and biochemical modifications related to myofibrillar structure, cytoskeleton, and membranes. As *rigor* progresses, the space for water to be held in the myofibrils is reduced, and fluid can be forced into the extra-myofibrillar spaces, where it is more easily lost as drip [48]. Therefore, it is quite possible that, at the time we performed treatment and analysis (ca. 48h *post-mortem*), the muscle fibre membranes were already significantly degraded [49] and so no further changes of

the electrical properties were detected following the application of electric pulses.

3.3 Magnetic Resonance Imaging and T_2 Mapping

Biological tissues present high complexity of structure and spatially dependent properties. It has been demonstrated that the modification of cell membrane permeabilization due to electroporation is also associated with modification of the structure (i.e. intra- and extracellular volume change), and ionic concentration variation due to leakage of intracellular content [41]. Hence, other techniques evaluating the effects of tissue electroporation have been suggested. Magnetic resonance imaging (MRI) technique has been applied to monitor the spatially dependent effect of PEF treatment in vegetable tissues [50], [51]. Transverse relaxation time T_2 has been employed as an indication of the redistribution of water and solutes in the tissue after the PEF treatment [52]. T_2 relaxation times (also know as *spin-spin* relaxation times) are a measure of the effectiveness of spin coupling with neighbouring spins and with other source of magnetic fields. Local magnetic fields and possibly also molecular motions are both associated with the change of chemical environment. Electroporation pulses that can induce structural changes, such as damages of cell membranes can therefore also alter the chemical environment, with a consequential influence on the NMR relaxation rates. In fact, the primary effect of electroporation is related to membrane disruption and to the consequential release of the intracellular content. Moreover, the MRI and magnetic resonance electrical impedance tomography (MREIT) were proposed as methods to monitor the electric field distribution in the tissue during the application of PEF treatment [51].

3.3.1 New Findings

By means of MREIT we obtained the electric field distributions in potato, apple and chicken samples when subjected to PEF treatment. For each biological sample considered, an optimal voltage amplitude was selected, to obtain a good signal-to-noise ratio of MR signals (i.e. potato 750 V; apple 1180 V; chicken 860 V). For more details regarding the methodology please refer to **Paper IX**. In addition to MREIT, T_2 mapping was also performed in the same samples to determine changes of tissue water content that would occur due to the application of electroporation pulses. In fact, T_2 relaxation value is correlated by the proton exchange between water and solutes, and by diffusion of water protons through internally generated magnetic field gradients, causing magnetic susceptibility differences in the tissue exposed to the magnetic field, such as interfaces between air and liquid filled pores. Therefore, T_2 values reflect to a certain extent the structure of the sample based on its water content [53].

As reported in **Paper IX**, the effect of PEF treatment on T_2 relaxation times were evaluated by measuring the T_2 times before and after the treatment. Moreover, changes of T_2 relaxation rates were related to the distribution of the electric field obtained by MREIT. In Figure 3.5 is depicted an example of subtracted T_2-weighted

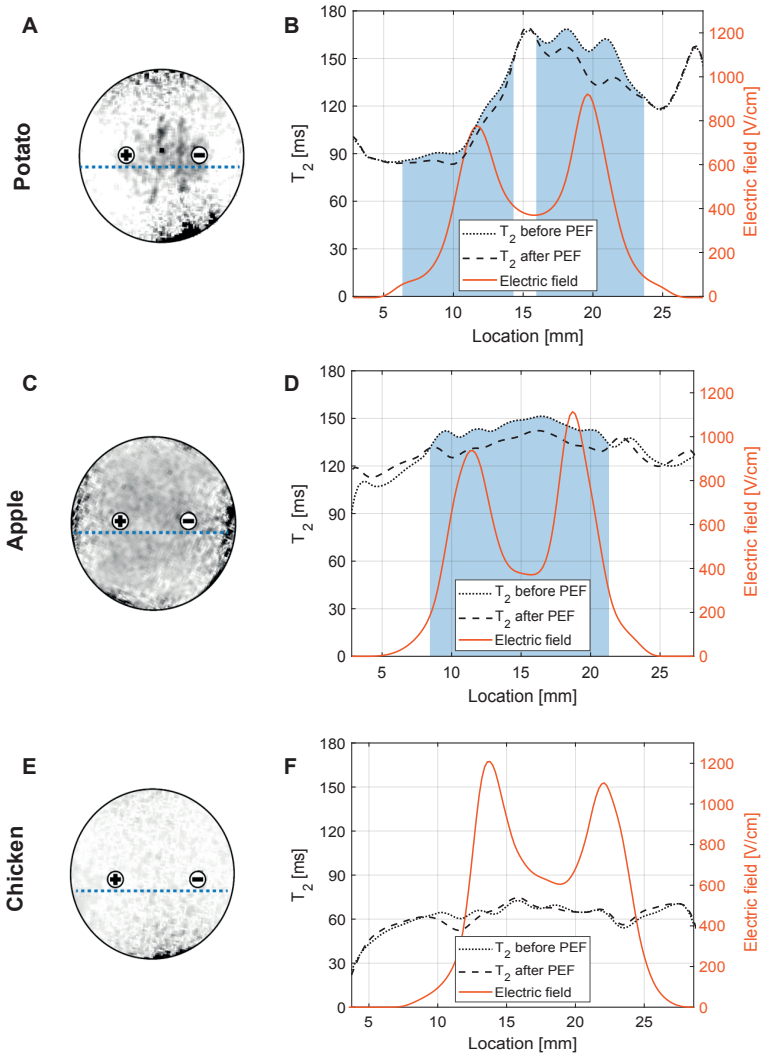


Figure 3.5: Subtraction of T_2–weighted images acquired before and after PEF treatment in potato tuber (A), apple tissue (C), and chicken breast (E). Location of electrodes are marked with + and – sign, while an evaluation line is depicted as blue dotted line. T_2 values acquired before (dotted lines) and after PEF treatment (dashed lines) in potato tuber (B), apple tissue (D), and chicken breast (F) along the evaluation line. Electric field (solid lines) was obtained by means of MREIT. Ranges of electric field corresponding to variations of T_2 value are marked by coloured background (adapted from Figure 6 in Paper IX).

images (i.e. obtained as (T_2-map after electroporation) - (T_2-map before electroporation)) of potato (A), apple (C) and chicken (E). T_2 changes and electric field distributions were evaluated taking into consideration an area crossing the middle of the sample. Figure 3.5 B,D,F shows a general decrease of T_2 relaxation times following the PEF treatment in both potato and apple tissue, while no considerable changes were induced in the skeletal muscle exposed to high-voltage pulses. The general decrease of T_2 in plant tissues could be ascribed to the loss of compartmentalization and diffusion of intracellular water and ion leakage through the tonoplast and the plasmalemma membranes, resulting in inner morphology modifications (e.g., overall volume shrinkage) and in different water-solutes interactions. On the contrary, no changes in bulk water mobility in chicken breast muscles were detected by means of MR imaging. In poultry the *rigor mortis* onset is more rapid than in bovine and porcine muscles since, chicken breast in particular, they are mainly composed of fast-twitching fibres that are associated with the anaerobic glycolysis [49]. As *rigor* progresses, the space for water to be held in the myofibrils is reduced, and fluid can be forced into the extra-myofibrillar spaces, where it is more easily lost as drip. Therefore, the absence of T_2 changes in this case study is reinforcing the conclusion that the skeletal muscle investigated, ca. 48h *post-mortem*, had fibre membranes already highly degraded, as reported in subsections 3.1.1 and 3.2.1, and so no release of intracellular water could be detected by MRI.

4 PEF pre-Treatment to Enhance Mass Transfers

This chapter will briefly describe the application of PEF technology in the food processes studied in this research project. PEF technology was used as a pre-treatment method to enhance the kinetics of mass transfers and modify the quality of both plant- and animal-based food products.

4.1 Toxicants Reduction in Deep-Fat Fried Products

Certain foods, more specifically certain food components such as asparagine and reducing sugars, could lead to the formation of toxicants, such as acrylamide, during heat treatment at temperatures above 120 °C as a result of the Maillard reaction [54]. Acrylamide has been identified as a contaminant in a range of fried and oven-cooked foods (e.g. French fries, potato crisps, bread and cereal) and drinks (e.g. coffee); and its classification as probably carcinogenic in humans has caused worldwide concerns. Recently, the Commission Regulation (EU) 2017/2158 of 20 November 2017 has established new 'mitigation measures and benchmark levels for the reduction of the presence of acrylamide in foods', which aim to ensure that food businesses put in place steps to mitigate acrylamide formation. [55] analysed European manufacturers' data on acrylamide in potato crisps from 2002 to 2016, and the study showed that, even though acrylamide levels in potato crisps in Europe have been levelled off in recent years, > 5% of samples exceeded the regulated benchmark level for potato crisps (0.75 mg kg^{-1}).

The application of PEF for potato snacks pre-treatment has been extensively studied, and many researchers have already reported high numbers of benefits that could be achieved by applying electric pulses to raw potatoes [56]–[58]. Although in literature it has been reported that PEF treatment of raw potatoes could assist and increase the release of sugars and amino acids that represent the main substrates for the Maillard reaction, consequently leading to formation of lower amounts of acrylamide [59], the effective reduction of acrylamide content in deep-fat fried potato products was still unclear.

4.1.1 New Findings

Paper I and **III** describe the effect of the application of PEF as a pre-treatment, and the effect of subsequent washing steps of treated potato slices, on the Maillard reac-

tion substrates and final acrylamide content, and on the quality of fried potato crisps. In particular, **Paper I** focused on the optimization of either PEF protocol and sample preparation scheme (a presentation of flow scheme in Fig. 4.1), in order to maximise the release of acrylamide precursors from the raw tissue. The efficiency of PEF as a non-thermal pre-treatment on the acrylamide reduction was compared to a conventional blanching in hot water usually used as a pre-treatment in the fried potato industrial lines.

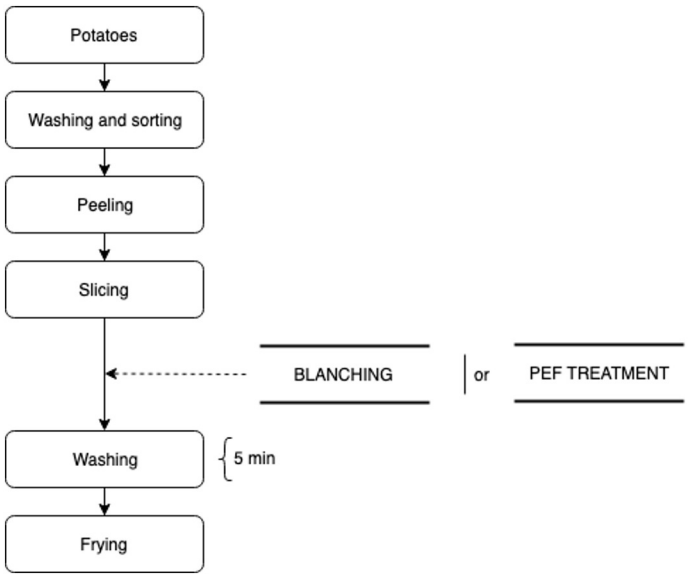


Figure 4.1: Scheme of experimental processing steps for the production of crisps (corresponding to Figure 4 in Paper I).

Thanks to preliminary trials based on the measurements of changes in the electrical properties of both the PEF-treated whole potato tuber and the PEF-treated potato slices, it was possible to observe a more efficient cell disruption due to the high-voltage treatment when applied after the slicing step of the processing flow. Supposedly, the larger surface-area-to-volume ratio exposed to the electric pulses resulted in a more efficient cell membrane permeabilization of potato slices. Moreover, to facilitate the leakage of ions/small molecules from intracellular compartments, the treated slices were subjected to a washing step. Washing of potato slices is a common practice in potato crisps production as it allows the removal of any surface starch residue prior to frying. This work demonstrated that the highest release of cell fluid into the aqueous media was achieved after washing the PEF-treated slices for 5 minutes in distilled water, as after this period of time the maximum variation of the electrical conductivity of the residual washing water was measured.

Following the production flow presented in Fig. 4.1, a consisted reduction of Maillard reaction substrates (i.e. reducing sugars and free asparagine) and acrylamide content was achieved in PEF-treated potato slices, as it is shown in Table 4.1.

Table 4.1: Maillard substrates (glucose, fructose and free asparagine) contents of raw potato slices untreated, after blanching and after PEF treatment, and acrylamide content of untreated, blanched and PEF-treated potato crisps. Results are expressed in mg kg^{-1} of dry weight. Reduction percentages are calculated in relation to the untreated sample (corresponding to Table 1 in Paper I).

Sample	Glucose			Fructose			Asparagine			Acrylamide		
	Mean	% RSD*	Reduction (%)	Mean	% RSD	Reduction (%)	Mean	% RSD	Reduction (%)	Mean	%RSD	Reduction (%)
Untreated	70.0	14.1	-	58.9	1.7	-	10,487.8	8.7	-	2.0	3.3	-
Blanched	50.9	11.9	11.9	56.1	3.5	4.9	6296.1	6.2	40	1.6	9.1	17
PEF-treated	75.4	10.6	n.a†	55.8	3.1	5.4	5416.9	9.3	48	1.4	7.5	31

∗ Percent relative standard deviation (n=3).

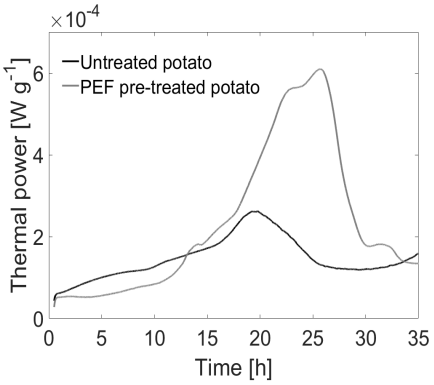
† Not applicable.

The PEF pre-treatment protocol and experimental conditions selected in this study resulted in a reduction of around the 30% of acrylamide content compared to the untreated product, while only the 17% of reduction was observed in potato slices subjected to the conventional blanching pre-treatment. The cell electroporation resulted, therefore, in an improvement of the diffusion of Maillard reaction substrates and so of acrylamide reduction in fried potato crisps compared to the applied heat pre-treatment (Table 4.1). The results of this study seem to indicate that the main reduction promoted by PEF is related to the free asparagine, which was found at concentrations more abundant than reducing sugars. Nevertheless, although the reduction of acrylamide content in PEF pre-treated crisps was significant, the final amount found was still higher than the recommended legislative limits (i.e. 0.75 mg kg^{-1}).

In **Paper III** a combined strategy was developed with the idea of reaching a further reduction of the acrylamide content in fried potato crisps. The PEF-treatment was, therefore, combined with a potato dipping step in *Aureobasidium pullulans* L1 yeast (patented procedure No. WO2019058248A1 [60]) water suspension, in order to investigate the possible synergistic effect of both innovative pre-treatments. In fact, [60] reported for the first time that the yeast *A. pullulans* L1 strain successfully assimilated the free asparagine in a potato homogenate after 30 minutes of contact, leading to a consistent decrease of the acrylamide content in the final fried potato crisps (83% of reduction). Despite the large reduction of acrylamide formation promoted by the enzymatic activity of the yeast (i.e. hydrolytic cleavage of L-asparagine), the exploitation of its efficacy was possible only after a certain enzyme-substrates contact time (i.e. $\geq$ 30 min), not always suitable with industrial production times. The application of a PEF treatment to potato slices before the dipping in the yeast suspension could, therefore, lead to a reduction of the total contact time needed. It was hypothesised that the increased membrane permeability of the potato cells induced by the PEF pre-treatment could lead to a higher release of cell content into the water, that in turn would become richer in nutrients exploitable by the yeast metabolism. This hypothesis was experimentally proven employing isothermal calorimetry to measure continuously and in real-time the heat flow produced by the yeast *A. pullulans* L1 in different substrates, untreated and PEF-treated raw potatoes.

Figure 4.2 displays the thermograms recorded at 25 °C for 35 hours and the total heat (integral of the thermograms) produced by the yeast activity. The heat flow signals can be considered as an index of the yeast growth rate [61], and results reported in Fig. 4.2B suggest that the PEF pre-treatment of the potato tuber had a positive effect on the yeast metabolism stimulating both its entity (i.e. higher heat produced) and its rate (i.e. higher slope of the curve, it is related to the exponential growth phase).

A



B

	Untreated potato	PEF pre-treated potato
Heat [J g^{-1}]	17.9 ± 0.7 ^{a}	31.6 ± 4.0 ^{b}
Slope [W (g·h)$^{-1}$]	$1{\cdot}10^{-5}$ ± 0.1 ^{a}	6.5 ± $4{\cdot}10^{-5}$ ^{b}

Figure 4.2: Thermal profiles (A) and calorimetric parameters (B) measured by isothermal calorimetry during growth of *A. pullulans* L1 strain in contact with untreated potato and PEF pre-treated potato. Different letters in the same line indicate significant differences among samples ($p < 0.05$) (adapted from Figure 4 and Table 2 in Paper III).

Although, the effectiveness of the yeast in reducing the acrylamide content in the fried crisps was enhanced by the PEF pre-treatment of raw potato (i.e. especially after 5 minutes of contact time), the PEF treatment of potato slices alone, followed by a washing step for 5 and 15 minutes, resulted the most effective in reducing the acrylamide formation in potato crisps (i.e. reduction of approx. 36% and 59% compared to the untreated product for 5 and 15 min of washing, respectively).

4.2 Dehydration and Drying of Fruits

Fresh fruits, together with fresh vegetables, are classified as highly perishable products, since their moisture content is generally more than 80% [62]. Dehydration and drying processes to obtain semi-dried and dried fruits offer benefits for the post-harvest management, in terms of preservation (i.e. increased shelf-life and safety), and also in terms of costs reduction for packaging and transport due to smaller mass and volume. The preservation of fruits and vegetables by the removal of water is usually accomplished by conventional convective drying. Nevertheless, there are numerous studies reporting on the drawbacks of the convective drying method, primarily related to the modification of important quality features of the products (e.g. color, texture, flavour, nutrients) and to the relatively high operating times and energy requirements [63]. Hence, it is obvious the reason why in the last 20 years a lot of effort has been put in finding new drying methods able to reduce the operational costs and minimally impact the quality of the food materials [64].

Osmotic Dehydration (OD) is known as partial dehydration process, and it is usually employed as a stand-alone treatment for the production of intermediate moisture fruits or as a pre-treatment prior to drying [65]. OD is a two-way mass transfer process, that causes the partial dewatering of the product due to the osmotic gradient

between the product and the osmotic solution. Together with the drying, the mass transfer is based mainly on diffusion processes, therefore the cellular structure of the food matrix plays an important role. In fact, the intensification of the dehydration and drying can be achieved by the disruption of the tissue cellular structures. PEF treatment, able to induce cell damage, has been reported to increase the diffusion rate of water and solutes when coupled with OD [66]. Moreover, the modification of cell membrane permeability that PEF causes can increase the velocity of water loss and, thus, affect the drying kinetics [23]. In light of these considerations, PEF pre-treatment, could be introduced in the food production sector as new hybrid drying technology aiming at the preservation of the physical and nutritional quality of the food products and contributing to the environmental sustainability.

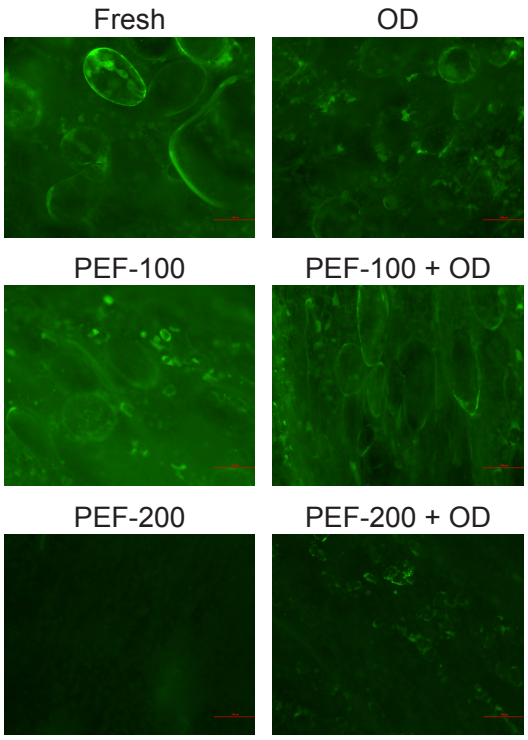
4.2.1 New Findings

PEF pre-treatment coupled with OD was applied to plant tissues for the production of semi-dried fruits or dried fruit snacks. In particular, **Paper II** aimed at evaluate the effect of PEF and OD on physiological changes of organic fruits; while **Paper VI** and **VII** aimed at assessing innovative pre-drying processes (applying PEF and OD prior to hot-air drying, alone or in combination) for the valorization of kiwifruit waste by the production of functional snacks.

The increased kinetic of water and solute transfers by the application of PEF-assisted osmotic dehydration has been extensively reported in literature [67]–[70]. Tough plant tissues are made up of biologically active tissues, the evaluation of the effect of the treatments on their physicochemical and physiological properties is of high importance. The main goal of the application of innovative pre-treatments to osmo-dehydrated tissues is related to the preservation of the fresh-like characteristic, which is often linked to the preservation of the cell viability and cell turgor pressure.

In **Paper II** the metabolic response of organic strawberries and kiwifruits subjected to PEF-assisted osmotic dehydration has been studied, and an example of the effect of the treatments applied to kiwifruits in terms of micro- (i.e. cell viability) and macro-structure (i.e. textural firmness) modifications is showed in Fig. 4.3. While the application of OD alone did not compromise the cell viability (viable cells are identified by the bright fluorescence in Fig. 4.3A), as expected the effect of PEF depended on the applied electric field. The PEF treatment at 100 V cm^{-1} alone or in combination with OD led to a retention of complete or partial cell viability, respectively; whereas when PEF treatment at 200 V cm^{-1} was applied alone or in combination with OD no viable cells were observed. It is important to consider the limitation of the method evaluating the cell viability by fluorescence microscopy as only a confined area is analysed and therefore only partial information can be deduced.

A



B

Sample	Firmness [N]
Fresh	3.10 ± 0.49 ^{a}
OD	2.15 ± 0.43 ^{b}
PEF-100	1.83 ± 0.52 ^{b}
PEF-200	0.77 ± 0.12 ^{d}
PEF-100 + OD	0.98 ± 0.24 ^{c}
PEF-200 + OD	0.96 ± 0.14 ^{c}

Figure 4.3: Representative images of kiwifruit samples stained with FDA (A) and textural firmness [N] (B) of fresh kiwifruits, after osmotic dehydration (OD), after PEF treatment at 100 V cm^{-1} (PEF-100) and 200 V cm^{-1} (PEF-200), and after the combined treatments (PEF-100 + OD and PEF-200 + OD). Different letters in the same column indicate significant differences among samples ($p < 0.05$).

Nevertheless, the results indicate that the higher electric field applied (i.e. 200 V cm^{-1}) could irreversibly electroporate the cell membranes of kiwifruit samples. Similarly, a loss of textural firmness (expressed as max penetration force in Newton) proportional to the applied electric field was observed (Fig. 4.3B), confirming the extensive cell structure breakdown promoted by the higher electric field.

In **Paper VI** and **VII** the impact of PEF pre-treatment, alone or in combination with OD, was assessed in kiwifruit waste (i.e. undersized kiwifruits, not complied with the Regulation (EC) No 1673/2004) by the production of fruit snacks with added nutritional value. Both pre-drying treatments (PEF and/or OD) influenced the hot-air drying kinetics at 50, 60 and 70 °C, contributing to the reduction of the total drying time compared to the untreated fruits. Moreover, the shortest drying times required for the pre-treated samples could be beneficial for the production of fruit snacks with high functionality (i.e. higher concentration of bioactive compounds, such as Vitamin C) and better overall quality (i.e. retention of the fresh-like colour).

4.3 Curing and Rehydration of Fish

Fish is a high perishable raw material where deterioration caused by biochemical phenomena and microorganisms begin soon after slaughtering, therefore, preservation practices to prolong its shelf-life are needed [71]. Curing is one of the oldest method used for preserving fish from spoilage and, among curing methods, salting process has remained almost unaltered to the present day. The conventional fish salting methods include dry-salting and wet-salting. Both methods preserve the products by rendering the medium an unsuitable environment for microbial propagation, and it is accomplished by either abstracting the water and by causing soluble substances to diffuse into the product [72]. The main ingredient used in salting is sodium chloride (NaCl) and, even at relatively low concentrations (< 5% w/w), sodium cations and chloride anions attract water molecules that become unavailable for use by microorganisms [73]. While the dry-salting is performed by applying the salt and other ingredients (e.g. sugars and spices) directly to the fish surface; during wet-salting, the fish muscle is submerged into, or injected by, a brine solution with concentration of NaCl between 5% and 15%. The concentration of salt in the brine affects the weight gain, water holding capacity and the commercial quality of the final fish product [74]. The weight gain, in turns, depends on the ability of the myofibrillars proteins to retain water inside the muscle. It is well known that salting variables (i.e. salt concentration and brining time) of fish alters the muscle protein solubility and denaturation, affecting the structure in the intra- and extra-cellular matrix [75], [76]. The fish brining time usually varies from 2 to 10 days, depending on the desired level of salt in the muscle. Sodium chloride diffuses through the fish flesh and water to the outside due to the osmotic gradient between the fish muscle and the brining solution. This process is not infinite, an equilibrium is reached when the chloride ions forming a water-binding complex with protein, that exerts an osmotic pressure itself, will eventually balance that due to the surrounding brine. The slow rate of salt diffusion into the fish tissue, and the exosmosis of water out of the tissue, has directed the scientific community towards finding methods able to accelerate the kinetics of salting (such as pulsed vacuum brining [77], vacuum tumbling [78], and high intensity ultrasound brining [79], to name a few).

Brine salting can be used individually for the preparation of lightly salted fish products, or as a preliminary step in the production of heavily salted or cured-salted fish. In the last case, due to the unpalatable high salt concentration in the fish muscle (approx. 16% to 20% w/w), salt-cured fish (such as cod, *Gadus morhua*) must be desalted and rehydrated before consumption (residual salt concentration in the range of 2 - 3% w/w). The desalting/rehydration step is usually performed by the consumer at home and by immersing the product in stagnant water for at least 24h at room temperature or refrigerated conditions [80]. At this stage, a two-way mass transfer takes place, resulting in the leaching out of sodium chloride ions from the tissue matrix and, contrariwise, in the water uptake, observing a tendency of cod weight to increase as well as volume, and in the re-solubilization of salt and proteins. Therefore, in a certain sense, the fish rehydration process could be considered as the opposite of the

one of salting [81]. The fish desalting/rehydration step, nowadays, has been implemented and included among the industrial operations. However, the industrial-scale desalting process presents many problems mainly linked to the long processing times (generally around 2-3 days, depending on the thickness of the fish pieces) and the quality of the final product. Large-scale rehydration is similar to the desalting procedure performed by consumers at home, and it employs handling large amounts of water, as several water renewals are necessary to improve the process efficiency (also in terms of water yields). Therefore, the industrial fish desalting/rehydration step presents criticisms related also to the wastewater management. The latter should be careful considered as an important variable for the industrial process optimization [82]. In fact, the residual brine is a polluting effluent characterised by dissolved and suspended solids (mainly Na^+ and Cl^- ions, and, in smaller portion, dissolved proteins), and it must be treated before its spill in the municipal sewage system [83]. For this reason, the industrial cod desalting step should be optimized yielding to a commercial product with higher process yield (high desalted fish - initial salted fish weight ratio) and limited waste.

PEF technology could theoretically enhance the kinetic of mass transfers in both salting and desalting processes of fish.

4.3.1 New Findings

The possibility of applying PEF treatment to accelerate the brining process of sea bass (*Dicentrarchus labrax*) was investigated in **Paper IV** and **V**. While **Paper IV** examined the effect of PEF pre-treatment and brine salting on lipid and protein oxidation in sea bass samples, **Paper V** focused on evaluating the mass transfer kinetics and the water and salt distribution within the fish muscle. Sea bass fillets, prior to brine salting, were subjected to PEF treatment at two different electric field strengths (300 V cm^{-1} and 600 V cm^{-1}), and held in the brine solution for 8 days at 0-4 °C (for more detailed methodology refer to **Paper V**). Figure 4.4 shows the mass fractions of total weight change (A), water (B) and NaCl (C) uptake of untreated and PEF treated (300 and 600 V cm^{-1}) sea bass samples during the brine salting (5 % of NaCl concentration). While the untreated sample showed an increased total weight, and water and salt uptake up to 120 hours of the brining process, followed by a dramatic drop at 192 hours of salting. The PEF treated sea bass fillets showed a constant increase of weight, water and salt during the entire brining period. The lowest weight yield in the untreated fish may possibly be explained by an inhomogeneous salt distribution within the inner and the outer parts of the muscle at the beginning of brining, leading to a disintegration of the fish muscle pieces in the last part of the experiment, as previously reported [84]. Applying PEF treatment to sea bass fillets allowed to reach a similar salt uptake after 48 hours of brining, instead of 120 hours needed for the untreated fish muscle, thus reducing the time necessary for the process.

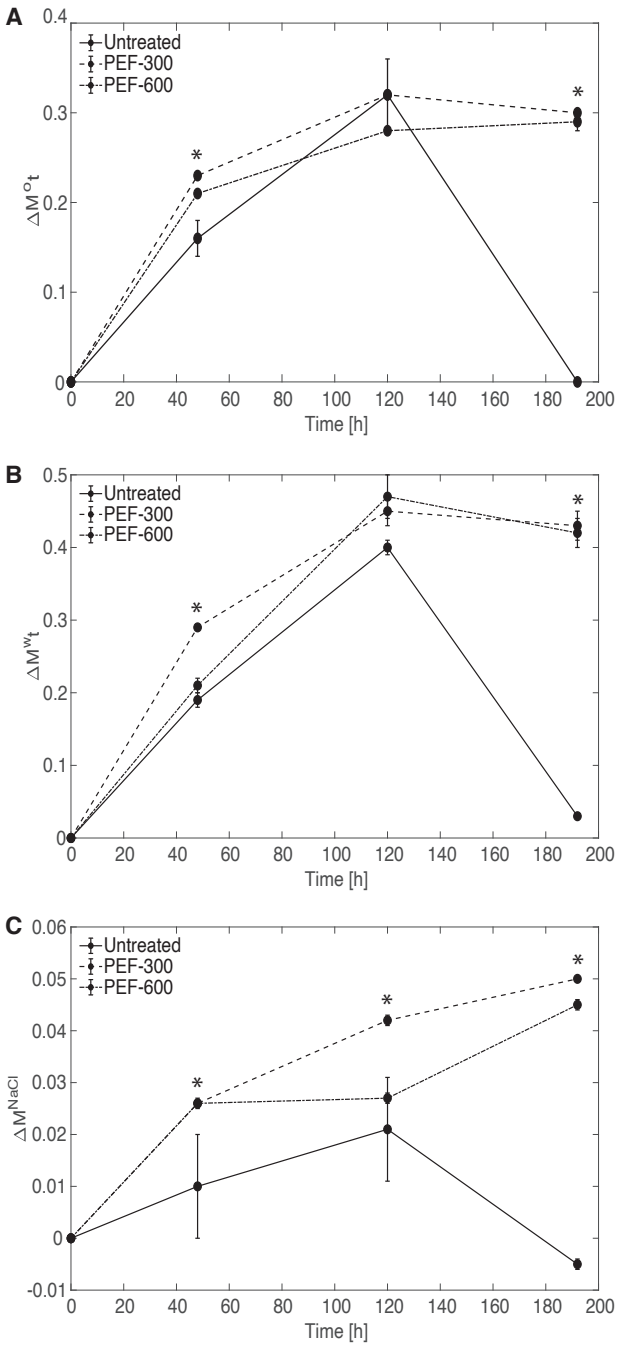
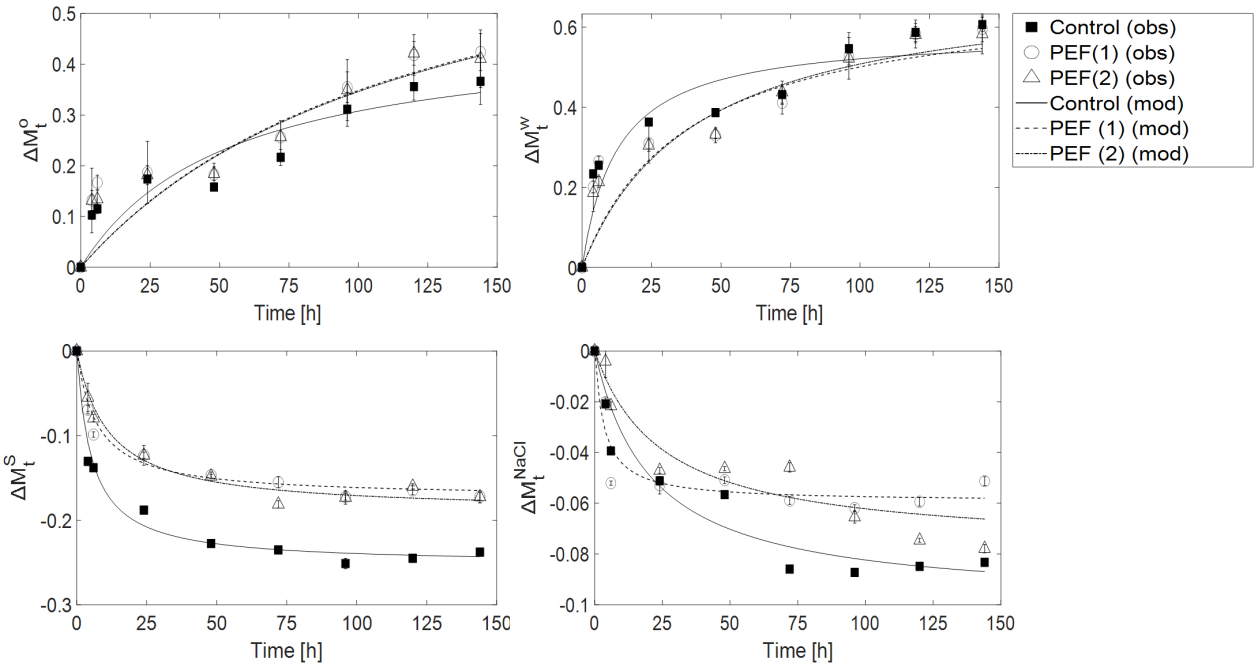


Figure 4.4: Total weight change (ΔM_t^o) (A), water uptake (ΔM_t^w) (B) and NaCl uptake (ΔM_t^{NaCl}) (C) of untreated, and PEF treated sea bass fillets at 300 V cm^{-1} (PEF-300) and 600 V cm^{-1} (PEF-600) during brining process with 5% salt concentration. Results are expressed as means ± standard deviations (error bars) of n = 5. Values marked with $*$ refer to significant differences ($p < 0.05$) between the PEF treated and the untreated samples at each brining time (adapted from Figure 1 and Paper V).

One of the possible explanation is that electroporation facilitated the salt uptake by the fish muscle through increasing the extra-cellular spaces in the muscle serving as additional channels for the diffusion of the brine. Moreover, we hypothesized that PEF treatment applied in this study promoted a more homogeneous distribution of NaCl within the inner and the outer parts of the fish muscle, facilitating the mass transfer and leading to enhanced diffusion of salt from the brine into the muscle. The obtained results suggested that PEF treatment could lead to a reduction of the time needed for brine salting (- 50% for the presented experimental plan). However, aspects related to the effect of the electric treatment on protein structure and functionality should be carefully considered.

Although, PEF is categorized as a non-thermal food processing technology, its application at high voltage and current could lead to a local temperature increase due to Joule effect, which must be considered for thermo-sensitive compounds such as lipids rich in polyunsaturated fatty acids (PUFAs) and proteins. In **Paper IV** it has been shown that PEF treatment of sea bass fillets prior to brine salting resulted in higher oxidation level of lipids and proteins (in terms of primary and secondary lipid and protein oxidation products) compared to the untreated fish muscle. However, the higher electric field applied (600 V cm^{-1}) showed significantly ($p < 0.05$) lower values of peroxides and thiobarbituric acid reactive substances (TBARS) compared to the fish muscle treated at electric field of 300 V cm^{-1}. The product treated with electric pulses of 600 V cm^{-1} of amplitude, together with the untreated one, during brine salting did not exceed the maximum level of lipid oxidation (5 meq active oxygen / kg of lipids) considered acceptable for consumption (established by Standard for fish oils CODEX STAN 329-2017). It has been hypothesized that, on one side electroporation could have resulted in a disintegration of cell membranes facilitating the exposure of various pro-oxidants (enzymes, free iron, heme-proteins, etc.) and, therefore, accelerating the lipid oxidation when 300 V cm^{-1} were applied. On the other side, higher electric field (600 V cm^{-1} in this case) could have inactivated the pro-oxidative endogenous enzymes, leading to a lower lipid oxidation.

The possibility of applying PEF technology to increase the velocity of desalting/rehydration of dry-salted cod was explored in **Paper VIII**. Two different PEF pre-treatments were selected, at two different electric fields (500 and 1000 V cm^{-1}), in order to understand if the increased permeabilization of cell membranes due to the electroporation process could lead to an increase of mass transfers phenomena that characterise the rehydration of salted cod (i.e. water uptake and leaching out of salt). The study of mass transfer kinetics (reported in the graphs in Fig. 4.5) highlighted some discrepancies between the untreated fish muscle (control) and the one subjected to the PEF pre-treatments (PEF (1) and PEF (2)) during the whole rehydration period considered (six days). Moreover, the empirical Peleg's model adequately described the cod desalting/rehydration kinetics (coefficients of determination, R^2, are given in the table in Fig. 4.5).



Sample	k_1	SE	k_2	SE	R^2	$1/k_1$	$1/k_2$
Mass variation							
Control	112.939	14.311	2.121	0.183	0.776	0.009 ^{a}	0.471 ^{b}
PEF (1)	158.749	15.068	1.282	0.178	0.656	0.006 ^{b}	0.780 ^{a}
PEF (2)	160.175	13.840	1.287	0.163	0.714	0.006 ^{b}	0.777 ^{a}
Water uptake							
Control	21.416	2.489	1.704	0.523	0.841	0.047 ^{a}	0.587 ^{a}
PEF (1)	54.872	5.305	1.447	0.079	0.707	0.018 ^{b}	0.691 ^{a}
PEF (2)	57.505	4.481	1.392	0.065	0.804	0.017 ^{b}	0.718 ^{a}
Solutes loss							
Control	20.994	1.314	3.966	0.423	0.955	0.048 ^{a}	0.252 ^{a}
PEF (1)	42.286	2.780	5.774	0.066	0.915	0.024 ^{b}	0.173 ^{b}
PEF (2)	59.695	3.646	5.254	0.074	0.925	0.017 ^{b}	0.190 ^{b}
Salt loss							
Control	209.338	18.746	10.049	0.322	0.901	0.005 ^{b}	0.100 ^{a}
PEF (1)	58.213	7.117	16.841	0.232	0.795	0.017 ^{a}	0.059 ^{b}
PEF (2)	321.131	33.178	12.873	0.544	0.785	0.003 ^{b}	0.078 ^{b}

Figure 4.5: Mass variation (ΔM_t^o), water uptake (ΔM_t^w), solutes loss (ΔM_t^S) and NaCl loss (ΔM_t^{NaCl}) of untreated (control) and PEF treated dry-salted cod samples at 500 V cm^{-1} (PEF (1)) and 1000 V cm^{-1} (PEF (2)) as a function of the rehydration time. Experimental data (obs) with the curve fitting (mod) are shown. Error bars correspond to the standard deviations of n = 5. The table below reports the coefficients of Peleg's kinetic model of mass and water uptake, solutes and salt loss during the rehydration of dry-salted cod. Different letters in the same column indicate significant differences among samples ($p < 0.05$) (corresponding to Figure 1 and Table 1 in Paper VIII).

It has been observed that PEF technology positively influenced the weight gain along the cod rehydration, showing an increased total mass variation compared to the untreated sample, independently of the amplitude of pulses applied (i.e. PEF (1) and PEF (2) reached similar values of mass variation, ΔM_t^o, after 144 hours of rehydration). The higher weight of the pre-treated fish muscle is caused by the poor solid loss from the food matrix, as both PEF treated samples displayed a higher retention of solutes than the untreated sample, and, in particular, a lower leaching of NaCl (Fig. 4.5). Nevertheless, at the end of the dealting process, salt levels of both untreated and PEF pre-treated fish muscles were comparable to the one of the commercialised product (around 1 - 2% of NaCl). Therefore, the results of this explorative study could indicate the advantageous application of the PEF technology in the industrial environment, since it could lead to higher process yields (higher weight gain) and to the possibility of reducing the water renewals, as less NaCl is lost in the waste brine, yielding to lesser water wastes.

Conclusions

The results presented in this PhD thesis contribute to expand scientific knowledge on the effects of electroporation phenomena in biological tissues of interest for food, and reveal the possible exploitation of PEF technology in many real food production systems for the enhancement of mass transfers.

In general, the analysis of changes of electrical properties (e.g. complex impedance) of the biological tissue exposed to high-voltage treatments is one of the well-established methods currently used for the determination of the degree of cell disruption followed by PEF. It must be taken into consideration, however, that additional phenomena connected with other physical changes of tissues can take place after the pulsation. In fact, research results to date suggest that the modification of cell membrane permeabilization due to electroporation is also associated with modification of the structure (i.e. intra- and extra-cellular volume change), and ionic concentration variation due to the leakage of intracellular content. Hence, the findings of this research activity demonstrated that other techniques, such as MRI, could be used to monitor the spatially dependent effect of PEF treatment. In particular, transverse relaxation time T_2 gave indications of the redistribution of water and solute in the tissues immediately after the PEF treatment. The research findings provided useful insights and calls for critical choice of electroporation assessment methods, also with respect to matrix characteristics (e.g. physiological state) in determination of appropriate PEF treatment conditions.

In this research study, it has been confirmed, also, the high potentiality of PEF technology for a wide range of applications in the food processing for the enhancement of quality and/or to accelerate the kinetic of mass transfers. By monitoring PEF treatment parameters and other manufacturing steps it was possible to achieve a consistent reduction of acrylamide and its precursors in deep-fat fried potato crisps. The drying kinetic of kiwifruit snacks was significantly reduced by the application of PEF pre-treatment alone or in combination with osmotic dehydration, suggesting the possibility of customize an hybrid drying system aiming at reducing the operational costs and at preserving the quality of the food materials. Finally, the effect of PEF on fish muscles to accelerate either the brining or the rehydration processes has been presented, and additional useful information about the feasibility of using PEF technology in this direction has been provided. However, the effect of electroporation on animal tissues is highly dependent also on *post-mortem* meat conditions (i.e. ageing time). Therefore, to introduce new potential industrial applications, further multi-disciplinary studies should focus on the exploration of the electroporation phenomenon in animal-based foods.

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Paper I

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Important factors to consider for acrylamide mitigation in potato crisps using pulsed electric fields

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ABSTRACT

This preliminary study aimed to compare the application of pulsed electric field (PEF) with a traditional blanching as pre-treatments before frying for the mitigation of acrylamide content in potato crisps.

Measuring the degree of cell disintegration index (p_o) and the changes in water electrical conductivity during washing of potato slices, PEF protocol and sample preparation scheme were optimized. Peeled potato slices (thickness 1.5 ± 0.2 mm) were subjected to PEF (1.5 kV/cm, pulse duration 10 µs, total treatment time 10 ms, pulse frequency 100 Hz) and to blanching (85 °C for 3.5 min) pre-treatments and then to washing in water, evaluating the reduction of acrylamide precursors (reducing sugars and free asparagine). After frying (175 °C, 3 min), product quality, in terms of colour, texture and acrylamide content were evaluated. Results showed that PEF promoted acrylamide precursors leaching followed by a reduction of the final acrylamide content of around 30%, significantly higher if compared to the reduction obtained with blanching, with only slight modifications of the final quality of the product, in terms of colour and texture.

Industrial relevance: The Commission Regulation (EU) 2017/2158 of 20 November 2017 has introduced new benchmark levels and mitigation strategies for the reduction of the presence of acrylamide in foods, directing food businesses to the research of measures to lower the acrylamide formation in foods. The actual industrial production process of fried potato crisps involves the use of many mitigation strategies, such as a blanching of raw potatoes. However, the traditional blanching treatment presents several practical drawbacks and leads to undesirable changes of the product quality. The application of PEF as a pre-treatment could reduce the acrylamide content in deep-fat fried potato crisps. This preliminary study gives important indications regarding the possibility of combining a PEF pre-treatment on raw potato slices with subsequent industrial processing steps for the production of potato crisps with low acrylamide concentration.

1. Introduction

Acrylamide has been identified as a contaminant in a range of fried and oven-cooked foods (e.g. French fries, potato crisps, bread and cereal) and drinks (e.g. coffee); and its classification as probably carcinogenic in humans has caused worldwide concerns (International Agency for Research on Cancer, 2014). Although most epidemiologic studies examining the relationship between estimated dietary consumption of acrylamide and specific cancer resulted inconclusive, experimental animal studies identified neurotoxicity, carcinogenicity, adverse effects on male reproduction, as possible critical endpoints for acrylamide toxicity (European Food Safety Authority, 2015).

Certain foods, more specifically certain food components such as asparagine and reducing sugars, could lead to the formation of acrylamide during heat treatment at temperatures above 120 °C as a result of the Maillard reaction (Mottram, Wedzicha, & Dodson, 2002). Among fried carbohydrate-rich foods, potato crisps contribute to a substantial proportion of the estimated intake of acrylamide in the European adult population (European Food Safety Authority, 2015). Statistical data revealed that in Europe the consumption of salty snacks, in particular potato chips/crisps, stands to an average of 1.5 kg per capita in 2018 (Statista, 2018).

National authorities, together with research institutes and food industries reported many strategies of controlling and minimizing the

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formation of acrylamide, with particular concern to fried potato-based products, due to the presence of large concentration of acrylamide precursors in the potato. In fact, levels up to 4000 ppb of this contaminant have been detected in potato crisps (Becalski, Lau, Lewis, & Seaman, 2003).

Recently, the Commission Regulation (EU) 2017/2158 of 20 November 2017 has established new "mitigation measures and benchmark levels for the reduction of the presence of acrylamide in foods", which aim to ensure that food businesses put in place steps to mitigate acrylamide formation (European Union, 2017).

Powers, Mottram, Curtis, and Halford (2017) analysed European manufacturers' data on acrylamide in potato crisps from 2002 to 2016, and the study showed that, even though acrylamide levels in potato crisps in Europe have been levelled off in recent years, > 5% of samples exceeded the regulated benchmark level for potato crisps (750 ng g^{-1}).

Several investigations have proposed mitigation ways to be applied at different stages of the manufacturing process of potato crisps, in order to reduce the concentration of acrylamide precursors. Among conventional mitigation strategies, hot water blanching of potato slices appeared to facilitate the extraction of Maillard reaction substrates, in addition to enzyme inactivation, also improving the colour uniformity and texture, and oil absorption reduction (Mestdagh et al., 2008; Pedreschi, Kaack, & Granby, 2004). However, this thermal pre-treatment presents the drawbacks of being time-intensive, high energy-consuming and of promoting considerable modifications of sensorial properties of final product.

Recent studies have proposed alternative non-thermal technologies in food processing and preservation. Pulsed electric fields (PEF) have been widely described as one of the most promising non-thermal novel technologies in the last decades, stimulating intensive research in several fields such as biotechnology, medicine and food processing. PEF treatment is based on the application of an external electric field applying short and intensive electric pulses. The application of PEF for potato snacks pre-treatment has been extensively studied, and many researchers have already reported high numbers of benefits that could be achieved by applying electric pulses to raw potatoes. Fauster et al. (2018) have recently described the impact of PEF treatment on potato structure and various potential advantages on quality and economic aspects of industrial French fries production, including reductions of cutting force, starch loss and oil uptake. Furthermore, the application of pulsed electric field treatments above a specific critical value of field strength is well known to enhance mass transfer from plant tissues, increasing the cell membrane permeabilization (Donsì, Ferrari, & Pataro, 2010). Following this principle, Jaeger, Janositz, and Knorr (2010) have stated that PEF treatment of raw potatoes could assist and increase the release of sugars and amino acids that represent the main substrates for the Maillard reaction, consequently leading to formation of lower amounts of acrylamide. Moreover, Janositz, Noack, and Knorr (2011) have reported a significant increase in the release of reducing sugars (fructose, glucose) and sucrose in potato slices after PEF application.

However, the effective reduction of acrylamide content in deep-fat fried potato products is still unclear, although many researchers observed a significant increase of acrylamide precursors extractability on PEF treated potatoes, reducing browning during frying (Ignat, Manzocco, Brunton, Nicoli, & Lyng, 2015). Besides, lab-scale investigations followed different trials' schemes, applying PEF as a pre-treatment of whole potato tubers before or after peeling, concentrating on the treatment itself, with low attention to the combination of other process operational units that could influence the outcome. It is necessary to understand how the rate of mass transfer promoted by applying certain electric field strengths could be influenced by other processes operations, and how the quality of the final product will be preserved.

This preliminary study aimed to evaluate the effect of the application of PEF as a pre-treatment, and the effect of time modulation of subsequent washing steps of treated potato slices, on the Maillard reaction substrates and final acrylamide content, and on the quality of fried potato crisps. Measuring the degree of cell disintegration index (p_o) (Angersbach, Heinz, & Knorr, 1999) and the changes in water electrical conductivity during washing of potato slices, it is possible to optimise either PEF protocol and sample preparation scheme, in order to maximise the release of acrylamide precursors from the raw tissue. The efficiency of PEF as a non-thermal pre-treatment on the acrylamide reduction was compared to a conventional blanching in hot water usually used as a pre-treatment in the fried potato industrial lines.

2. Materials and methods

2.1. Sampling

Potato tubers (*Solanum tuberosum L.*) of the Lady Claire variety (suitable for industrial processing), were purchased at a local market one month after harvesting and stored in the dark at 10 ± 2 °C for a maximum of two weeks before trials. The storage temperature was chosen according to Pinhero, Coffin, and Yada (2009). The initial moisture content of potato tubers was 81.92 ± 0.58%, evaluated by drying 5 g of fresh potato tissue in a convection oven at 105 °C until a constant weight was achieved. Before pre-treatment, only tubers of a similar size and shape were selected, manually peeled and sliced (1.5 ± 0.2 mm in thickness) using a stainless-steel electric slicer machine (Mod. KAFPL0922N CAD, Italy). Both whole tubers and slices were rinsed for 1 min in tap water (water temperature: 18 ± 2 °C) and subsequently submitted to blanching or PEF treatment, followed by washing as better detailed in Section 2.4.

2.2. Pre-treatments

2.2.1. Blanching

Blanching was performed by immersing potato slices in hot distilled water at 85 °C and for 3.5 min stirring with a product-to-water ratio of around 1:2 (*w/w*), according to the method used by Pedreschi, Mariotti, Granby, and Risum (2011). The use of distilled water, according to the same authors, allowed to maximise the diffusion of acrylamide precursors from potato tissue. Hence, although the use of distilled water is not industrially relevant, in this study distilled water blanching was chosen in order to compare pulsed electric fields achievements with the best performances of blanching.

2.2.2. Pulsed electric fields (PEF) treatment

PEF pre-treatments were performed using a lab-scale PEF unit delivering a maximum output current and voltage of 60 A and 8 kV, respectively (Mod. S—P7500, Alintel, Italy). The generator provides monopolar rectangular-shape pulses and adjustable pulse duration (5–20 μs), pulse frequency (50–500 Hz) and total treatment time (1–600 s). The treatment chamber (50 mm length x 50 mm width x 50 mm height) consisted in two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. Output voltage and current were monitored using a PC-oscilloscope (Picoscope 2204a, Pico Technology, UK). Samples were treated at room temperature in tap water, with an initial electrical conductivity of 542 ± 2 μS cm^{-1} at 25 °C, measured using an EC-meter (Mod. Basic 30, Crison, Spain). Trials were conducted delivering $n = 1000$ pulses at fixed pulse width (10 ± 1 μs), frequency (100 Hz) and repetition time (10 ± 1 ms). An electric field strength of 1.5 kV/cm was selected in order to achieve irreversible electroporation (Faridnia, Burritt, Bremer, & Oey, 2015). Temperature changes due to PEF treatments were negligible.

Fig. 1 shows a schematic diagram of the PEF apparatus used. The same PEF protocol was applied on both whole tubers (Fig. 1A) and potato slices (Fig. 1B), in order to understand if the different exposed surface-to-volume ratio could have affected the efficiency of mass transfer. In both cases, the treatment chamber was filled with a

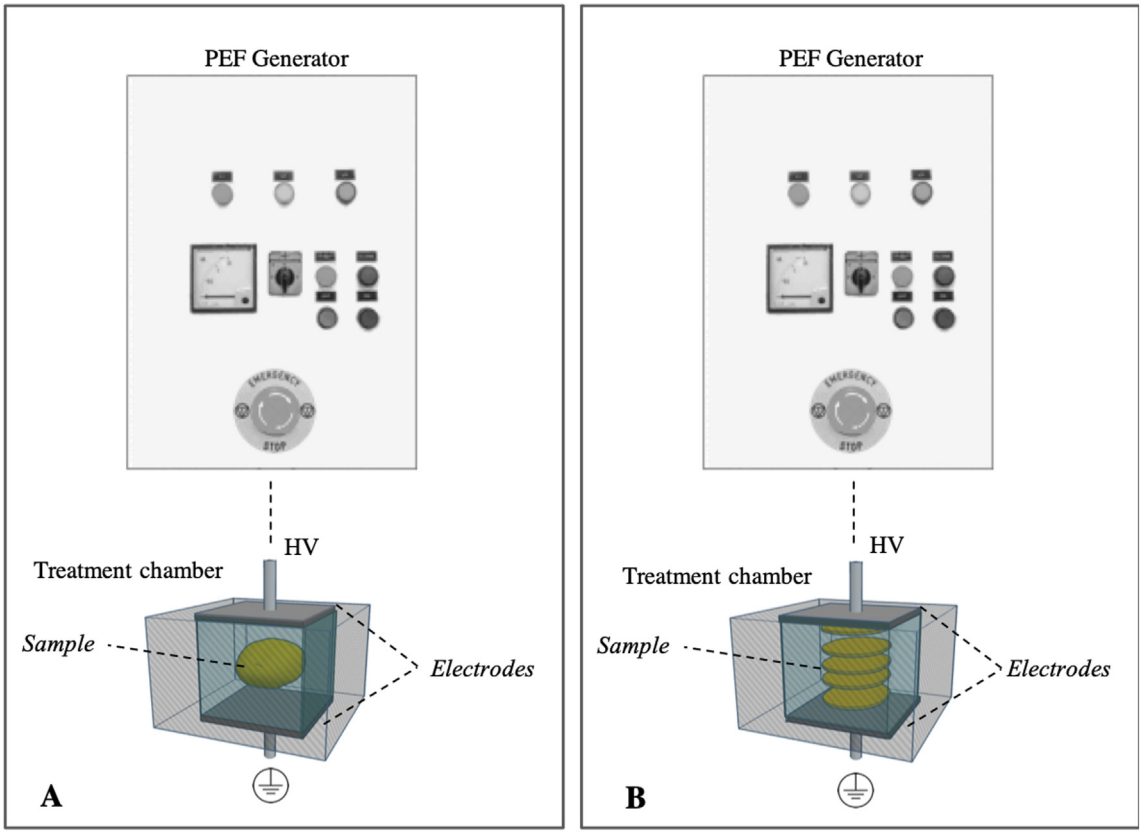

Fig. 1. Schematic diagrams of experimental apparatus: (A) whole potato tuber PEF treatment; (B) potato slices PEF treatment.

product–to–water ratio of around 1:5 (*w*/w).

2.3. Determination of cell disintegration index (p_o)

Cell disintegration index (p_o) was analysed, according to Angersbach et al. (1999), for PEF treated whole tubers and slices. The method is based on changes in the electrical properties of an intact and permeabilized biologic membrane (considered equal to a resistor and a capacitor). The electrical conductivity (σ) for intact and processed samples was obtained by impedance measurements at low (1 kHz) and high (5 MHz) frequencies. The impedance spectra were acquired using a precision impedance meter (Mod. LCR-8105G, GW Instek, Taiwan) connected to a parallel plate measuring cell with adjustable gap. The cell disintagration index (p_o) was calculated by the following equation:

$$p_o = \frac{(\sigma_h^i/\sigma_h^s)\sigma_l^s - \sigma_l^i}{\sigma_h^i - \sigma_l^i} \quad (1)$$

where σ_l^i and σ_l^s indicate the electrical conductivity of untreated and treated cell material at low frequency (1 kHz), respectively; and σ_h^i and σ_h^s indicate the electrical conductivity of untreated and treated material at high frequency (5 MHz), respectively. The parameter p_o ranges from 0 for intact tissue to 1 for complete disrupted tissue.

2.4. Washing in water

Potato slices, either untreated or subjected to PEF or blanching were soaked and stirred in tap water (18 ± 2 °C), with an initial electrical conductivity of 319 ± 4 μS cm^{-1} at 25 °C, and with a product-to-water ratio around 1:1.5 (*w*/*w*). In order to select the optimal soaking time as a result of maximum release of intracellular compounds into the external aqueous phase due to PEF-induced electroporation, washing times of 5, 10, 15, 20 min were tested on PEF-processed potato slices before frying. For each dipping time, changes in water electrical conductivity were registered using an EC-meter (Mod. Basic 30, Crison, Spain). The selected washing times, based on the highest water electrical conductivity variation recorded, were applied also to untreated (control) and blanched potato slices before frying, in order to obtain comparable results for further analysis.

2.5. Frying conditions

Untreated (control), blanched and PEF-treated potato slices were deep-fried in high-oleic sunflower oil using an electrical fryer (Mod. MFR280R, Fama Industrie, Italy) at 175 °C as initial oil temperature. Potato-to-oil weight ratio was around 1:10 and slices were fried for 3 min until a final moisture of ~2% (wet basis) was reached. Temperatures of frying oil and frying potatoes were monitored using thermocouples sensors type K connected to a data logging system (Mod. 9211A, National Instruments™, Texas).

2.6. Acrylamide precursors in raw potatoes

2.6.1. Reducing sugars analysis

The concentrations of glucose and fructose in raw potatoes were quantified using the method described by Rodriguez-Saona, Wrolstad, and Pereira (1997) with few modifications. A 2 g sample of freeze-dried potato slices was dissolved in 20 mL of distilled water using ultrasonic bath (Elmasonic, Germany). The sample was centrifuged (Centrifuge Thermo Electron, USA) 15 min at 3500 rpm, and the supernatant was collected. A 1 mL aliquot of the solution was passed through C18 cartridges (1000 mg, 6 mL; Phenomenex) for purification. Subsequently, the sample was resuspended in 0.25 mL of deionised distilled water before injection into HPLC. Glucose and fructose were determined with HPLC Agilent Infinity 1260 (Agilent Technologies, Santa Clara, CA, USA) coupled to ELSD PL-ELS 1000 (Agilent, Santa Clara, CA, USA) as detector. The analytical column was a SphereClone NH2

(250 mm × 4.60 mm i.d.; 5 µm particle size) (Phenomenex, Torrance, CA, USA); the elution was in isocratic mode using a mixture of water:acetonitrile 70:30 (*v*/v) as mobile phase at a flow rate of 0.6 mL/min. The sample injection volume was 10 µL. All samples were analysed in triplicate.

2.6.2. Asparagine analysis

The concentration of free asparagine in raw potatoes was quantified dissolving 5 g sample of freeze-dried potato slices in 50 mL of distilled water using ultrasonic bath (Elmasonic, Germany) for 10 min. The sample was centrifuged (Centrifuge Thermo Electron, USA) 15 min at 3500 rpm, the supernatant collected, micro-filtered (0.22 µm) before HPLC analysis. Free asparagine was determined with HPLC Agilent Infinity 1260 (Agilent Technologies, Santa Clara, CA, USA) coupled to a diode array detector (UV wavelength set at 338 and 262 nm). In order to obtain the derivatization sample, a 200 µL sample was added to 400 µL borate buffer with 50 µL o-phtalaldehyde-3-mercaptopropionic acid (OPA) reagent. Chromatographic conditions are described in Plata-Guerrero et al. (2009). All samples were analysed in triplicate.

2.7. Analysis of fried potato crisps

2.7.1. Computer Vision System (CVS) for colour determination

The surface colour of potato crisps was measured using a Computer Vision System (CVS) consisting of an illumination source, a colour digital camera (CDC), and an image processing software. Potato crisps samples were placed inside a dark box to exclude external light, and RGB images were acquired by a CDC (Mod. D7000, Nikon, Japan) with a 105 mm lens (Mod. AF-S Micro Nikkor), located vertically over the sample at a distance of 35 cm and connected to a PC. The lighting system consisted of four daylight fluorescent lamps (60 cm in length) connected to an electronic ballast to ensure uniform illumination, with a colour temperature of 6500 K and sited at an angle of 45° with the CDC. For each sample, untreated, blanched and PEF treated, 12 images were captured, each of one side of potato crisps. The pre-processing of RGB images, segmentation and colour quantification were performed with ImageJ analysis software (NIH, USA). The average value of the segmented pixels in the CIE L^* a^* b^* colour space was registered as the colour of the sample. From numerical values of a^* (green/red) and b^* (yellow/blue) chromatic parameters, hue angle (h°) was calculated by the following equation and used to describe colour variations between samples:

$$h° = tan^{-1}(b^*/a^*) \quad (2)$$

2.7.2. Texture

Texture analysis of crisps were performed at room temperature (~20 °C) using a Texture Analyser TA-XT2 (Mod. HDi 500, Stable Micro System, Surrey, UK) equipped with a 5 kg load cell. A puncture test was selected to evaluate samples firmness and crispness. Crisp samples selected on the basis of uniform size and shape, were placed on a support rig (HDP/CFS, Crisp Fracture Support Rig and corresponding Heavy Duty Platform) and compressed for 3 mm distance using a spherical probe (P/0.25S) of ¼ - inch diameter (Salvador, Varela, Sanz, & Fiszman, 2009). Force vs distance curves were obtained using a test speed of 1.0 mm s^{-1} and the results obtained from 12 slices for each sample were expressed as firmness, calculated by means of maximum force values and as crispness, calculated from means of linear distance (the length of a line joining all fracture points in the force-deformation curve) between the first and the last facture peaks registered.

2.7.3. Acrylamide

2.7.3.1. Sample extraction and SPE purification. Potato chips samples were finely ground before the extraction; 1 g of sample was weighted into a polypropylene conical tube, and 100 µL 10 µg/mL internal standard solution ($^{13}C_3$-labelled acrylamide in MeOH) followed by 10 mL 0.1% (*v*/v) formic acid were added. After mixing with Vortex for 10 min, the extract was centrifuged at 4500 rpm for 15 min. A 2 mL portion of clarified solution was removed, avoiding collection of top oil layer when present, and filtered through paper filter. A solid-phase extraction (SPE) was performed using C18 cartridges (1000 mg, 6 mL; Phenomenex). Cartridges were first conditioned with 5 mL methanol followed by 5 mL water; 1 mL of filtered sample was loaded and washed with 1 mL water. Eluition was performed with 1 mL acetone. Acetone was removed under nitrogen flow and sample was dissolved in 1 mL 0.1% (*v*/v) formic acid before injection. SPE clean-up was performed on 3 extracts for each sample.

2.7.3.2. HPLC-ESI-MS/MS analysis. LC-ESI-MS/MS in positive ion mode (ESI^+) analyses were performed by an Agilent 6420 triple quadrupole (Agilent, Santa Clara, United States) coupled to an Agilent 1290 Infinity LC Pump equipped with an autosampler and a thermostated column oven, according to Calbiani, Careri, Elviri, Mangia, and Zagnoni (2004) with some modifications. The analytical column was a Poroshell 120 C18, 3.0 × 100 mm, 2.7 µm (Agilent, USA) maintained at 20 °C. The elution was in isocratic mode using a mixture of 0.1% (*v/v*) aqueous formic acid and methanol (99.5/0.5, *v/v*) as mobile phase at a flow rate of 0.3 mL/min. The sample injection volume was 10 µL. Full-scan analyses were performed in the 40–100 Da mass range, acquiring the following transitions: extracted ion at *m/z* 55, due to the transition 72 > 55, and at *m/z* 58, due to the transition 75 > 58 were used for the quantitative analysis. A calibration curve was made for the quantification diluting stock solution of acrylamide with water in the 1.5–200 µg/L range. For acquisition and processing data, the Agilent MassHunter Workstation software was used.

2.8. Statistical analysis

Significant differences between results were calculated by paired samples Student's *t*-test, parametric analysis of variance (ANOVA) and Tukey multiple comparison, with a significance level of 95% ($p < 0.05$). If Shapiro-Wilk test for normality and Levene's test for homoscedasticity of data resulted statistically significant ($p < 0.05$), non-parametric multiple range test Kruskal-Wallis and Holm stepwise adjustment were used, with a significant level of 95% ($p < 0.05$) (R Foundation for Statistical Computing, Austria). All treatments were conducted in triplicate and results were expressed as mean ± standard deviations of replications.

3. Results and discussion

3.1. Experimental design set up

3.1.1. Effect of PEF treatment on cell disintegration index and degree of mass transfer

As reported by Angersbach et al. (1999), impedance measurements of plant tissues allow the evaluation of cell membrane permeabilization after applying PEF treatments.

To understand the efficiency of selected PEF protocol on the degree of cell disruption, and so on the level of mass transfer, cell disintegration index of PEF-treated potato tubers and potato slices was calculated from Eq. (1). Results are shown in Fig. 2; as expected, higher surface dimension exposed to electric pulses, being related to slices, resulted in higher cell disintegration, explaining the better efficiency of the pre-treatment if applied after the slicing step of the experimental scheme.

It is well established that measurements of the changes in electrophysical properties of untreated and treated cell tissues represent a reliable method to correlate PEF processing protocol and the cell damage degree in biological systems. Moreover, it has been widely reported that PEF-induced membrane permeabilization has the potential to effectively enhance mass transfer from the inner part of biological tissues, increasing the diffusion of cell compounds/metabolites (Donsì

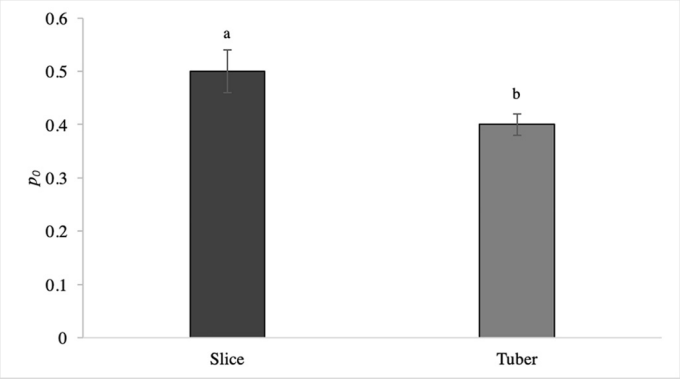

Fig. 2. Cell disintegration index (p_0) of PEF treated potato slices and tubers (E = 1.5 kV/cm; n = 1000). Results are expressed as means ± standard deviations (error bars) of n = 20. Values with different letters differ significantly ($p < 0.05$).

et al., 2010).

Another useful method to assess the intactness of cell membranes, reported by many researchers, is the measurement of ions/small molecules leakage from intracellular compartments. Washing of potato slices is a common practice in potato crisps production and allows to remove any surface starch residue prior to frying. In this work, different washing times of PEF-treated potato slices were evaluated, measuring the changes in electrical conductivity of residual washing water. Results are shown in Fig. 3; the maximum water conductivity variation was achieved after 5 min of washing, highlighting the period of time subsequent to PEF-treatment that permitted the highest release of cell fluid into the aqueous media.

Faridnia et al. (2015) reported that by suspending a PEF-treated potato tuber into an isotonic solution of mannitol (0.2 M) it was possible to monitor the electrolytes leakage from cell tissue by measuring changes in electrical conductivity of the surrounding media. Furthermore, it has been demonstrated that cell fluid leakage due to electroporation is function of time, as many transition processes induced by PEF (e.g. moisture and air redistribution among microscopic extracellular channels, mass transfer, partially or completely resealing of cell membranes) could last from seconds to hours (Oey, Faridnia, Leong, Burritt, & Liu, 2017). On the basis of this concept, it is clear that subsequent unit operations for potato crisps manufacturing need to be assessed in order to maximise mass transfers.

Thanks to the aforementioned introductory studies, in this work the experimental plan for the production of lab-scale deep-fat fried potato crisps was designed applying the different pre-treatments on raw potatoes directly after slicing and by selecting 5 min as the preferred time for potato slices washing step. A scheme with the experimental processing steps is shown in Fig. 4.

3.2. Deep-fat fried potato crisps analysis

3.2.1. Colour

Colour is one of the most important parameters to control during frying, being strongly related to consumer perception (Scanlon, Roller, Mazza, & Pritchard, 1994). Visual quality is associated with physical, chemical and sensorial evaluation and it is an important driver for buying being associated by consumers with flavour, safety, storage time, nutritional aspects and taste (Pedreschi, Kaack, & Granby, 2006).

Colour of potato crisps is often measured using a colorimeter in L*a*b* units. According to Pedreschi, León, Mery, and Moyano (2006), the use of a computer vision system (CVS) technique instead of the conventional colorimeter for monitoring the development of colour in potato crisps has different advantages, such as the possibility to analyse the whole surface of the product and to identify the presence of brown spots and other defects.

Fig. 5 shows images of control (5A), blanched (5B) and PEF treated (5C) potato crisps after frying. After blanching and PEF treatment, changes are noticeable in the appearance of potato slices compared to the control. The slices pre-treated by PEF showed a more uniform and lighter surface colour. Images have been converted from RGB into L*a*b* channels. The calculated values of L* and h° (Eq. (2)) of the three samples are reported in Fig. 6.

Development of colour during frying is the result of the Maillard reaction, that involves reducing sugars and the amino acid asparagine and has been related to the formation of toxic compounds such as acrylamide. The extent of the Maillard reaction depends on the presence of reaction substrates and on frying parameters such as temperature and time (Romani, Bacchiocca, Rocculi, & Dalla Rosa, 2008; Romani, Rocculi, Mendoza, & Dalla Rosa, 2009).

The evolution of colour during frying is generally indicated by a decrease in L* and/or an increase of the redness parameter (a*) and of

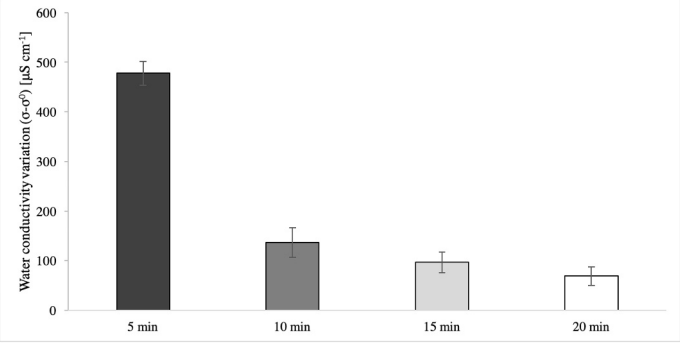

Fig. 3. Variations of water electrical conductivity affected by different washing times of PEF treated potato slices. Results are expressed as means ± standard deviations (error bars) of n = 5. Values with different letters differ significantly ($p < 0.05$).

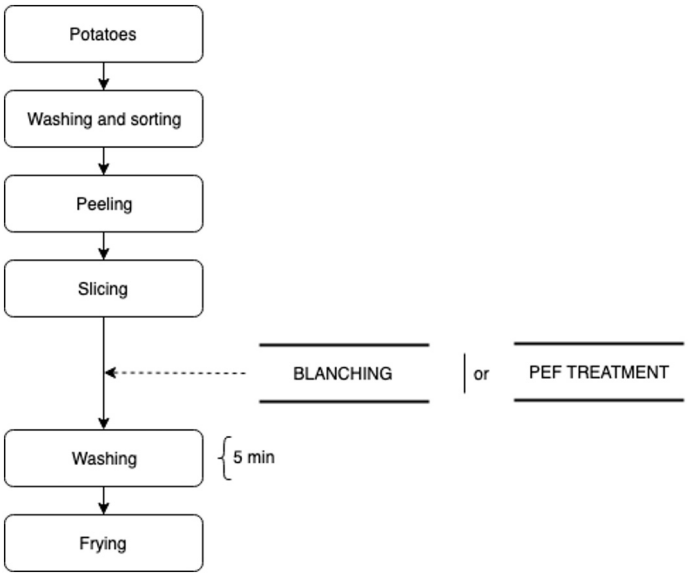

Fig. 4. Scheme of experimental processing steps.

the hue (h°). Variation of colour parameters observed in the present study allowed the discrimination among samples. The three samples did not show significant differences in terms of L*. On the other side, hue values increased for both pre-treated samples compared to the control, the highest values being related to the PEF pre-treated sample. Ignat et al. (2015) found similar results comparing the colour development during frying of blanched and PEF-treated potato slices. The increase of hue values testified a change from red to yellow colour, indicating the decrease of non-enzymatic browning reactions entity. Pre-frying treatments, such as blanching or dipping, are steps generally used in potato snacks manufacturing to stabilise the colour (Pedreschi et al., 2004). In fact, hot water blanching of raw potatoes could ease the leach out of superficial reducing sugars, main substrates or Maillard browning reactions. Moreover, the evolution of potato chips colour during frying has shown a good correlation ($R^2 = 0.9569$) with the acrylamide concentration, as reported by Pedreschi, Moyano, Kaack, and Granby (2005). Lighter and less red chips are related to a lower concentration of non-enzymatic browning reactions substrates and so to a lower content of acrylamide. The possibility of the release of the Maillard substrates from potato tissue treated with PEF has been already observed by various authors (Jaeger et al., 2010; Janositz et al., 2011). The increase of mass transfer upon PEF treatment is due to the effect of electroporation and cell permeabilization, that allow an increasingly diffusion of intracellular components across the membranes, as confirmed by the increase of conductivity of the washing water observed above.

3.2.2. *Firmness & crispness*

Texture is one of the main characteristics that influence the sensorial properties of potato-based products, and a delicate and crispy texture is recommended for potato crisps (Kita, 2014). Texture changes in potato crisps during frying result in the initial tissue softening and further crust development (Pedreschi, Moyano, Santis, & Pedreschi, 2007).

The changes of firmness and crispness of untreated, blanched and PEF treated potato crisps subjected to frying are shown in Fig. 7A and B, respectively. A slight, but significant reduction of both parameters was observed for blanched and PEF treated samples in comparison to the untreated one.

Structural changes of potato crisps could be influenced by many factors, e.g. firmness depends on the degree of starch gelatinization, on changes in the cell walls structure, mainly related to an increase in their permeability and on the reduction of intercellular adhesion between neighbouring cells (Moyano, Troncoso, & Pedreschi, 2007). Crispness instead is influenced mainly by dry matter content and oil uptake during frying (Abong, Okoth, Imungi, & Kabira, 2011).

Blanching at high temperature (80–100 °C), contrary to that at low temperature, has been already reported to promote potato tissue softening, by starch modification (hydration, swelling and gelatinization) along with β eliminative cleavage and pectin solubilization (Botero-Uribe, Fitzgerald, Gilbert, & Midgley, 2017). Moreover, high temperature blanching decreases polyphenol oxidase activity, responsible for enzymatic browning (Bingol, Wang, Zhang, Pan, & McHugh, 2014).

Recently, some studies have been performed showing the effect of PEF pre-treatment on texture and other quality parameters of potato tissue before frying. Fauster et al. (2018) observed softening of the potato tissue and therefore the improvement of cutting behaviour (smoother surface and lower feathering). PEF treatment (0.3–1.2 kV/

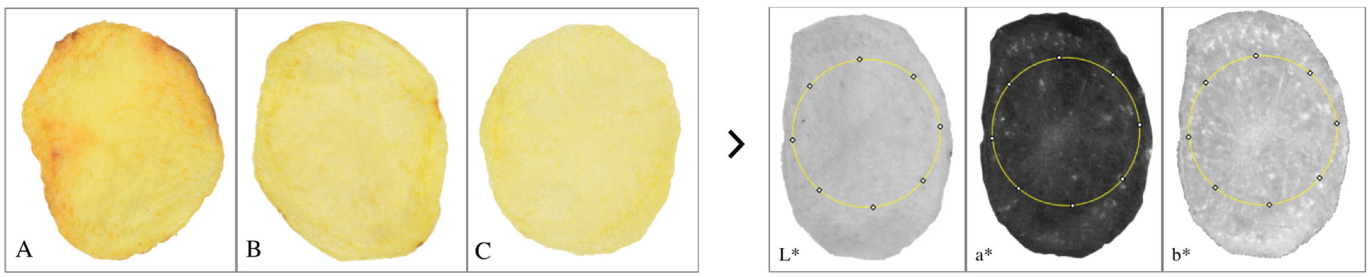

Fig. 5. Examples of RGB images of untreated (a), blanched (b) and PEF-treated (c) potato crisps (left), and image conversion from RGB into L*a*b* channels (right). Pixels areas analysed are highlighted in yellow.

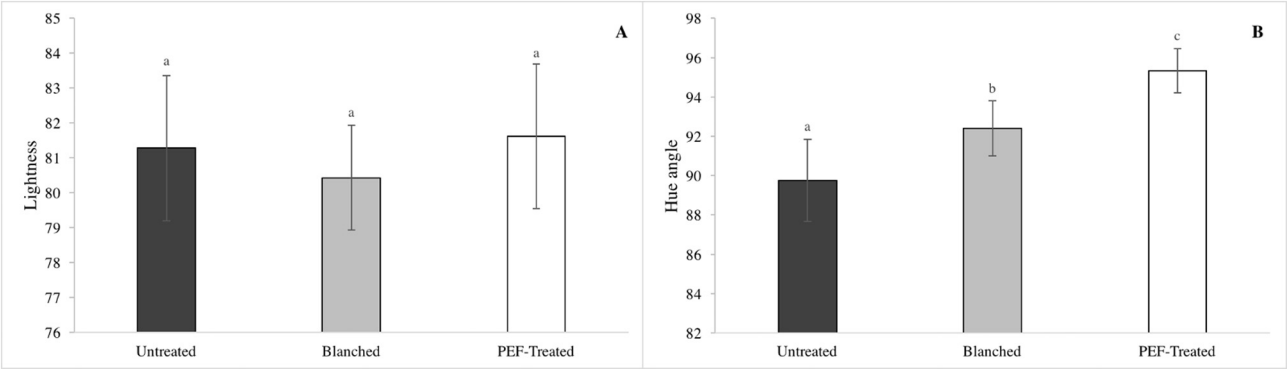

Fig. 6. Lightness (A) and hue angle (B) of untreated, blanched and PEF-treated potato crisps. Results are expressed as means ± standard deviations (error bars) of $n = 20$. Values with different letters differ significantly ($p < 0.05$).

cm) caused also a significant softening of the ground tissues of sweet potato, resulted into lower force necessary for cutting (Liu et al., 2017). The softening of the tissue upon PEF treatment is probably due to the cell structure modification, mainly the increase in the membrane permeability and the irreversible cell breakdown, that in turn increase the water transfer, which is very important during potato-based product frying (Botero-Uribe et al., 2017). However, there is a lack of information in the literature about the effect of PEF pre-treatment on final products texture. Ignat et al. (2015) observed no differences in texture of PEF treated potato cubes (0.75 kV/cm and 2.50 kV/cm) in comparison to blanched and untreated ones. In our work, PEF-treated samples presented lower firmness and crispness in comparison to the untreated one, while no significant differences were observed between PEF-treated and blanched samples. The discrepancy could be due to the different shape of potato samples, indeed Ignat et al. (2015) performed their study on potato cubes (2 × 2 × 2 cm), while the present work was focused on 1.5 mm potato slices, as well as to different PEF process parameters and frying temperature.

3.2.3. Maillard reaction substrates and acrylamide content

As previously demonstrated, reducing sugars and asparagine represent the main limiting substrates of acrylamide formation in potato products (Amrein et al., 2003).

Table 1 shows the glucose, fructose and free asparagine content (mg kg^{-1}) in raw potato slices untreated and submitted to conventional and innovative pre-treatments (blanching and PEF, respectively), all followed by a washing step of 5 min.

Free asparagine was found at concentrations more abundant than reducing sugars, and its reduction in treated potato slices were higher than those found for glucose and fructose. In fact, PEF treatment allowed a reduction of 48% of free asparagine compared to the untreated sample, higher than the reduction reached by blanching (40%). Both pre-treatments, blanching and PEF, allowed just a slight reduction of the fructose initial content (4.9% and 5.4%, respectively). No glucose reduction was observed in PEF-treated potato slices; on the contrary the 27% of its reduction was shown in blanched potato samples.

It is well established that acrylamide precursors, reducing sugars and amino acids, are leached out by blanching treatment of raw potatoes (Zhang, Kahl, Bizimungu, & Lu, 2018); on the other hand, PEF treatment has been mentioned as a potential alternative method to assist the removing of Maillard reaction substrates, and consequently reducing the acrylamide content in cooked potato-based products (Jaeger et al., 2010). While according to Janositz et al. (2011), PEF promoted a significant decrease in the reducing sugars content, the results of the present study seem to indicate that the main reduction is related to free asparagine.

The acrylamide content (mg kg^{-1}) of untreated, blanched and PEF-treated potato crisps is displayed in Table 1. For the potato crisps pre-treated by PEF, the acrylamide content appeared lower compared with those pre-treated by conventional blanching. The PEF pre-treatment protocol and experimental conditions applied in this study resulted on a reduction of around 30% of acrylamide content compared to control (untreated), while only around the 17% of reduction was observed on blanched samples compared to control (untreated). Similar results of acrylamide reduction due to hot water blanching of potato slices were previously reported by other authors (Pedreschi et al., 2011). The cell electroporation phenomenon induced by the application of the selected PEF protocol on raw potato slices resulted in a further improvement of the diffusion of Maillard reaction substrates and so of acrylamide reduction in fried potato crisps compared to the applied conventional pre-treatment.

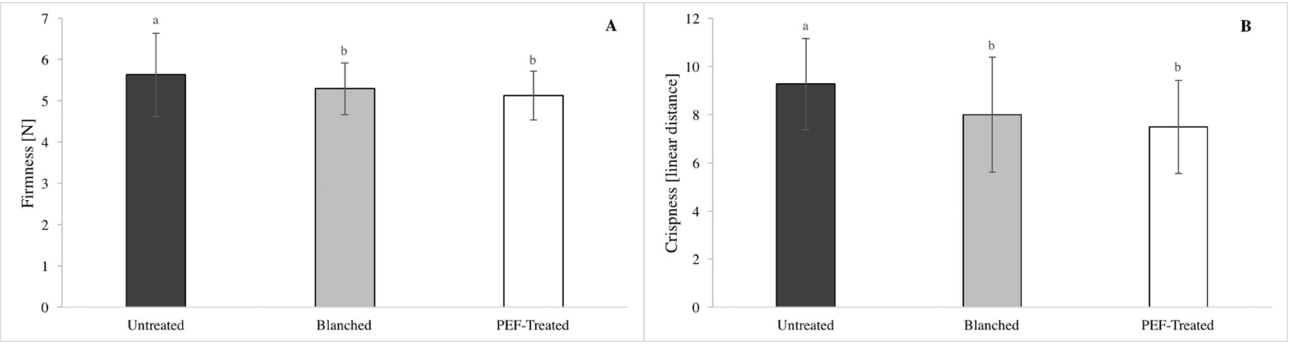

Fig. 7. Firmness (A) and crispness (B) of untreated, blanched and PEF-treated potato crisps. Results are expressed as means ± standard deviations (error bars) of n = 20. Values with different letters differ significantly (p < 0.05).

Table 1
Maillard substrates (glucose, fructose and free asparagine) contents of raw potato slices untreated, after blanching and after PEF treatment, and acrylamide content of untreated, blanched and PEF-treated potato crisps. Results are expressed in mg kg^{-1} of dry weight. Reduction percentages are calculated in relation to the untreated sample.

Sample	Glucose			Fructose			Asparagine			Acrylamide		
	Mean	%RSD[a]	Reduction (%)	Mean	%RSD	Reduction (%)	Mean	%RSD	Reduction (%)	Mean	%RSD	Reduction (%)
Untreated	70.0	14.1	–	58.9	1.7	–	10,487.8	8.7	–	2.0	3.3	–
Blanched	50.9	11.9	27	56.1	3.5	4.9	6296.1	6.2	40	1.6	9.1	17
PEF-treated	75.4	10.6	n.a.[b]	55.8	3.1	5.4	5416.9	9.3	48	1.4	7.5	31

[a] Percent relative standard deviation ($n = 3$).
[b] Not applicable.

4. Conclusions

Overall this preliminary study confirmed the high potentiality of the application of pulsed electric fields as a pre-treatment to improve the release of acrylamide precursors in raw potatoes and so to reduce the acrylamide content in deep-fat fried potato crisps. Moreover, important indications regarding the possibility of industrial application of PEF pre-treatment for the production of potato crisps were given. By monitoring PEF treatment parameters and other manufacturing steps, it was possible to achieve a consistent reduction of acrylamide due to its precursors leaching during the washing step, with only slight modifications of the final quality of the product, in terms of colour and texture.

Although PEF pre-treatment led to a significant reduction of acrylamide content in potato crisps if compared to untreated and blanched samples, the final amounts found were still higher than recommended legislative limits (0.75 mg kg^{-1}). In this direction other possible combined strategies need to be developed for industrial applicability. The combination of PEF and a mild blanching of raw potatoes and the monitoring of subsequent manufacturing operational units could enhance the extraction of reducing sugars and free asparagine and consequently the reduction of the acrylamide formation in potato crisps.

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Paper II

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Metabolic response of organic strawberries and kiwifruit subjected to PEF assisted-osmotic dehydration

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ABSTRACT

This research aims to evaluate the effect of pulsed electric field (PEF) pre-treatment for 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 were 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) and then subjected to OD in hypertonic solutions (40% w/w) of sucrose or trehalose, both added with calcium lactate 1%. 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 after 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 the intensity of osmotic dehydration of fruits. However, it is important to understand the implication of the treatment on the tissue metabolism and structure 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.

1. Introduction

Since the mid-1990s the organic food market has been rapidly expanding and organic fruits and vegetables have been growing the fastest. The worldwide total area used for organic fruit production by 2015 has been recorded as 288 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, high quality, healthy, and environmentally friendly food products.

In the processing of organic strawberries and kiwifruits to obtain semi-dried products with longer shelf-life and fresh-like characteristics, it is fundamental to select treatments that are chemical additives free and that allow the retention of nutritional and sensorial properties. These characteristics can be obtained by the application of non-thermal processing (Tylewicz et al., 2017).

Osmotic dehydration (OD) is often coupled with other non-thermal technologies, such as ultrasound and pulsed electric fields (PEF) in order to accelerate the mass transfer and decrease processing times (Dermesonlouoglou, Zachariou, Andreou, & Taoukis, 2016; Nowacka 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 to 600 V/cm (Phoon, Galindo, Vicente, & Dejmek, 2008; Tylewicz et al., 2017) to 20–80 kV/cm, depending on the desired effect (Barba, Galanakis, Esteve, Frigola, & Vorobiev, 2015). PEF application can lead to reversible or irreversible cell membrane permeabilization, depending on both the intensity of the applied electric field and the characteristics of the raw materials. Reversible electroporation occurs when cell membranes can recover their structure and functionality after removing the electrical field and it is used to assure the survival of the electrically stimulated cells. The irreversible permeabilization causes permanent membrane damage and consequently the cell death (Donsì, Ferrari, & Pataro, 2010).

Because fruits are made up of biologically active tissues, evaluating the effect of different technologies on the tissue metabolic response and cell integrity and vitality is extremely important. The metabolic

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response of vegetables has been widely studied by isothermal calorimetry (Dellarosa et al., 2016; Dymek et al., 2016; Nowacka et al., 2018; Panarese et al., 2014; Panarese, Tylewicz, Santagapita, Rocculi, & Dalla Rosa, 2012; Rocculi et al., 2012; Tappi et al., 2017; Yusof, Wadsö, Rasmusson, & Gómez Galindo, 2017). The decrease in metabolic heat production of different plant tissues could be due to the loss of cell viability caused by different treatments (Mauro et al., 2016; Nowacka, Fijalkowska, et al., 2018; Tylewicz et al., 2017).

For example, OD promoted the reduction of metabolic heat in kiwifruits (Nowacka et al., 2018; Panarese et al., 2012), and apples (Tappi et al., 2017), due to a partial loss of cell vitality. PEF treatments have also been 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).

Electrolyte leakage (EL) is used to assess the degree of permeability and integrity of the membranes, while texture measurements reflect the loss of turgor pressure, the softening of cell walls or the 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 coupled with osmotic dehydration (OD) on physiological changes of 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 moisture content) and kiwifruits (*Actinidia deliciosa*) var. *Hayward* (12 ± 1°Brix, 83 ± 2% of moisture content) from organic farming were purchased from the local market in Cesena (Italy). The fruits were stored at 4 ± 1 °C for no longer than one week. The strawberries were washed, hand stemmed and cut into halves along the central axis of the fruit. The kiwifruits were washed, peeled and cut into slices of 10 mm width, which were further divided into four triangular pieces.

2.2. Pulsed electric field (PEF) treatment

Approximately 35 g of samples (about six strawberry halves and 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 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). The conductivity value was chosen as the average conductivity for strawberry and kiwifruit samples. PEF treatments were applied using a lab-scale pulse generator S-P7500 60A 8 kV (Alintel srl., Bologna). Treatment conditions were selected on the basis of previous experiments (Traffano-Schiffo et al., 2016; Traffano-Schiffo et al., 2017; Tylewicz et al., 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 1000 rectangular pulses of fixed 10 µs width at the frequency of 100 Hz were applied (Fig. 1). The specific energy input, calculated as suggested by Raso et al. (2016), was 0.96 and 1.92 kJ/kg for samples treated at 100 and 200 V/cm respectively.

2.3. Osmotic dehydration (OD) treatment

The OD treatment was carried out by immersing the samples in 40% (w/w) sucrose or trehalose solution. Sucrose was selected as the most commonly used sugar for OD, while trehalose was selected because of its known ability to protect cell membrane from physical stresses (Crowe et al., 2001). Calcium lactate (CaLac) 1% (w/w) was added to osmotic media as structuring agent (Tylewicz et al., 2017). The treatment was performed at 25 °C with continuous stirring and maintaining fruit:OD solution ratio of 1:4 (w/w) to avoid changes in the solution concentration for the entire duration of the treatment (120 min). All obtained samples with related abbreviations are reported in Table 1.

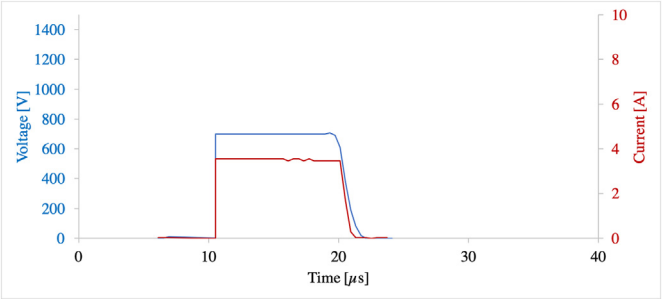

Fig. 1. Representative pulses applied to the samples reporting Voltage and Current.

2.4. Analytical determinations

2.4.1. Metabolic activity by isothermal calorimeter (TAM)

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 ampoules, sealed with a teflon coated rubber seal 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 power ($mW \cdot g^{-1}$) was calculated according to Gómez Galindo, Wadsö, Vicente, and Dejmek (2008). The average metabolic heat production was calculated by integrating the metabolic heat profiles. The first 4 h of analysis was excluded 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.

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 International, 2002).

2.4.2. Cell viability

The cell viability test was performed using fluorescein diacetate (FDA, Sigma-Aldrich, USA, λex = 495 nm, λem = 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 solution prepared with an isotonic

Table 1
Abbreviations of analysed samples.

Sample code	Electric field (V/cm)	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

sucrose concentration, and then rinsed with distilled water. 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 (EL)

Two pieces of strawberries (or kiwifruit) for each sample (about 10 g), were placed in distilled water in a ratio of 1:4 (w/v) and kept under constant agitation at room temperature (25 ± 1 °C). EL was determined by measuring the electrical conductivity of water medium with a conductivity meter (EC-Meter basic 30 +, Crison), immediately (T0) and after three hours of stirring (T3 h) at room temperature, and after ten minutes of boiling (TC), to ensure the complete tissue disintegration.

2.4.4. Texture analysis and texture disintegration index

Firmness (N) of strawberry halves and kiwifruit slices was evaluated at room temperature (25 ± 1 °C) performing a penetration test using a TA-HDi500 Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell and a stainless-steel probe of 8 mm diameter. The test was carried out by penetrating the samples for 90% of their thickness. 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, 2002; Wiktor et al., 2018). To characterise the extent of tissue damage, the texture disintegration index (Z_t) 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, 2016). 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.

2.5. 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 their respiratory activity. However, heat production can be altered by different types of tissue damage, as reported in various studies (Rocculi et al., 2012; Tappi et al., 2017; Wadsö, Gomez, Sjöholm, & Rocculi, 2004). This parameter also represents an indirect index of cell viability, useful to understand if the applied treatments have determined the loss of the tissue viability. 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 treatments. As an example, Fig. 2 shows calorimetric signals obtained by strawberries samples submitted to the different treatments.

Considering only the first 20 h at the chosen temperature of 10 °C, the production of heat due to microbial growth on the sample can be considered negligible, as shown by previous works (Galindo, Rocculi, Wadsö, & Sjöholm, 2005; Riva, Fessas, & Schiraldi, 2001). Therefore the recorded metabolic heat can be exclusively attributed to the endogenous metabolism of the tissue, which is the sum of the basal metabolism and the tissue response to cutting ('wounding response') and/or the applied treatment (Wadsö et al., 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 than values measured by Panarese, Tylewicz, et al. (2012). However, in this work the measurements were carried out at a higher temperature (20 °C), justifying the detected 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 reduction in water content was significant (4% and 3% for sucrose and trehalose, respectively). The effect of PEF, as expected, depended on the applied electric field strength. 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 from samples treated only with 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 around 3% and 5% for sucrose and trehalose, respectively, 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 (2.4% and 2.7% for sucrose and trehalose, respectively), the heat production 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 endogenous heat production was observed, while in the 200 V/cm treated samples this parameter was even lower than the 100 V/cm treatment, with no significant differences between the used sugars. The application of 100 V/cm electric field in combination with OD resulted in an increase of dehydration only when trehalose was used (a further 2%), while the higher treatment allowed to increase water loss (between 1% and 2%) with both sugars.

3.2. Cell viability

Cell viability was assessed using the dye fluorescein diacetate (FDA), which can actively penetrate through the cell membrane, where it is hydrolysed 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 representative photos of kiwifruit samples stained with FDA subjected to the different treatments compared to the fresh one. The application of OD alone did not compromise cell viability. The effect of PEF, as expected, depended on the applied electric field strength: 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 partially retained. When 200 V/cm and OD were combined no viable cells were observed.

Fig. 4 shows representative photos of strawberries samples stained with FDA subjected to the different treatments compared to the fresh

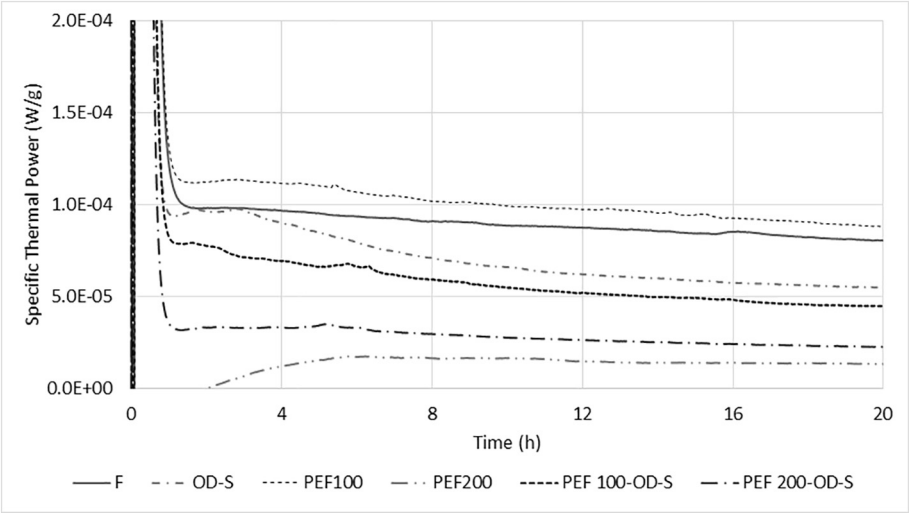

Fig. 2. Example of calorimetric signals obtained from strawberries as fresh (F), after OD with sucrose (OD-S), after PEF treatment at 100 (PEF100) and 200 (PEF200) V/cm, and after the combined treatments (PEF100 OD-S; PEF200 OD-S),

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

Sample	Heat (J/g)	Water content (%)	EL (%)	Firmness (N)	Z_t
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.

one. The cell viability was similar to the fresh fruits when the lower PEF voltage and OD were used singularly, while the application of 200 V/cm electric 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 200 V/cm treated samples, cell viability was completely compromised.

3.3. Electrolytes leakage

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

Unfortunately, results obtained for this fruit were not satisfactory. Fresh samples already showed a very high EL of about 52%, and the PEF treatment decreased or maintained these values, respectively, for the 100 V/cm and the 200 V/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 an opposite behaviour; the samples treated with trehalose presented a higher EL at the end of the process than those 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 they were both significantly lower than the samples treated with only OD. The physiological inhomogeneity in ripening degree of the samples used could have determined alterations in the permeability of cell membranes (Antunes & Sfakiotakis, 2008).

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

Sample	Heat (J/g)	Water content (%)	EL (%)	Firmness (N)	Z_t
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.

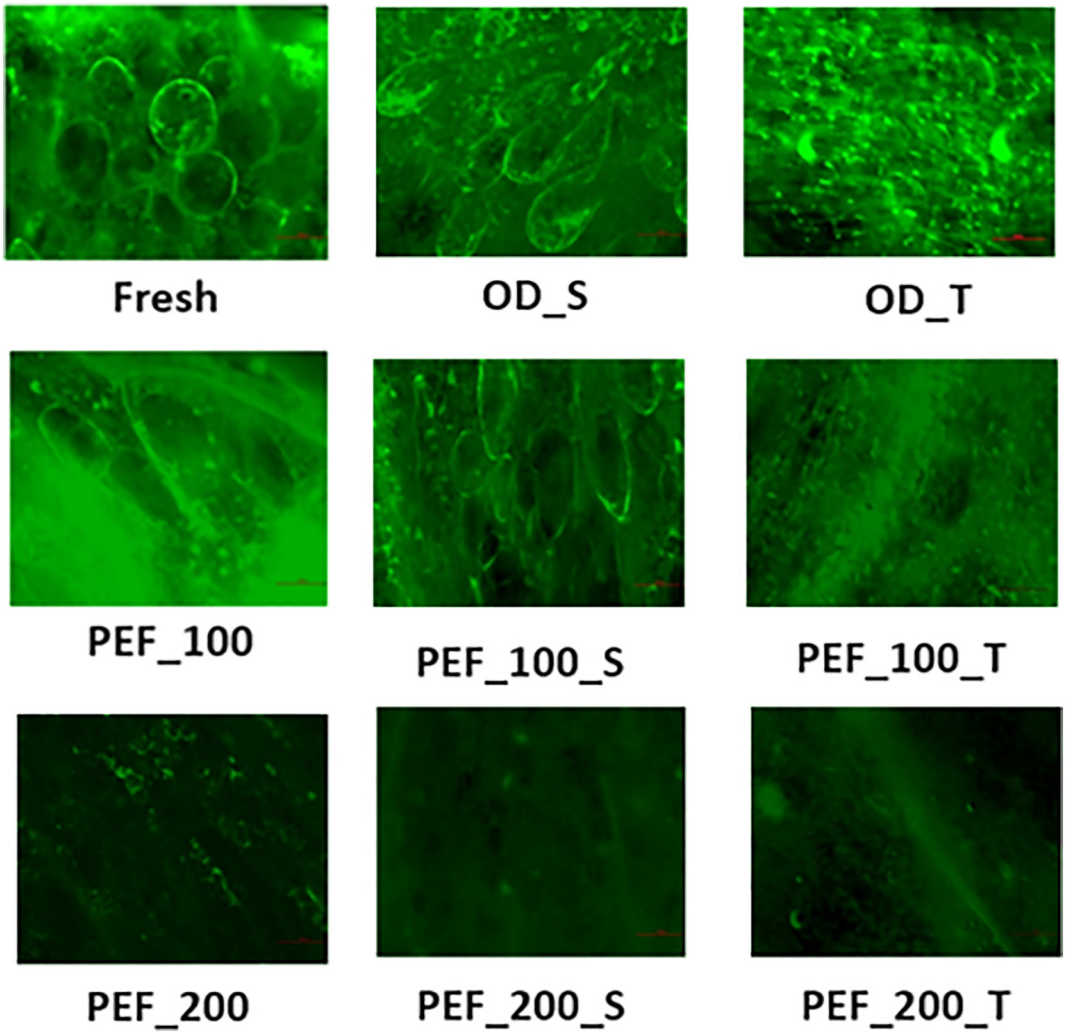

Fig. 3. Representative photos of kiwifruit samples stained with FDA subjected to the different treatments compared to the fresh one.

The loss of electrolytes in the strawberry samples is shown in Table 3**.** The fresh sample presented the lowest value of EL. The PEF treatment alone seemed to slightly increase the EL, but differences were not significant. The OD treatment with both sugars increased the EL of about 15%. In combined treatments, a further reduction in the ability to retain the solutes and the consequent increase of released electrolytes was observed, which was proportional to the intensity of the PEF treatment 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 Tables 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 texture disintegration index (Z_t), as an indicator of tissue disruption. Values are reported in Tables 2 and 3, for kiwifruit and strawberries, respectively. In both fruits, the increase of Z_t was proportional to the voltage applied, although they were generally lower in strawberries. For the 200 V/cm treatment, Z_t reached values of 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, the assessment of various parameters to understand the effect of OD, PEF and their combination on two different fruit tissues has been performed.

The effect of OD on fruit tissue metabolism has been studied by different authors. Castelló, Fito, and Chiralt (2010), Moraga, Moraga, Fito, and Martínez-Navarrete (2009) and Torres, Castelló, Escriche, and 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, due to the removal of oxygen from tissues. Mauro et al. (2016) observed that the use of

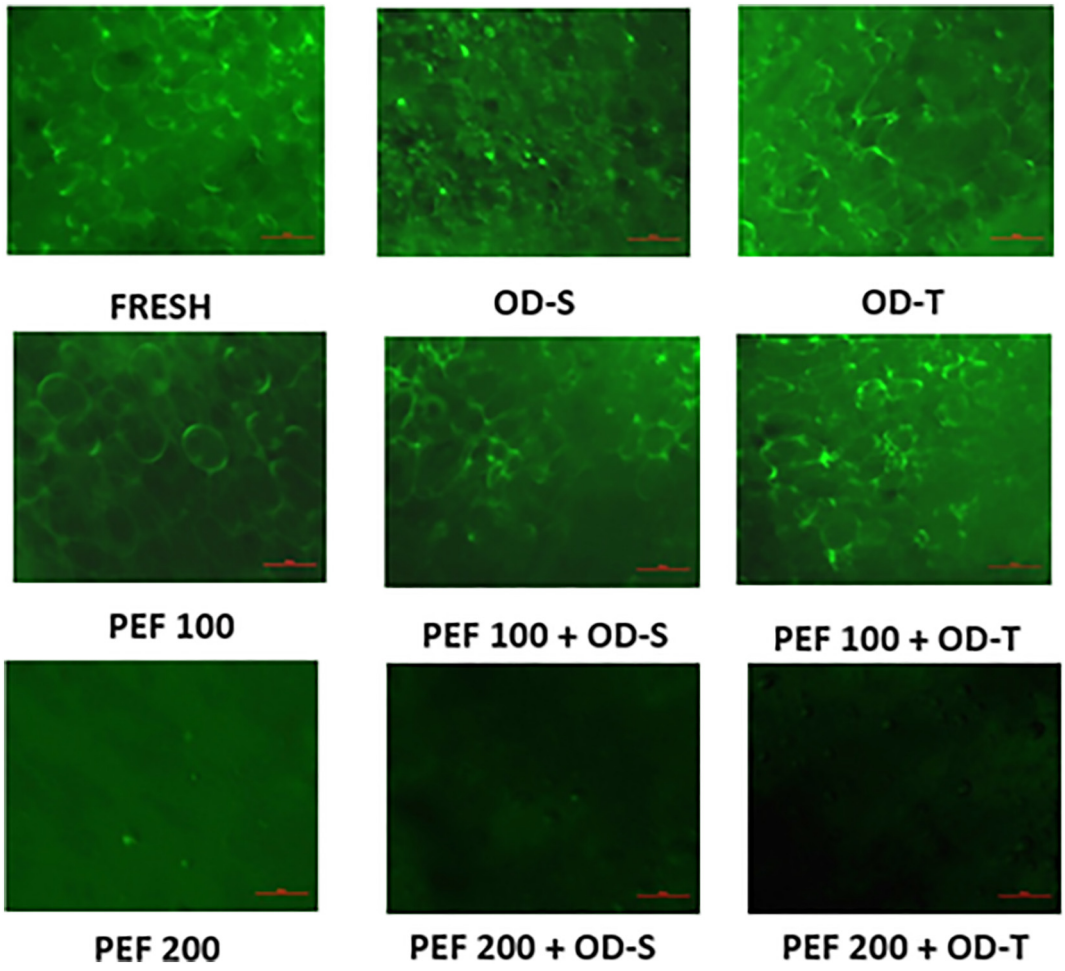

Fig. 4. Representative photos of strawberries samples stained with FDA subjected to the different treatments compared to the fresh one.

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 osmo-dehydrated in sucrose at 61.5%, and by Tylewicz et al. (2017) for strawberries treated in solutions of sucrose and trehalose, both at 40%.

However, a reduction of metabolic heat production upon osmotic dehydration has been observed for kiwifruit and apple tissues (Panarese, Laghi, et al., 2012; Tappi et al., 2017). Moreover, the addition of calcium to the osmotic solution was shown to further decrease the tissue metabolic activity, that was attributed to a decrease of respiration rate (Lester, 1996; Luna-Guzmán et al., 1999; Castelló et al., 2010; Tappi et al., 2017).

Some authors (Blum and Tuberosa, 2018; Mavroudis, Dejmek, & Sjoholm, 2004) reported that the cell survival depends on the water status and content of the products, showing a progressive loss of the cell survival with the increase of the dehydration rate.

In the present study, for both fruits, OD allowed to keep cell viability and did not seem to alter in a significant way the metabolism of the tissues, despite the decrease of water content in OD treated samples (Tables 2 and 3). Mavroudis et al. (2004) suggested that death in the outer layer of apples occurs when the osmotic medium concentration is 50% or above, which is higher than that used in the present research. The process time and the structural properties of tissue have an influence on the survival rate as well. Panarese, Laghi, et al. (2012) observed that the physiological response to OD is also influenced by the ripening degree.

Electrolytes leakage generally increased after OD with both sugar solutions, however these differences were significant only in kiwifruit samples treated with sucrose. This may indicate that a partial damage of cell membranes occurred, more pronounced in kiwifruit than in strawberries. When fruits are subjected to the OD several phenomena could take place, like plasmolysis, shrinkage of the vacuole, changes in the structure of the cell walls, which could cause the softness of the tissue (Panarese, Laghi, et al., 2012). While in sucrose dehydrated kiwifruit samples evidenced a slight firmness loss, in trehalose dehydrated samples textural parameters were unaffected. Strawberries showed a similar trend, but not statistically significant differences were found in textural properties after OD treatment. Moreover Tylewicz et al. (2017) observed a less marked changes in the texture of strawberry samples dehydrated in trehalose than in sucrose solution, probably due to the protective effect of trehalose on the cellular structure (Velickova et al., 2013).

The study of Tylewicz et al. (2017) also showed that the PEF treatment itself caused cell death only when a field strength of 200 V/cm or higher was used, while lower electric field strengths (100 V/cm) allowed to preserve the tissue viability, indicating that cell membranes were able to retain their structure and functionality after the treatment. Ersus and Barrett (2010) studied the effect of PEF at different intensities on cellular integrity of onion cells by staining them with neutral red dye, which is able to color the intact vacuoles. They observed that, at the electric field strength applied (167 V/cm), no cell rupture appeared, regardless the number of used pulses (10 or 100). They suggested that the applied electric field was lower than the critical threshold, which is necessary to electroporate cell membranes.

Fincan & Dymek (2002) developed a method to visualise single permeabilised cells in onion tissue upon PEF treatment showing how

their distribution in the tissue is not homogeneous and that a time scale of internal transport and mixing exists because of the heterogenicity of the permeabilised tissue.

However, the effect of PEF on vegetable tissue metabolism is less known. Dellarosa et al. (2016) characterized the effect of PEF treatments on the metabolism of apple tissues; results showed that 100 V/cm field strength allowed to maintain cell viability but determined an increase in the production of heat compared to the fresh sample. A similar effect was also observed for strawberries in the present study. According to the authors, this effect may have been caused by a tissue response to the stress generated by the electroporation of membranes; the increased heat production reflected the energy expended by the tissue to compensate for reversible changes in the membrane. According to Gomez-Galindo (2016), the transient permabilisation of the membrane and the struggle of the cells to recover normal functionality promote changes on cell metabolism and tissue properties.

According to Gómez Galindo et al. (2008), the formation of pores and their resealing, caused by reversible electroporation, induces a physiological response in the tissue that involves the oxygen consuming pathway and may last up to several hours after the treatment.

In the study of Dellarosa et al. (2016), the application of field strengths of 250 V/cm and 400 V/cm promoted damages to the apple cells, causing their death and bringing the metabolic heat production very close to zero. In the present study, cell metabolism was evaluated by calorimetric measurements and microscopic observations. It must be noted that the evaluation of cell viability by FDA analysis is limited to a restricted area and can therefore give only partial information that should be considered complementary to the calorimetric results. Although a complete loss of viability was observed by fluorescent microscopy in sample treated at 200 V/cm, a residual metabolic heat production was observed. These results indicate that the field strength applied lead to a strong reduction of cell viability in the tissue, probably due to irreversible electroporation.

This hypothesis was confirmed by the EL in strawberries and by the texture measurements. Ions leakage in strawberries increased proportionally to the electric field applied. Faridnia et al. (2015) investigated the ions leakage from potato tissue following PEF treatment at different intensities and duration. They observed a higher ions leakage when electric field strength 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 Z_t index in samples treated at 200 V/cm indicates an extensive cell structures breakdown, more pronounced in kiwifruit in which it was very close to the tissue subjected to freezing and thawing (0.934).

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

Contrasting results were found for the two sugars: trehalose seemed to further reduce metabolic heat production and to better maintain samples structure than 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 during drying (Crowe et al., 2001). This effect has been observed on both meat (Dovgan, Barlič, Knežević, & Miklavčič, 2017) and vegetable (Phoon et al., 2008) products. For this reason, OD with trehalose has been used as a pre-treatment before drying, thus improving the characteristics of the re-hydrated products (Aktas, Yamamoto, & Fujii, 2004). Nevertheless, ions leakage seemed to increase when using trehalose, while mechanical properties showed different results but without a clear trend in relation to both the applied field strengths and the type of fruit. Therefore, the effect of the sugar used should be better clarified.

5. Conclusions

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

The application of OD alone to organic strawberries and kiwifruits allowed to preserve the functionality of the cell membranes causing only a slight decrease in the endogenous heat production and a higher electrolytes leakage.

The application of the lower strength PEF (100 V/cm) generally did not alter the metabolic and structural indexes, although it seemed to promote a physiological reaction in strawberry tissues, evidenced by an increase of metabolic heat production. The combination with OD led to a further decrease in metabolic heat production and an increase of textural breakdown. On the contrary, the application of 200 V/cm PEF caused total loss of cell vitality and tissue breakdown, probably due to the irreversibility of the electroporation. For a possible industrial application, it would be important to evaluate the effect of the increased textural breakdown on the final products quality, in accordance with the intended use (e.g. minimal processing, further drying, freezing etc.)

Further clarifications are needed on the effect of the substitution of sucrose with trehalose.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Paper III

Schouten, M.A., **Genovese, J.**, Tappi, S., Di Francesco, A., Baraldi, E., Cortese, M., Caprioli, G., Angeloni, S., Vittori, S., Rocculi, P. and Romani, S. (2020). Effect of innovative pre-treatments on the mitigation of acrylamide formation in potato chips. *Innovative Food Science & Emerging Technologies*, 64, p.102397.

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Effect of innovative pre-treatments on the mitigation of acrylamide formation in potato chips

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ABSTRACT

The aim of this research was to evaluate the reduction of acrylamide (AA) formation in potato chips applying innovative pre-treatments. Raw potato slices were subjected for 5 and 15 min to: dipping in water; dipping in *Aureobasidium pullulans* L1 yeast water suspension; dipping in water or in yeast water suspension after pulsed electric fields (PEF) in order to investigate a possible synergistic effect of pre-treatments. The raw potato samples were analysed for AA precursors and, after frying, for AA by using HPLC-MS/MS. In addition, the final potato chips main quality parameters were evaluated.

Compared to untreated potato chips, yeast treatment promoted a reduction of AA formation mainly at the longest dipping time (15 min). PEF treatment followed by water dipping was the most effective in reducing AA for both the studied treatment times. The combination of PEF and yeast treatments led only a slight reduction of AA formation.

Industrial relevance: The Commission Regulation (EU) 2017/2158 has established new acrylamide (AA) benchmark levels in different foods due to its negative classification as "probably carcinogenic to human". For this reason, food industries are interested in developing different processing methods to reduce the AA formation and at the same time to maintain an acceptable quality of final products. Fried potatoes (French fries and chips) are the most vulnerable foods in terms of high content of AA formation, being rich in the main Maillard reaction substrates, such as asparagine and reducing sugars, and characterized by a high surface to volume ratio. Among the strategies recently suggested for the reduction of AA in potato chips, pulsed electric fields (PEF) and yeast pre-treatments are very promising, having the potentiality to reduce AA precursors in raw potato tissues. In this study the possibility to apply yeast and PEF pre-treatments on raw potato slices with suitable processing times for a possible industrial application were evaluated.

1. Introduction

Acrylamide (AA) is an undesired food toxic compound classified as "probably carcinogenic to human" (group 2A), due to its neurotoxic and genotoxic proprieties on the basis of animal studies (IARC, 1994). In the last few years, authorities and regulations have been more and more restrictive concerning the maximum AA levels allowed in popular heat-treated foods and beverages (European Commission, 2017; Food Drink Europe, 2019; Palermo et al., 2016). The latest Commission Regulation (EU) 2017/2158, although not introducing a legal limit, contains stricter measures to reduce AA levels in different food groups and subgroups such as fried potato, bakery products and roasted coffee (European Commission, 2017).

AA is principally formed during food thermal processing (> 120 °C) by the reaction of free amino acids (e.g. asparagine) with reducing sugars (D-glucose and D-fructose) as part of the Maillard reactions, also known as non-enzymatic browning reactions (Stadler & Scholz, 2004). On the other hand, Maillard reactions result in physical and chemical

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changes, important for the development of some peculiar food characteristics like the desirable taste, colour and flavour (Medeiros Vinci, Mestdagh, & De Meulenaer, 2012; O'Connor, Fisk, Smith, & Melton, 2001). For this reason, the challenge for food industries and researches is to develop methods to reduce this toxicant, without changing the desired final properties.

Potato is the second major crop for human consumption in Europe and the fourth in the world, being considered an excellent source of energy, carbohydrates and nutritional fibres (Dourado et al., 2019). Nevertheless, fried potato products (French fries, chips, etc.) are highly susceptible to AA formation due to asparagine and reducing sugars content as well as the high temperatures applied during the frying process (Parker et al., 2012) and the high surface to volume ratio. Due to the large consumption of fried potato products worldwide, the reduction of undesirable AA, without compromising the sensory characteristics is essential (Dourado et al., 2019).

The principal strategies proposed to reduce AA in potato products are the selection of cultivar and storage conditions, the control of time and temperature of heat treatment, the application of different frying techniques (e.g. under vacuum), the use of asparaginase enzyme and hot water blanching as pre-treatments (Amrein et al., 2003; Foot, Haase, Grob, & Gondé, 2007; Medeiros Vinci et al., 2012; Pedreschi, Mariotti, Granby, & Risum, 2011; Romani, Bacchiocca, Rocculi, & Dalla Rosa, 2009). However, these strategies present different disadvantages such as long processing times, high costs, negative sensory modifications and difficult implementation for industrial scale. Therefore, it is necessary to find alternative methods to reduce the formation of AA in potato products.

Recently, the application of biocontrol agents as asparaginase producers (Di Francesco et al., 2019) and non-thermal treatments such as pulsed electric fields (PEF) (Genovese et al., 2019) were proven useful to reduce the AA precursors in the potato tissues and hence the subsequent AA formation. The metabolic activity of microorganisms could reduce the asparagine concentration through the activity of the asparaginase enzyme, which hydrolyses asparagine to aspartic acid and ammonia. Di Francesco et al. (2019) reported for the first time that the yeast *Aureobasidium pullulans* L1 strain can successfully assimilate asparagine in "Primura" var. potato homogenate after 30 min of contact, leading to a great decrease in the AA content in the final fried potato chips (−85%). The yeast ability to produce enzymes has attracted considerable biotechnological interest because these hydrolytic enzymes have a potential commercial value in various industries (Deshpande, Rale, & Lynch, 1992). As demonstrated by Dunlop and Roon (1975), *A. pullulans* showed a hydrolytic cleavage of L-asparagine with intact whole cells. Generally, the liberation of enzymic activity from intact protoplast cells is considered the single conclusive proof for the existence of an extracellular enzyme (Dunlop & Roon, 1975), subsequently able to act on the potato tissue composition. Moreover, *A. pullulans* has demonstrated to be able to ferment sugars, among which sucrose covers an important role because proved as the carbon source for pullulan synthesis (Sheng, Tong, & Ma, 2016). Furthermore, An, Ma, Chang, and Xue (2017) showed how potato starch can promote *A. pullulans* enzyme production and pullulan biosynthesis. PEF technology consists in an electrical treatment of short time (from nanoseconds to milliseconds) that promotes the temporary or permanent loss of the semi-permeability of cell membranes in biological tissues, favouring mass transfer phenomena (Fincan & Dejmek, 2003; Puértolas, Koubaa, & Barba, 2016; Vorobiev & Lebovka, 2010). Recently, Genovese et al. (2019) described the possibility to reduce AA precursors in raw potatoes by the application of PEF followed by a 5 min water dipping, which led to a reduction of around 30% of AA content in fried potato chips.

The aim of present research was to evaluate the possibility to reduce AA in potato chips by applying for different times the above-mentioned innovative pre-treatments, *A. pullulans* L1 strain and PEF, alone or in combination.

The AA and its precursors (e.g. reducing sugar and asparagine) content, quality parameters such as moisture, oil content, colour and texture of potato chips were also evaluated.

2. Materials and methods

2.1. Potato

Fresh potato tubers (*Solanum tuberosum* cv Lady Claire) were purchased at the local market (Emilia-Romagna, Italy) and stored for a maximum of 15 days in the dark at 15 °C and 90% relative humidity (RH). Potato slices were prepared by reproducing as much as possible the main industrial production steps and conditions. Tubers were washed in running water, manually peeled and cut in slices of 1.5 ± 0.2 mm thickness by using an electric cutter machine mod. KAFPL0922N (CAD Italy, Italy). Potato slices were rinsed immediately after slicing for approximately 1 min in tap water (18 ± 2 °C) in order to eliminate part of the starch material on the surface.

2.2. Aureobasidium pullulans L1 strain

Aureobasidium pullulans L1 strain is a potential biocontrol agent mainly active against fruit postharvest pathogens, representing a promising alternative to common fungicides in the control of postharvest diseases (Di Francesco et al., 2018; Di Francesco, Ugolini, D'Aquino, Pagnotta, & Mari, 2017). The yeast was maintained on Nutrient Yeast Dextrose Agar (NYDA), consisting of nutrient broth 8 g, yeast extract 5 g, dextrose 10 g and technical agar 15 g (all from Oxoid, Basingstoke, UK) in 1 L of distilled water, and stored at 4 °C until use. Two days before the experiments, the yeast was cultured in 250 mL conical flasks, containing 50 mL of Nutrient Yeast Dextrose Broth (NYDB: NYDA without Agar) on a rotary shaker at 200 rpm for 48 h at 25 °C. Cell suspension was prepared by centrifugation of the liquid culture samples at 6000 rpm for 10 min at 4 °C. Yeast cells were suddenly washed twice with sterile distilled water to remove the growth medium and suspended in sterile distilled water to reach a concentration of 10^8 cells/mL by using a hemocytometer. Yeast suspension was used for the pre-frying treatments and isothermal calorimetry assay.

2.3. Chemicals and reagents

Acrylamide (for molecular biology, ≥99% (HPLC), C_3H_5NO, molecular weight 71.08 g/mol, CAS No 79-06-1) and its precursors namely, L-asparagine (≥98% (HPLC), $C_4H_8N_2O_3$, molecular weight 132.12 g/mol, CAS No 70-47-3), D-(−)-fructose (≥99%, $C_6H_{12}O_6$, molecular weight 180.16 g/mol, CAS No 57-48-7) and D-(+)-glucose (analytical standard, $C_6H_{12}O_6$, molecular weight 180.16 g/mol, CAS No 50-99-7) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Individual stock solutions of AA, fructose and glucose, at a concentration of 1000 mg/L, and asparagine, at 500 mg/L, were prepared by dissolving the pure standard compounds in water and storing them in glass-stoppered bottles at −18 °C. Afterwards, standard working solutions at various concentrations were prepared daily by appropriate dilution of the stock solution with water. HPLC-grade acetonitrile was supplied by Sigma-Aldrich (Milano, Italy). HPLC-grade formic acid (99%) was obtained from Merck (Darmstadt, Germany). C18 octadecyl silica sorbent was purchased from Phenomenex (Castelmaggiore, Bologna, Italy). Deionized water (> 18 MΩ cm resistivity) was further purified using a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). All solvents and solutions were filtered through a 0.2 μm polyamide filter from Sartorius Stedim (Goettingen, Germany). Before HPLC analysis, all samples were filtered with Phenex™ RC 4 mm 0.2 μm syringeless filter, Phenomenex (Castelmaggiore, Bologna, Italy).

2.4. Pre-treatments

The raw potato slices were subjected to different pre-treatments

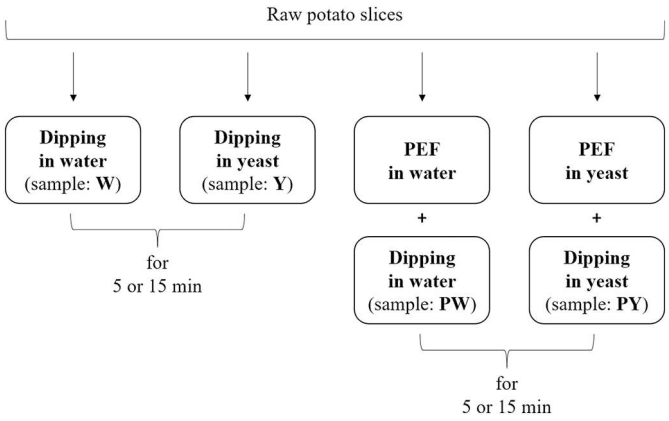

Fig. 1. Scheme of processing pre-treatments steps studied for the acrylamide reduction in fried potato chips and corresponding samples codes.

before frying, obtaining different samples as showed in Fig. 1. In detail, the raw potato slices were subjected to: dipping in water for 5 and 15 min (samples named respectively W5 and W15); dipping in *A. pullulans* L1 yeast water suspension for 5 and 15 min (Y5 and Y15 samples); dipping in water after PEF in water (PW5 and PW15 samples) and dipping in yeast water suspension after PEF in the same yeast suspension (PY5 and PY15 samples). Raw potato slices that have not undergone any pre-treatment were considered as control sample (C).

2.4.1. Aureobasidium pullulans L1 treatments

The pre-treatment by using L1 strain suspension was carried out according to the patented procedure No. WO2019058248A1 (authors: M. Mari, A. Di Francesco and L. Ugolini, *Alma Mater Studiorum*, Bologna and CREA), with slight modifications. Each treatment was carried out at room temperature (~25 °C) by dipping 80 g of potato slices in 200 mL of yeast suspension concentrated 10^8 cells/mL for 5 (Y5) and 15 (Y15) min. The yeast untreated samples were represented by 80 g of potato slices dipped in 200 mL of tap water at room temperature for the same times (5 min: W5, 15 min: W15). After dipping, the potato slices were collected, rinsed with tap water and carefully dried with absorbent paper. Each treatment for each sample (W5, W15, Y5, Y15) was performed in triplicate.

2.4.2. Pulsed electric fields (PEF) treatments

PEF pre-treatments were performed using a lab-scale PEF unit delivering a maximum output current and voltage of 60 A and 8 kV respectively (mod. S−P7500, Alintel, Italy). The generator provides monopolar rectangular-shape pulses and adjustable pulse duration (5–20 μs), pulse frequency (50–500 Hz) and total treatment time (1–600 s). The treatment chamber (50 mm length × 50 mm width × 50 mm height) was a prototype built by Alintel (Italy) and consisted of two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. The outputs of the generator, tension and current, were monitored using a PC-oscilloscope mod. Picoscope 2204a (Pico Technology, UK). Raw potato slices (20 g) were treated at room temperature in tap water (100 mL) with an initial electrical conductivity of 536 ± 23.2 μS/cm at 25 °C (EC-meter mod. Basic 30, Crison, Spain) and delivering n = 1000 pulses at fixed pulse width (10 ± 1 μs), frequency (100 Hz), time interval between pulses (10 ± 1 ms) and applying an electric field strength of 1.5 kV/cm, resulting in a specific energy input of 105 ± 5.5 kJ/kg, calculated according to Raso et al. (2016). For more details regarding the PEF-treatment protocol and the measured cell disintegration refer to Genovese et al. (2019).

The PEF treatment was repeated four times in order to obtain one batch (80 g) of treated product for each sample; temperature changes due to PEF treatment, measured with a temperature probe mod. TESTO 445 (Testo GmbH & Co, Milano, Italy), were negligible. After the PEF treatments, 80 g of potato slices were collected and left dipped in 200 mL of water for 5 (PW5) and 15 (PW15) min. After dipping, the potato slices were collected, rinsed with tap water and carefully dried with absorbent paper. Each dipping treatment was performed in triplicate for each sample (PW5, PW15), consequently the preliminary PEF treatment was repeated 12 times for each sample ((20 g × 4) × 3).

2.4.3. Combination of treatments

The combination of PEF and yeast pre-treatments was performed filling the PEF treatment chamber with about 100 mL of yeast aqueous suspension (10^8 cells/mL) and 20 g of raw potato slices. The initial electrical conductivity of the yeast aqueous suspension measured using an electrical conductivity meter (EC-meter mod. Basic 30, Crison, Spain), was 536 ± 23.2 μS/cm at 25 °C (comparable with the tap water initial electrical conductivity used for PEF treatment in water). The selected PEF conditions and applied energy input were the same as mentioned in Section 2.4.2. After the PEF treatments, 80 g of potato slices were collected and left immersed in 200 mL of the PEF-treated yeast suspension for 5 (PY5) and 15 (PY15) min. Subsequently, the potato slices were collected, rinsed with tap water and carefully dried with absorbent paper. Each dipping treatment in the PEF-treated yeast suspension was performed in triplicate for each sample (PY5, PY15); consequently the preliminary PEF treatment in the yeast aqueous suspension was repeated 12 times for each sample ((20 g × 4) × 3).

2.5. Frying conditions

Untreated (C) and pre-treated potato slices were deep-fried in 6 L of high-oleic sunflower oil (potato to oil ratio 1:20 w/w) at 175 °C for 3 min, by using an electrical fryer mod. MFR280R (Fama Industrie, Italy). Temperatures of frying oil were monitored using K-type thermocouples sensors with an accuracy of 0.1 °C (Chromel/Alumel, Tersid Came, Italy), connected to a data logging system (mod. 9211A, National Instruments™, Texas).

2.6. Analytical determinations

2.6.1. Isothermal calorimetry

Isothermal calorimetry was used to evaluate the best combination of PEF pre-treatment and dipping in aqueous yeast suspension, by monitoring and determining the development of metabolic heat of the *A. pullulans* L1 yeast. The effect of PEF was investigated in different substrates. NYDB medium was used as ideal substrate for the yeast growth, while tap water and potato tissue in a 1:1 (w/w) ratio was used to simulate a real substrate. Moreover, because PEF could also increase the release of solutes from the potato tissue that could influence the yeast activity, tap water with potato tissue subjected to PEF was also considered. A schematic representation of the combination of samples and substrate is reported in Fig. 2.

In each vial, 2 g of potato (raw or PEF-treated), 1 mL of yeast (10^8 cells/mL) (control and PEF-treated) and 2 mL of substrate (NYDB or tap water) were placed. Vials were sealed with Teflon caps and aluminium screw lids.

For each sample three replicas were performed. The extent of metabolic heat production by the yeast was measured continuously with a TAM Air isothermal calorimeter (TA Instruments/Thermometric, Sweden) with a sensitivity of ± 10 μW. This instrument contains eight twin calorimeters, each of which has its own reference to increase sensitivity and accuracy. As a reference, an ampoule with distilled water was used. The amount of distilled water was determined according to Panarese, Tylewicz, Santagapita, Rocculi, and Dalla Rosa (2012). Isothermal calorimetric measurements were performed at a constant temperature of 25 °C, chosen as the optimal one for yeast growth, for 48 h. Baselines were recorded before and after each measurement for 30 min. The heat produced was continuously recorded with dedicated software (TAM Air assistant, TA Instruments/

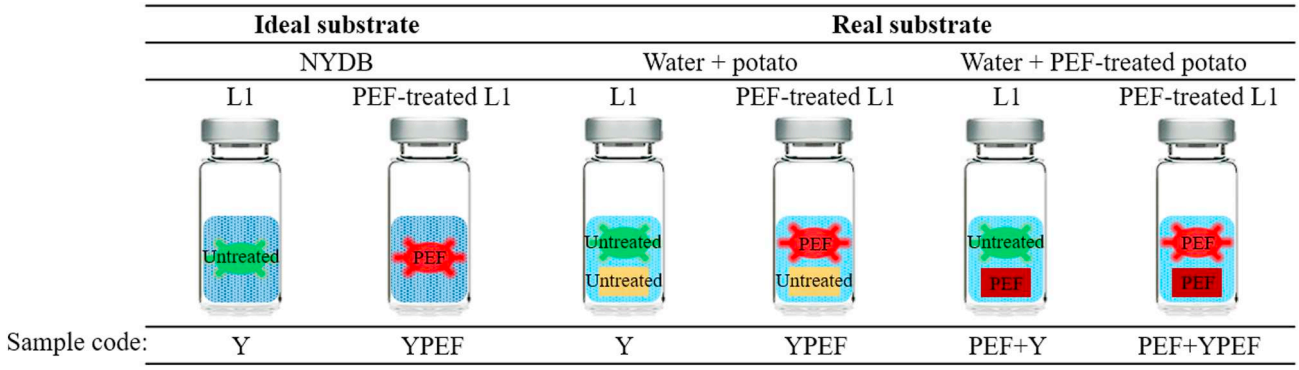

Fig. 2. Schematic representation of the combinations of sample and substrate evaluated by isothermal calorimetry as preliminary study to assess the possibility of combining PEF and yeast dipping pre-treatments (NYDB: dark blue; water: light blue; untreated yeast: green; PEF-treated yeast: light red; untreated potato: yellow; PEF-treated potato: dark red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Thermometric, Sweden). The thermograms obtained were normalized for the weight of the sample.

2.6.2. HPLC-MS/MS analysis: quantification of asparagine, reducing sugars and acrylamide

The freeze-dried raw potatoes and potato chips samples were crumbled finely in mortar and 2 g of sample were weighed in a 50 mL conical flask. The extraction was performed with 20 mL of Milli-Q water firstly by 1 min of agitation in a vortex mixer and secondly by 10 min of ultrasound-assisted extraction at room temperature. After pouring into a 50 mL centrifuge plastic tube, the sample was centrifuged at 5000 rpm for 10 min and the supernatant was collected and stored overnight at −18 °C to precipitate starch and facilitate the separation and removal of fat fraction. Later, the sample was thawed at room temperature, once again centrifuged at 5000 rpm for 10 min and then 1 mL of water supernatant was transferred to a 1.5 mL microcentrifuge tube containing 100 mg of C18 sorbent. Before centrifugation at 13,300 rpm for 15 min, the sample was vortexed for 1 min. Finally, an aliquot of supernatant was collected and diluted 1:100 in mobile phase for asparagine analysis and 1:2 in acetonitrile for AA, fructose and glucose analysis. Before high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) injection, the diluted samples were filtered with a 0.2 µm syringeless filter.

The quantitation of AA, asparagine, fructose and glucose, is frequently performed by different methods and using various analytical instruments. For instance, sugars are often analysed by high performance liquid chromatography-refractive index detector (HPLC-RID) (Coelho et al., 2018; Vivanti, Finotti, & Friedman, 2006), asparagine using high performance liquid chromatography with fluorescence detection (HPLC-FD) (Amrein et al., 2003) and AA by high performance liquid chromatography-mass spectrometry (HPLC-MS) (Elmore et al., 2015; Zhou, Wang, Chen, & Zhang, 2015). Few works have reported the simultaneous analysis, in starchy foods, of these four molecules using a single approach (Nielsen, Granby, Hedegaard, & Skibsted, 2006). In the present research we introduced a simple method for simultaneous quantification of AA, asparagine, glucose and fructose by using HPLC-MS/MS.

The extraction process was optimized by taking the cue from previous data (Nielsen et al., 2006) with some modifications. Water was used as the extraction solvent for the high solubility of target molecules and a dispersive solid-phase extraction (DSPE) using C18 sorbent was chosen for sample clean-up, because was a simple and fast technique able to remove non-polar molecules which could act as interferences (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003).

HPLC-MS/MS studies were performed using an Agilent 1290 Infinity series and a Triple Quadrupole 6420 from Agilent Technology (Santa Clara, CA) equipped with an electrospray ionization (ESI) source operating in positive ionization mode. The HPLC-MS/MS parameters of each analyte were optimized in flow injection analysis (FIA) (1 µL of a 10 mg/L individual standard solution) by using optimizer software (Agilent). The separation of target compounds was achieved on a Kinetex Hilic analytical column (100 mm × 4.6 mm i.d., particle size 2.6 µm) from Phenomenex (Torrance, CA, USA) preceded by a KrudKatcher ULTRA HPLC In-Line Filter (2.0 µm Depth Filter × 0.004 in ID). The mobile phase for HPLC-MS/MS analysis was a mixture of 15% water (A) and 85% HPLC-grade acetonitrile (B), both with 0.1% formic acid. The separation was obtained by flowing at 0.8 mL/min with this gradient elution: isocratic condition until 2.5 min (85% B), 3.5 min (70% B), 5.5 min (70% B), 6.5 min (85% B) and then constant until the end of the run (15 min). All solvents and solutions were filtered through a 0.2 µm polyamide filter from Sartorius Stedim (Goettingen, Germany). The injection volume was 2 µL. The temperature of the column was 25 °C and the temperature of the drying gas in the ionization source was 350 °C. The gas flow was 12 L/min, the nebulizer pressure was 45 psi and the capillary voltage was 4000 V. Detection was performed in the multiple reaction monitoring (MRM) mode. The MRM peak areas were integrated for quantification and the most abundant product ion was used for quantitation, and the rest of the product ions were used for qualification. The selected ion transitions and the mass spectrometer parameters are reported in Table 1.

As an example, Fig. 3 shows the HPLC-MS/MS chromatogram of a standard mixture of the monitored compounds plotted as overlapped multiple reaction monitoring (MRM) transition of each compound. Results were expressed as µg/kg (dry matter) for AA and mg/kg (dry

Table 1
HPLC-MS/MS acquisition parameters (MRM mode) adopted for the quantification of acrylamide, asparagine, and sugars.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor (V)	Collision energy (V)	Retention time (min)	Polarity
Acrylamide	72	55	45	8	1.52	Positive
Fructose	203	203	80	0	1.96	Positive
Glucose	203	203	80	0	2.18	Positive
Asparagine	133	74[a]	64	16	5.17	Positive
		98		16		

[a] This product ion was used for quantitative analysis; the rest for confirmatory analysis.

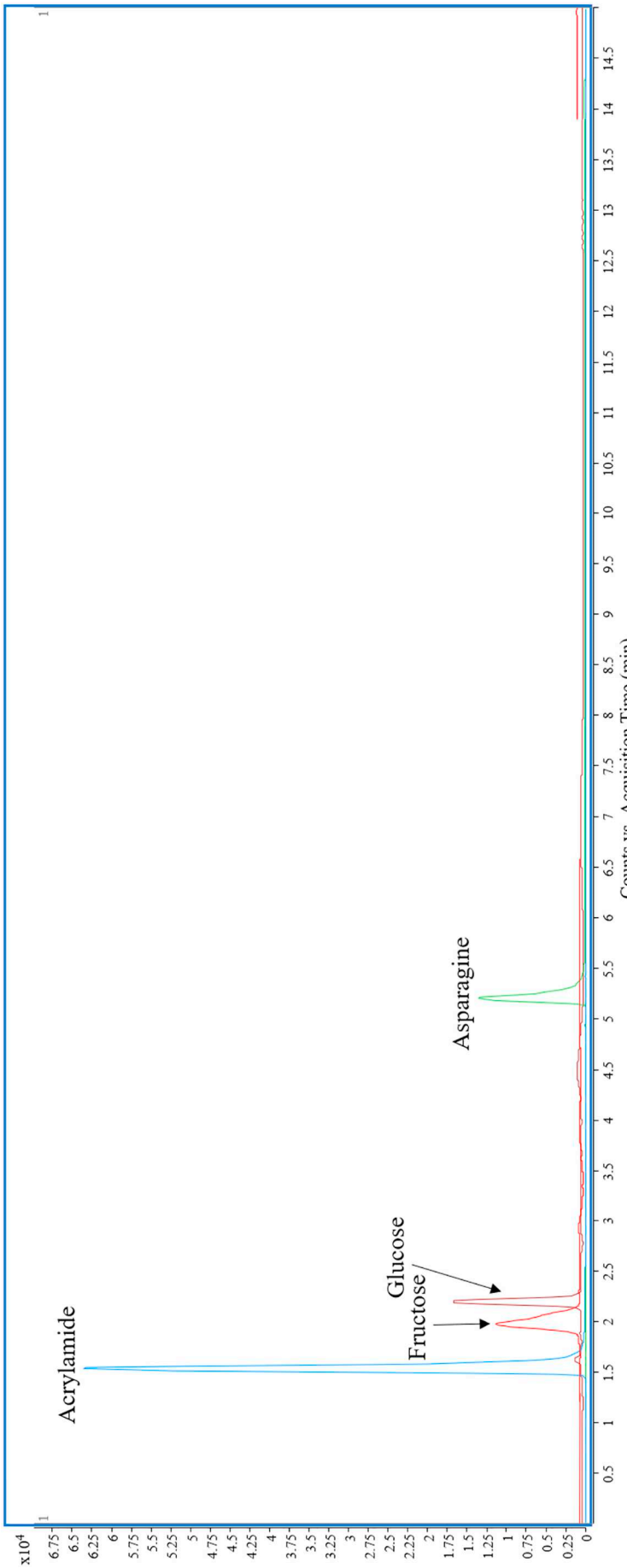

Fig. 3. HPLC-MS/MS chromatogram of a standard mixture of acrylamide, asparagine and sugars plotted as overlapped multiple reaction monitoring (MRM) transition of each compound.

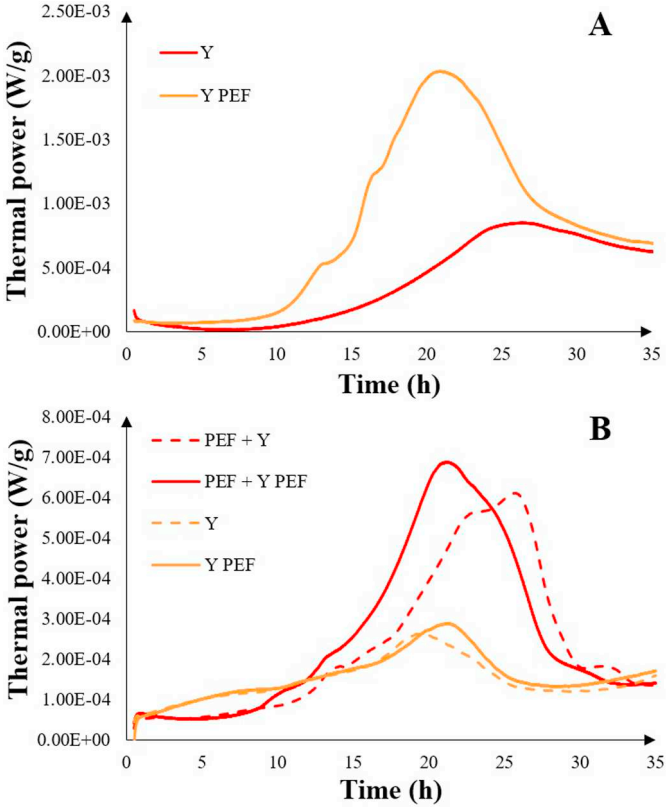

Fig. 4. Thermal profiles measured by isothermal calorimetry during growth of *A. pullulans* L1 strain subjected to PEF treatment, compared to its control growth in an ideal (A) and in real substrate (B).

matter) for AA precursors.

2.6.3. Moisture

The moisture content was determined on all types of raw and fried potato samples by gravimetric method. The samples were dried to constant weight in a thermo-regulated laboratory stove (mod. UF110, Memmert, Schwabach, Germany) at 70 °C (around 48 h) and 105 °C, (around 24 h) respectively for raw and fried potatoes. Results were expressed as percentage of water. The analysis was carried out in triplicate for each sample.

2.6.4. Oil content

The oil content of potato chips after frying was determined by Soxhlet extraction, performing the procedure with petroleum ether as a solvent at 60 °C for 3 h (AOAC, 1990). Oil content was reported as a percentage on dry matter. The analysis was carried out in triplicate for each sample.

2.6.5. Colour

The surface colour of whole potato chips was determined using a computer vision system (CVS). The samples were placed inside a dark chamber over a white background in controlled lighting conditions that consisted in four daylight fluorescent lamps (TL-D Deluxe, Natural Daylight, 18 W/965, Philips, USA) with a colour temperature of 6500 K (D65 standard). The fluorescent tubes (60 cm long) were located 35 cm above the sample and inclined at an angle of 45°. The RGB images of the samples were acquired using a colour digital camera mod. D7000 (Nikon, Japan) equipped with 105 mm lens (mod. AF-S Micro Nikkor, Nikon, Japan), positioned vertically and connected to display and capture the images directly by the computer. For each sample, untreated and pre-treated, 10 images were captured on both sides of potato slices.

The pre-processing of RGB images and colour quantification in CIE $L^*a^*b^*$ scale was performed with ImageJ analysis software (NIH, USA). From numerical values of a^* (green-red) and b^* (yellow-blue) chromatic parameters, hue angle (h°) was calculated by the following equation (McGuire, 1992) and used to describe colour variations between samples:

$$h^\circ = \tan^{-1}(b^*/a^*) \quad (1)$$

The colour measurements were carried out on the two surfaces of 5 potato chips for each sample.

2.6.6. Texture

The texture measurement on chips samples was performed at room temperature using a Texture Analyser mod. TA-HDi500 (Stable Micro System, UK) equipped with a 5 kg load cell and a spherical probe in stainless steel with a diameter of 6 mm at 1.0 mm/s test speed. The samples, selected on the basis of uniform size and shape, were placed on a support rig (HDP/CFS) and compressed for 3 mm distance. The acquired results were expressed as hardness, calculated by means of maximum force values, and as index of crispness, calculated by means of linear distance between the first and the last peaks registered (Tylewicz et al., 2019). Force vs distance curves were obtained from 12 potato chips for each sample.

2.7. Data analysis

The results were reported as mean value ± standard deviation of replications. The software STATISTICA 8.0 (Statsoft Inc., UK) was used for the statistical analysis of the data. Significant differences between results were calculated by parametric analysis of variance (ANOVA) and Tukey multiple post-hoc comparison, with a significance level of 95% ($p < 0.05$).

3. Results and discussion

3.1. Preliminary tests on ideal substrate for the optimization of combined treatments

Isothermal calorimetry, that has been successfully used in the past to describe the growth ability of microorganisms (Braissant, Wirz, Beat, & Daniels, 2009), was employed to measure continuously and in real time the heat flow produced in different substrates by the yeast *A. pullulans* L1, untreated and subjected to the chosen PEF treatment protocol.

At first, the ideal growth substrate (NYDB) was used to evaluate the only effect of the selected PEF conditions on the yeast activity, in terms of heat produced. Fig. 4A shows the heat flow, recorded at 25 °C during 35 h, produced by *A. pullulans* after PEF treatment (YPEF) compared to the untreated one (Y). The thermograms, as suggested by Morozova et al. (2017), are relative to the kinetic profile of the fermentative process. The heat flow signal has been considered as an index of the yeast growth rate. The shape of the signal suggests the presence of a series of consecutive processes that occur in the vial.

Initially, in both samples, a constant thermal power was recorded, of about $8.4{\cdot}10^{-4}$ and $2.0{\cdot}10^{-3}$ W/g for sample Y and YPEF respectively, indicating the lag phase. Typically, the lag phase corresponds to the period of time in which yeasts synthetize the enzymatic pool necessary for their catabolism during which the multiplication is negligible (Morozova et al., 2017). After 10 h, the signal started to increase exponentially. This sudden change is due to the exponential growth phase of the yeast. The signal increased until reaching a maximum, in different times for the 2 samples, and then, because of various factors limiting the growth (e.g. reduced oxygen concentration in the head space, reduction of carbon and nitrogen sources in the growth medium, production of ethanol or increased pressure in the vial), the signal started decreasing, reaching almost a stationary phase, the

Table 2
Calorimetric parameters (heat and slope) measured during *A. pullulans* L1 growth in ideal (NYDB) and real (water + potato) substrates after PEF treatment compared to the control (Y: untreated yeast; YPEF: PEF-treated yeast; PEF: PEF-treated potato).

	Ideal substrate	
	Y	YPEF
Heat (J/g)	24.7 ± 2.8^{a}	45.8 ± 0.7^{b}
Slope (W/g·h)	5.5 ± 0.7·10^{-5a}	2·10^{-4} ± 0.1^{b}

	Real substrate			
	Y	YPEF	PEF + Y	PEF + YPEF
Heat (J/g)	17.9 ± 0.7^{a}	19.0 ± 0.9^{a}	31.6 ± 4.0^{b}	33.2 ± 2.3^{b}
Slope (W/g·h)	1·10^{-5} ± 0.1^{a}	1.5 ± 7·10^{-5a}	6.5 ± 4·10^{-5b}	5.0 ± 1·10^{-5b}

Different letters in the same line indicate significant differences among samples according to Tukey multiple post-hoc comparison ($p < 0.05$).

extension of which depends on the ability of the yeast to survive (Morozova et al., 2017).

Table 2 reports the average values of the heat produced by the yeast according to the different conditions investigated, expressed as either total heat (integral of the heat flow) and slope of the curve related to the exponential growth phase. Results obtained suggest that PEF treatment had a positive effect on the yeast metabolism stimulating both its entity and rate. Different literature reports indicated that low intensity electrical fields (0.1–1 kV/cm) and short treatment times (μs-s) did not bring damage to the cell membranes functionality, but actually enhanced microbial reactions and activities (Mattar et al., 2014; Schottroff, Krottenthaler, & Jaeger, 2017). According to these authors, low intensity PEF allows to improve enzymatic synthesis, frequency of cell division, probability of survival of daughter-cells, increases tolerance to inhibitors (e.g. ethanol) and yeasts fermentation ability. Moreover, electroporation induced by PEF can modify the cytoplasmic membrane and hence the nutrients transportation due to the formation of pores or the activation of transport proteins. However, such mechanisms have not been fully clarified yet and PEF effect depends also on other factors such as growth substrate, dimension and specific resistance of microbial cells (Mattar et al., 2014). In relation to the different susceptibility of different microorganisms and to the entity of PEF treatment applied, the cells can be in three different states: intact, dead or damaged (sub-lethal stress) (Wang et al., 2018).

Fig. 4B shows the heat flow signals recorded at 25 °C during the yeast growth process, subjected to PEF (YPEF) compared to the control (Y), placed in a substrate simulating the real conditions, consisting of water and untreated potatoes or water and potatoes treated with PEF (PEF + Y and PEF + YPEF). In Table 2 the corresponding data of heat produced, and slope of the curve were reported. As expected, the extent of yeast growth in water-potato medium was significantly reduced compared to its optimal growth medium (NYDB). However, when placed in water-potato medium, no difference was observed for the yeast treated by PEF (PEF + YPEF) compared to its untreated control (PEF + Y) for both heat and slope produced. However, when the potato in the substrate was pre-treated by PEF (PEF + Y and PEF + YPEF), the heat produced almost doubled and the slope increased 5–6-fold in comparison to water-potato medium not treated with PEF (Y and YPEF). While total heat is an index of the metabolism entity, the slope indicates the rate of metabolic reactions. Our hypothesis is that PEF pre-treatment increased the membrane permeability of the potato tissue inducing a higher release of cell content into the water, that in turn became richer in nutrients exploitable by the yeast metabolism.

In addition, in the water-potato medium (real substrate) no differences in heat and slope were observed between PEF-treated (YPEF) and control (Y) yeast in both potato medium treated with PEF or not (Table 2). It seems that the positive effect of PEF on *A. pullulans* metabolism is visible only when the growth substrate is NYDB (Fig. 4A), while in the case of the water-potato substrate, the limiting nutrient availability flattened the differences (Fig. 4B).

Following these preliminary results, it was decided to PEF-treat the potato slices in the yeast water suspension in order to verify the combined effect of the two pre-treatments on AA formation.

3.2. Application on real substrate

3.2.1. Acrylamide and precursors analysis

The levels of AA in fried potato chips and the concentrations of AA precursors in untreated raw potato (control C) and in differently pre-treated raw potato samples are reported in Table 3. The AA concentration of untreated potato chips was 1384.3 μg/kg, value that exceeds the reference level set by the Commission Regulation (UE) 2017/2158 (750 μg/kg). The untreated potato tubers were characterized by a glucose, fructose and asparagine content of 33.6 mg/kg, 6.8 mg/kg and 8826.3 mg/kg respectively.

Each applied treatment, especially after 15 min of dipping, has led to a reduction of AA in final potato chips; however, not always proportional to the content of evaluated AA precursors.

Dipping in water (W5, W15) had a positive effect in reducing AA levels in fried potatoes due to the promotion of precursors release from the food matrix to the water for leaching effect. Potato samples dipped in water for 5 and 15 min (W5, W15) have presented a significant AA reduction respectively of 6.6% and 44.0%. The decrement of AA in these samples can be attributed to the reduction of glucose and fructose, while the asparagine content did not undergo any reduction. It is worth to note that the sample W15 resulted in AA content in compliance with the limit set by the EU Regulation. However, the concentration of AA precursors, and so the potential concentration of AA formation, is linked to intrinsic properties of the food matrix, i.e. potato cultivar, post-harvesting conditions. Therefore, AA levels in fried potatoes can be

Table 3
Acrylamide levels in fried potato samples and acrylamide precursors contents in raw potato samples, untreated (C: control) and differently pre-treated at both 5 and 15 min (W: dipping in water; Y: dipping in yeast aqueous suspension; PW: PEF in water + dipping in water; PY: PEF in yeast aqueous suspension + dipping in yeast aqueous suspension).

Sample	Time (min)	Acrylamide (μg/kg)	Glucose (mg/kg)	Fructose (mg/kg)	Asparagine (mg/kg)
C		1384.3 ± 65.0^{a}	33.6 ± 6.8^{abc}	8.4 ± 1.0^{c}	8826.3 ± 576.0^{b}
W	5	1292.4 ± 96.2^{bc}	19.6 ± 0.4^{d}	6.9 ± 0.7^{d}	12,952.0 ± 1341.9^{a}
	15	775.3 ± 81.5^{d}	29.50 ± 5.0^{c}	7.8 ± 1.1^{cd}	12,036.4 ± 2142.5^{a}
Y	5	1375.9 ± 9.9^{ab}	39.5 ± 4.1^{a}	16.4 ± 0.7^{a}	12,945.3 ± 441.7^{a}
	15	676.4 ± 42.3^{e}	18.6 ± 0.9^{d}	3.8 ± 0.2^{f}	5957.5 ± 135.6^{c}
PW	5	886.8 ± 9.9^{d}	36.7 ± 0.88^{ab}	5.0 ± 0.1^{ef}	4108.9 ± 571.3^{c}
	15	572.0 ± 8.8^{f}	17.8 ± 3.0^{d}	5.4 ± 0.4^{e}	5875.0 ± 695.9^{c}
PY	5	1211.9 ± 4.2^{c}	33.53 ± 1.2^{abc}	5.7 ± 0.2^{e}	4188.0 ± 69.9^{c}
	15	1193.2 ± 20.1^{c}	31.1 ± 1.2^{bc}	14.1 ± 0.5^{b}	4360.8 ± 87.9^{c}

Different letters in the same columns indicate significant differences among samples according to Tukey multiple post-hoc comparison ($p < 0.05$).

much higher than those measured in this study, explaining the necessity of assessing new AA mitigation measures suitable for industrial processing conditions.

The yeast treatment after 5 min (Y5) did not lead to a significant reduction in AA compared to the control (C) and to the water dipped sample for the same time (W5). On the other hand, after 15 min (Y15) a significant AA reduction of 51.1% was reached, highlighting that the yeast requires contact times > 5 min. Nevertheless, in comparison to the sample dipped in water for the same time (W15) the additional reduction after 15 min yeast treatment (Y15) was only 7%. This slight, but significant reduction of AA after 15 min yeast dipping could be attributed to enzymatic and metabolic activity of the yeast able to reduce the levels of glucose, fructose and asparagine. In a recent study with the same yeast strain, a reduction of AA in potato chips, equal to 83.7%, was obtained after a dipping of 30 min (Di Francesco et al., 2019). However, industrial application often requires shorter times.

The samples subjected to PEF treatment followed by dipping in water (PW) led to the highest AA reduction for each treatment time, equal to 35.9% and 58.7% respectively for 5 and 15 min. The cell electroporation phenomena induced by the PEF treatment on raw potato slices resulted in a reduction of AA precursors leading to lower AA formation. This result confirms a recent study in which a significant reduction in the AA content (30%) was found in potato chips treated with PEF, compared to untreated samples (Genovese et al., 2019). Moreover, it is interesting to notice that, although precursors' concentration was similar for Y15 and PW15 samples, AA levels were lower in PW15. This could be attributed to the possible modification of other molecules and pathways which can participate to AA formation. In fact, it is known that AA is mainly formed by Maillard reaction from asparagine and α-carbonyl sources such as reducing sugars, but other mechanisms can contribute to the final AA level. For example, the acrolein, which can be formed from lipid oxidation and degradation of amino acids, carbohydrate and protein, the acrylic acid derived from aspartic acid, and the β-alanine can participate to AA formation (Stadler & Studer, 2016).

The combination of PEF and yeast treatments (PY) resulted in a slight AA reduction (12.4% after 5 min and 13.8% after 15 min), similar to the results obtained with 5 min of dipping in water, but significantly lower compared to the reductions obtained with the singular pre-treatments (Y15, PW5 and PW15 samples). This result could be related to the direct effect of PEF on yeast cells that can notably influence their propriety and activity. In fact, as reported by Stirke et al. (2014) and Mattar et al. (2015) PEF can influence yeast cell viability, dimensions, and consequently their enzymatic activity. Although isothermal calorimetry results showed an increase of the overall yeast metabolic activity, the specific alterations of metabolic pathways induced by PEF are still unknown (Mattar et al., 2015). In order to elucidate how PEF can affect *A. pullulans* L1 strain activity additional experiments have to be performed in the future.

3.2.2. Qualitative analysis

The intense deep fry heat treatment is responsible for the development of non-enzymatic browning reaction (Maillard reaction) that can lead to undesired AA formation, but also to desired changes in physical attributes, such as colour, texture, flavour, moisture and oil content (Anese et al., 2009; Pedreschi, Moyano, Kaack, & Granby, 2005; Romani et al., 2009; Yang, Achaerandio, & Pujolà, 2016).

The main quality characteristics of fried potato samples were analysed in order to evaluate the possible effect of different AA mitigation pre-treatments applied (Table 4).

The development of a brown-gold colour during potato frying is one of the most important quality parameters demanded by customers (Medeiros Vinci et al., 2012). The use of the CVS allowed to evaluate the colour of whole potato chips surface. Example of RGB images of potato chips samples untreated and differently treated are shown in Fig. 5A, each RGB image was processed to extract the numerical CIE $L^*a^*b^*$ data (Fig. 5B). The W15, Y15, PW5, PW15 and PY15 potato chips presented significant ($p < 0.05$) higher L^* values compared to the other samples, highlighting a brighter colour (Table 4). The h° values increased significantly for W15, Y15, PW5 and PW15 samples, underling a colour variation from the red-orange (dark) zone to the green-yellow (light) one compared to untreated sample (C).

The texture is another important sensorial characteristic of potato chips that should be monitored (Yee & Bussell, 2007). The various pre-treatments studied led to a slight and not always significant reduction of hardness and crispness index of potato chips compared with the untreated sample (C). To our knowledge, very few studies were carried out on the structural characteristics of the potatoes after similar pre-treatments. Zhou et al. (2015), using a different potato variety, found that the untreated potato samples were darker than yeast-treated ones characterized by a low AA content. However, no significant ($p > 0.05$) differences in texture was found among the treated fried potato samples and untreated ones. The colour and texture results were also confirmed by the study of Di Francesco et al. (2019) who found that the potato untreated samples appeared darker in colour than yeast treated ones. Regarding the effect of PEF treatment, Ignat, Manzocco, Brunton, Nicoli, and Lyng (2015) observed significant differences only in L^* and a^* chromatic values for PEF-treated fried potato cubes, while the hardness was similar to the control sample. These results were confirmed also by Genovese et al. (2019) who found similar colour development during frying of PEF-treated potato slices. Moreover, the authors observed a slight and significant reduction of texture parameters in PEF-treated samples in comparison to the untreated one as in this case.

In the raw potato samples the water dipping treatments led to a significant moisture content increase for all treatment times (W5, W15) compared to untreated control sample (C). This increase is probably due to capillary and osmotic phenomena. No significant ($p > 0.05$) differences were found between the moisture values of the samples subjected to dipping in water and in yeast water suspension for all treatment times. The raw samples subjected to PEF pre-treatment and subsequent dipping (PW5, PW15, and PY5, PY15) showed similar and lower moisture values than those treated only in dipping (W5, W15 and Y5, Y15). This result is probably explained by the increase of mass transfers from the sample to the dipping solution due to the modification of the cell membrane permeability induced by the PEF treatment. Similarly, in the fried potato chips, all PEF pre-treated samples showed a lower moisture content than the only dipping pre-treated ones. All fried potatoes subjected only to dipping pre-treatment presented moisture value not significantly different compared to the untreated one (C). Nevertheless, the reduction of moisture due to frying was similar between the samples when compared with the respective raw samples and equal to 95–96%. This and the low values of standard deviation data demonstrate the uniformity of frying process conducted on the different samples and repetitions.

Regarding the oil content, all treatments (W, Y, PW and PY) promoted an increase of oil uptake (between 34 and 41%) compared to the fried untreated sample (C). This increase is probably due to the slightly higher water content in the treated raw samples, especially for W and Y ones. In fact, during frying the evaporation of the water leads to an absorption of frying oil (Aguilera & Gloria-Hernandez, 2000). However, in the PW and PY samples, subjected to PEF pre-treatment, which before frying showed the lowest water content compared to those subjected only to dipping (W and Y), the highest oil content was found (between 38 and 41%). This result suggests that in the PEF-treated samples the absorption of oil does not depend only on the initial water content, but also on the induced tissue electroporation phenomenon.

In the literature there are conflicting results regarding oil absorption in potato after PEF pre-treatments. According to Fauster et al. (2018), Ignat et al. (2015) and Janositz, Noack, and Knorr (2011), PEF treatment on potatoes led to a reduction in oil content of the final fried product in the shape of cubes or sticks (French fries). This result was

Table 4
Colour parameters (L^*, h°), texture values (hardness, index of crispness), moisture and oil content of untreated (C: control) and treated potato chips (W: dipping in water; Y: dipping in yeast aqueous suspension; PW: PEF in water + dipping in water; PY: PEF in yeast aqueous suspension + dipping in yeast aqueous suspension).

Sample	Time (min)	Raw potato	Fried potato					
		Moisture (%)	Moisture (%)	L^*	h°	Hardness (N)	Index of crispness (linear distance)	Oil (%)
C		74.1 ± 0.5^{b}	4.1 ± 0.1^{ab}	84.5 ± 1.1^{b}	93.2 ± 1.7^{ef}	3.9 ± 1.1^{a}	9.3 ± 2.6^{a}	30.3 ± 1.3^{d}
W	5	78.9 ± 0.4^{a}	4.2 ± 0.1^{a}	84.5 ± 1.2^{b}	94.6 ± 0.9^{de}	2.9 ± 0.4^{bc}	8.0 ± 1.7^{ab}	37.3 ± 0.1^{b}
	15	79.9 ± 0.8^{a}	4.1 ± 0.1^{ab}	87.1 ± 0.2^{a}	94.9 ± 0.6^{cd}	3.6 ± 0.8^{ab}	8.8 ± 2.6^{ab}	34.3 ± 0.6^{c}
Y	5	78.1 ± 0.7^{a}	4.0 ± 0.4^{ab}	84.3 ± 1.2^{b}	92.8 ± 0.8^{f}	3.7 ± 0.8^{ab}	9.7 ± 1.5^{a}	34.7 ± 0.8^{c}
	15	79.2 ± 0.2^{a}	3.7 ± 0.1^{b}	86.0 ± 1.3^{ab}	96.4 ± 1.1^{bc}	3.3 ± 0.6^{abc}	9.7 ± 2.4^{a}	33.5 ± 0.6^{c}
PW	5	74.5 ± 1.3^{b}	2.8 ± 0.1^{c}	86.1 ± 0.7^{ab}	98.9 ± 0.3^{a}	2.6 ± 0.7^{bc}	6.6 ± 2.1^{b}	41.3 ± 0.9^{a}
	15	75.6 ± 0.9^{b}	2.7 ± 0.3^{c}	86.4 ± 1.1^{ab}	97.8 ± 0.9^{ab}	3.1 ± 0.9^{bc}	9.1 ± 2.2^{a}	37.5 ± 0.8^{b}
PY	5	74.5 ± 0.9^{b}	2.5 ± 0.2^{c}	84.7 ± 1.3^{b}	93.5 ± 0.2^{def}	2.9 ± 0.7^{bc}	7.6 ± 2.9^{ab}	41.9 ± 0.8^{a}
	15	75.0 ± 0.4^{b}	2.5 ± 0.4^{c}	85.2 ± 0.8^{ab}	93.4 ± 0.2^{def}	3.0 ± 0.8^{bc}	8.8 ± 2.8^{ab}	38.4 ± 0.5^{b}

Different letters in the same columns indicate significant differences among samples according to Tukey multiple post-hoc comparison ($p < 0.05$).

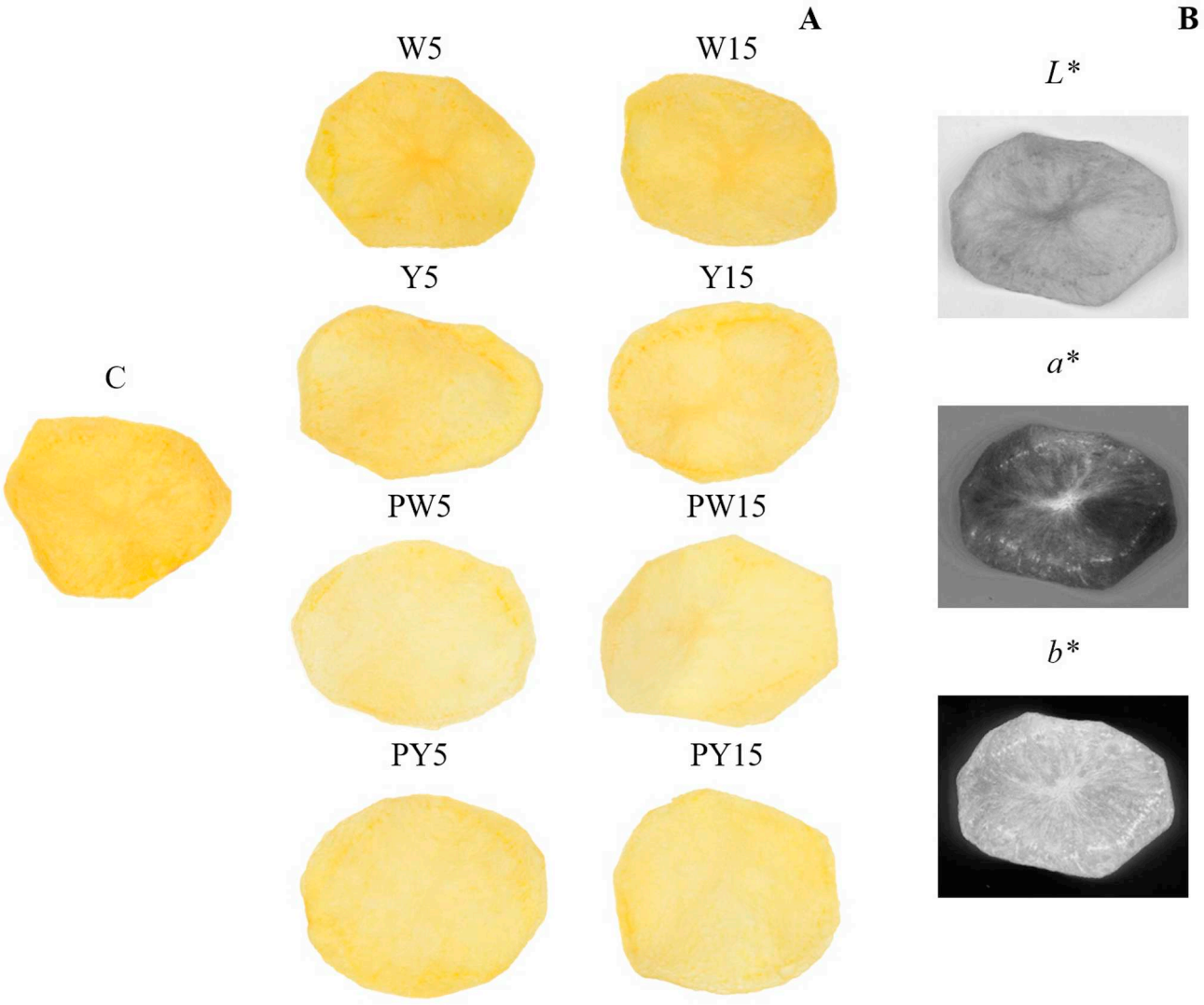

Fig. 5. Examples of RGB images of potato chips samples untreated and differently treated (A) and of images conversion from RGB into CIE $L^*a^*b^*$ channels (B).

ascribed to the transfer of water and intracellular substances to the potato surface due to the PEF-induced electroporation that, creating a barrier, reduced the oil uptake. This phenomenon is probably less evident in fried potato chips than in French fries. Moreover, in the present study the frying phase was not modulated as a function of the final reached moisture, while in the cited researches frying times were reduced after PEF pre-treatment and thus allowed to obtain a reduction in the oil absorption.

4. Conclusions

From the results obtained in this research work, the following conclusions can be drawn:

- the use of the yeast *A. pullulans* L1 strain in water suspension (patented procedure No. WO2019058248A1) as pre-treatment of potatoes to be fried as chips, was confirmed to be able to promote the reduction of AA in the finished product at dipping time longer than 5 min;
- the electroporation induced on raw potato slices by PEF treatment led to the highest reduction of AA formation in potato chips at each tested dipping time (5 and 15 min);
- the effect of the PEF treatment on the reduction of the AA formation in frying has been reduced when combined with dipping in yeast-water suspension for both tested dipping times;
- all the studied pre-treatments did not substantially influence the main final quality characteristics of potato chips.

The proposed strategies seem promising for the reduction of AA formation in fried potato products that will allow to comply with the current EU legislation, without causing detrimental effects on their final quality. However, further optimizations of tested pre-treatments are needed for industrial applications.

Moreover, the effect of PEF on the yeast activity should be further elucidated, in order to better exploit the yeast metabolism for AA reduction. Particularly, the time needed for the metabolic response of yeast to PEF treatment has to be carefully considered in order to optimize the studied pre-treatments.

Author statement

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Jessica Genovese: Methodology, Investigation.

Silvia Tappi: Methodology, Investigation.

Alessandra Di Francesco: Investigation, Resources.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Paper IV

Cropotova, J., Tappi, S., **Genovese, J.**, Rocculi, P., Dalla Rosa, M. and Rustad, T. (2021). The combined effect of pulsed electric field treatment and brine salting on changes in the oxidative stability of lipids and proteins and color characteristics of sea bass (*Dicentrarchus labrax*). *Heliyon*, 7(1), p.e05947.

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Research article

The combined effect of pulsed electric field treatment and brine salting on changes in the oxidative stability of lipids and proteins and color characteristics of sea bass (*Dicentrarchus labrax*)

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ABSTRACT

A combined effect of pulsed electric field application and salting in a brine with 5 and 10% w/w NaCl on oxidative stability of lipids and proteins, as well as color characteristics of sea bass samples, was assessed in the study. The applied intensity of the current was set at 10 and 20 A corresponding to 300 and 600 V cm^{-1}, respectively. Pulsed electric field (PEF) treatment led to a significant ($p < 0.05$) increase in primary and secondary lipid oxidation products expressed as peroxide value, conjugated dienes and 2-thiobarbituric acid reactive substances in PEF-treated samples compared to untreated ones. Conjugated dienes, as unstable primary oxidation products, correlated with b*-value ($p < 0.05$, $R = 0.789$), suggesting their contribution to the yellowness of the fish flesh due to fast decomposition and conversion into secondary oxidation products yielding yellow pigmentation.

However, none of the fish samples treated at the higher current intensity of 20 A exceeded the acceptable level of 5 meq active oxygen/kg lipid according to the requirements of the Standard for fish oils CODEX STAN 329–2017, suggesting acceptable oxidative status quality of sea bass samples after the treatment. PEF-treated fish samples also showed a significant increase in Schiff bases and total carbonyls on day 5 and day 8 of brine salting compared to non-treated samples, revealing a strong effect of electroporation on protein oxidation.

1. Introduction

Salting is one of the oldest techniques used for fish preservation. Nevertheless, despite the emergence of other effective preservation methods, where freezing is the most common, salting of fish still remains popular among food producers and consumers. Scandinavian countries export large volumes of salted fish products to Italy, Spain, Portugal and Latin America (Thorarinsdottir et al., 2004). However, a wide variety of salted fish products including anchovies, sea bass, sardines, etc are currently produced and marketed in the Mediterranean Basin countries as well. Due to high palatability and acceptable price in the market, these product commodities have become popular and highly appreciated in many European and non-European countries. At the same time, due to global trends directed towards a healthy lifestyle and policies to overall reduction of salt in foods (WHO, 2016), consumers are increasingly demanding lightly salted fish products with good sensory properties (Fan et al., 2014). Therefore, lightly salted fish products have recently started to gain increased popularity in Europe.

Brine salting can be used individually for preparation of lightly salted products, or as a preliminary step in the production of heavily salted or cured-salted fish. The main ingredient used in brine salting is sodium chloride (NaCl). Even at relatively low concentrations (<5% w/w), it acts as a preservative inhibiting bacterial growth and deactivating enzymes by dehydration and osmotic pressure (Lupín et al., 1981). Other ingredients such as spices, herbs, sugar or antioxidants can also be used in the brine salting to improve sensory characteristics of the final product after salting. During brine salting, fish is submerged in a brine with concentration of salt 5–15% NaCl for 1–8 days. After this step, the fish fillets can be removed from the brine and placed with alternate thin layers of salt into stacks for 10–12 days to perform dry salting (Thorarinsdottir et al., 2004).

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Many factors may affect the quality of the end product, including the type and quality of the fish raw material, concentration of salt in the brine and duration of brine salting (Thorarinsdottir et al., 2004). The salting method also influences the salt uptake by the muscle and affects its structural and mechanical properties. Thus, the rate of salt penetration into the fish muscle is higher during brine salting compared to dry salting (Akse et al., 1993).

Different pre-treatments have previously been studied to accelerate salt uptake by the muscle, including high intensity ultrasound brining (Chemat et al., 2011), pulsed vacuum brining (Andres et al., 2002), and vacuum tumbling (Mathias et al., 2003). At the same time, to the best of our knowledge, no studies have so far been performed on pulsed electric field (PEF) applications for brine salting of pelagic fish. PEF, as an emerging non-thermal technology, has high potential to contribute to enhanced diffusion of salt into the fish muscle through cell permeabilization leading to increased mass transfer (Hafsteinsson et al., 2000). PEF is based on the application of short duration pulses (few μs to ms) of an electric field (0.1–50 kV/cm) to a sample placed between two electrodes. Through a phenomenon known as electroporation, PEF allows to modify, in a reversible or irreversible way, the permeability and functionality of cell membranes (Toepfl et al., 2014). The two main applications of PEF to food products are the microbial inactivation (electric field in the range of 5–50 kV/cm) and the enhancement of mass transfer (electric field in the range of 0.1–5 kV/cm). In the present study, PEF pre-treatment was applied prior to brine salting to enhance mass transfer and increase salt uptake by the muscle while decreasing the duration of the process (data on salt content shown in Cropotova et al. (2021)).

Even if PEF is categorized as a non-thermal food processing technology, application of PEF-treatment can lead to a temperature increase due to the Joule effect, which must be considered with thermo-sensitive compounds such as lipids rich in polyunsaturated fatty acids (PUFAs) and proteins (Barba et al., 2015). Depending on the PEF-treatment conditions and processing parameters, the side effects of electroporation may include product discoloration due to damage to pigments, oxidation and denaturation of proteins (myoglobin), degradation and oxidation of PUFAs, bioactive peptides and vitamins, and consequently decrease of sensory characteristics and nutritional value of the end product in both plant and animal tissues (Gómez et al., 2019). Also, despite numerous studies performed on application of PEF to various food products (Chernomordik, 1992; Toepfl et al., 2014; Faridnia et al., 2015; Gómez et al., 2019), there is still a need to study the effect of this technology on oxidation reactions in muscle foods. Moreover, no studies investigating the effects of electroporation on lipid and protein oxidation reactions have to our knowledge been published.

It is very important to determine the common effects of PEF-treatment and brine salting on lipid and protein oxidation in sea bass samples. PEF-treatment prior to salting can lead to an increase in temperature during the treatment and a potential formation of free radicals acting as initiators of various oxidation reactions generating off flavors and various oxidation products causing a decrease in nutritional value through degradation of PUFAs and essential amino acids, as well as reduction of protein digestibility (Gómez et al., 2019). Electroporation can induce lipid oxidation through the formation of free radicals on unsaturated chains of fatty acids, as well as protein oxidation starting from the abstraction of a hydrogen atom from the carbon next to the amino group of the side chains. Lipid oxidation can produce rancid flavor and off-odors, thus aggravating the sensory profile of salted fish and decreasing its nutritional value, while oxidation in a side chain of amino acids can produce carbonyl groups leading to protein aggregation and loss of solubility (Rowe et al., 2004) which may also result in a decrease of water holding capacity and changes in texture of the fish muscle (Lund et al., 2011). The formation of Schiff bases can also decrease the quality of salted fish due to progressive cross-linking and polymerization reaction, including impaired functionality of myofibrillar proteins and loss of water-holding capacity (Estévez, 2011). Fish samples subjected to PEF-treatment, may exhibit greater sensitivity to lipid and protein oxidation due to higher exposure to various pro-oxidants such as heme-proteins (hemoglobin and myoglobin), transition metals and enzymes released during rupture of cell membranes (Gómez et al., 2019). The secondary lipid oxidation products may also react with primary amino groups on proteins resulting in protein carbonylation (Hematyar et al., 2019). Furthermore, a nucleophilic reaction between carbonyl groups and saturated lipid aldehydes can lead to the production of Schiff base products (Metz et al., 2004). Detection of carbonyl groups and Schiff bases are among the most common methods to detect and quantify protein oxidation in fish products (Hematyar et al., 2019).

Therefore, the aim of the present study was to investigate the combined effect of PEF-treatment and brine salting on lipid and protein oxidation in sea bass samples, as well as changes in color characteristics of the fish flesh.

2. Materials and methods

2.1. Materials

Sea bass (*Dicentrarchus labrax*) were supplied by TAGLIAPIETRA E FIGLI S.R.L. (Venice, Italy) in May 2019. The day after catch, the fish were delivered to L'ECOPESCE - ECONOMIA DEL MARE (Cesenatico, Italy) where they were gutted, filleted and deskinned. Immediately after processing, the sea bass fillets were placed on ice in Styrofoam boxes and transported to the CIRI-Agroalimentare laboratory in Cesena (Italy) where the experiment was carried out on the same day (24 h after catch).

Commercial salt "Sale alimentare di Sicilia" from ITALKALI S.RL. (NaCl ~98%) was used for preparation of brines.

2.2. PEF pre-treatment and brine salting

A total of 38 sea bass fillets were used for this experiment. From each fillet, 5 small pieces (8.3 ± 0.2 g each) with the dimensions of length 2.3 ± 0.2 cm, width 3.1 ± 0.4 cm and height 1.3 ± 0.5 cm were obtained (a total of 190 pieces) and randomly divided for the experimental samples. 10 pieces were used for the control sample, while 90 for each salting regime. For each salting regime, 3 sub-samples of 30 pieces were obtained and subjected to PEF pre-treatment or used as untreated ones.

The PEF pre-treatment, prior to salting, was performed using a lab scale PEF unit delivering a maximum output current and voltage of 60A and 8kV, respectively (Mod. S–P7500, Alintel, Italy). The generator provides monopolar rectangular-shape pulses and adjustable pulse duration (5–20 s), pulse frequency (50–500 Hz) and total treatment time (1–600 s). The treatment chamber (50 mm length x 50 mm width x 50 mm height) consisted of two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. Output voltage and current were monitored using a PC-oscilloscope (Picoscope 2204a, Pico Technology, UK). Sea bass pieces (approximately 7–8 g each) were treated at room temperature in tap water (water's initial electrical conductivity of 517 ± 20.4 μS/cm at 25 °C (EC-meter mod. Basic 30, Crison, Spain)), and delivering $n = 1000$ pulses at fixed pulse width (10 ± 1 μs), frequency (100 Hz), repetition time (10 ± 1 ms) and selecting two different current intensities, 10A and 20A, corresponding to values of electric field strengths of 300 V cm^{-1} and 600 V cm^{-1} and a total energy of 0.25 ± 0.01 and 1.01 ± 0.03 kJ/kg, respectively. The process parameters were chosen based on preliminary experimental trials, that allowed to confirm an increase in mass transfer rate halving the time necessary to reach the same salt content. The sea bass pieces were randomly distributed into the two experimental groups (PEF-treated and control samples) and salted by immersion into a brine with two different salt (NaCl) concentrations in tap water (5% and 10% w/w) at a ratio of 4:1 w/w brine/fish. Control samples were not subjected to PEF pre-treatment prior to brine salting. Samples for each sampling category were brined in one closed plastic container (5 fish pieces per one container, 500 ml). The salting process was carried out in a cold room at 0–4 °C for 2, 5 and 8 days according to the experimental plan displayed in Table 1. A flow chart of the process

Table 1. Experimental plan.

No sample	Sample code	NaCl concentration, %w/w	Duration of salting, days	Current intensity, A	Electric field intensity, V cm^{-1}
1	C-5-2	5	2	0	0
2	C-5-5	5	5	0	0
3	C-5-8	5	8	0	0
4	C-10-2	10	2	0	0
5	C-10-5	10	5	0	0
6	C-10-8	10	8	0	0
7	5-PEF-10-2	5	2	10	300
8	5-PEF-10-5	5	5	10	300
9	5-PEF-10-8	5	8	10	300
10	10-PEF-20-2	10	2	20	600
11	10-PEF-20-5	10	5	20	600
12	10-PEF-20-8	10	8	20	600
13	5-PEF-20-2	5	2	20	600
14	5-PEF-20-5	5	5	20	600
15	5-PEF-20-8	5	8	20	600
16	10-PEF-10-2	10	2	10	300
17	10-PEF-10-5	10	5	10	300
18	10-PEF-10-8	10	8	10	300

and a representation of the treatment chamber are shown in Figures 1A and 1B, respectively.

At each sampling day, sea bass samples were randomly collected and analyzed. Color measurements of the fish muscle were performed directly after each sampling day at University of Bologna (Cesena, Italy). The remaining experimental samples from each of the treatment were frozen at -80 °C and transported to Norwegian University of Science and Technology (Trondheim, Norway) for determination of lipid and protein oxidation.

Analyses were performed in 2–6 replicates for each PEF pre-treatment and salting regime.

2.3. Chemical and physical analyses

2.3.1. Lipid extraction

Lipids were extracted from raw (control) and brine salted sea bass samples by a mixture of chloroform-methanol-water by the Bligh and Dyer (1959) method. The fish samples were minced with a kitchen blender (Bosch 600W, Gerlingen, Germany) and a sample of 10 g was taken for extraction of lipids. After centrifugation, the upper layer of lipids in chloroform extract was separated, collected and further used in primary and secondary lipid oxidation analysis.

2.3.2. Primary and secondary products of lipid oxidation

The determination of primary and secondary lipid oxidation products included the quantification of peroxide value (PV), conjugated dienes (CDs) and 2-thiobarbituric acid reactive substances (TBARS), as described below.

The standard iodometric titration method (Cd 8b-90) (AOCS, 2003) was applied for PV determination. To assess the end point of titration, an automatic titrator (TitroLine 7800, Xylem Analytics, Mainz, Germany) coupled with a platinum electrode (Pt 62), was used. The titration was performed potentiometrically in duplicate and the results were expressed in meq active oxygen/kg lipids as a mean value ± SD.

CDs were determined spectrophotometrically according to the method described by Cropotova et al. (2019) as a modification of methods by Aubourg (1998) and Mozuraityte et al. (2017). Briefly, the absorbance of 1 mL chloroform extracts of lipids was measured against the solvent (chloroform) with a spectrophotometer GENESYS 12S UV-VIS (Thermo Scientific, USA) at 233 nm. The results were expressed as CD values in ml/g. The analysis was performed in two replicates for each sample, and the average with standard deviation was calculated.

TBARS were determined according to the method of Ke and Woyewoda (1979), as detailed described by Cropotova et al. (2019). For TBARS quantification, the pink-colored water phase was taken after the final step of centrifugation and its optical density was measured at 538 nm using a GENESYS 10S UV-VIS spectrophotometer (Thermo Scientifc, Pittsburgh, PA, USA). The analysis was performed in duplicate and the results were expressed in μmol TBARS/g lipids as a mean value ± SD.

2.3.3. Schiff bases

Schiff bases measurements were performed according to Buege and Aust (1978) with some modifications as follows. Briefly, the fluorescence of a chloroform extract of lipids (3 mL) obtained after Bligh and Dyer (1959) extraction was measured using a luminescence spectrometer LS 50B PerkinElmer (Waltham, Ma, USA) at 360 nm excitation and 430 nm

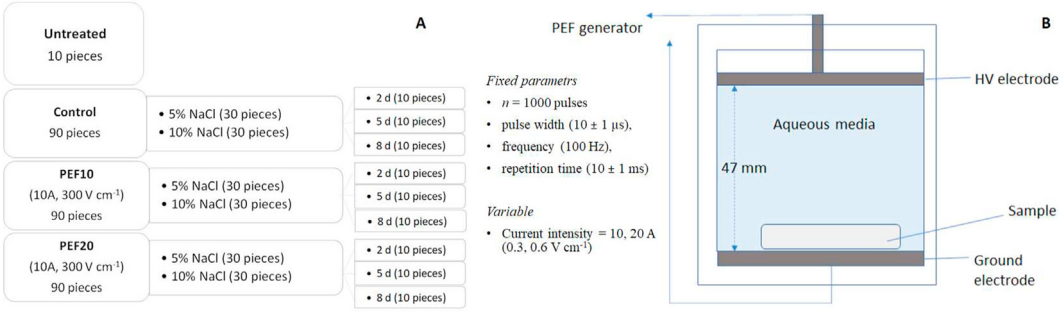

Figure 1. Flow chart of the process (A) and schematic representation of the treatment chamber (B).

emission wavelengths. The assay was carried out in two replicates for each sample, and the results were expressed in ml/mg as a mean value ± SD.

2.3.4. Protein oxidation

Protein oxidation was determined by measurement of protein carbonyls in sarcoplasmic and myofibrillar protein extracts by a DNPH based enzyme-linked immunosorbent assay (ELISA) in a 96-well polystyrene plate as described by Cropotova and Rustad (2019). The indirect ELISA kit, STA-310 OxiSelectTM, was purchased from Cell Biolabs, Inc. (San Diego, CA, USA). Sarcoplasmic (water-soluble) and myofibrillar (salt--soluble) protein extracts were prepared by a modification of the method of Licciardello et al. (1982), as previously described by Hultmann and Rustad (2002). Carbonyl groups were determined in the six parallels for each sarcoplasmic and myofibrillar protein extract, and the average value with standard deviation were calculated. The results were expressed in nmol carbonyls per mg protein.

2.3.5. Color parameters

Color parameters of sea bass samples were measured instrumentally using a spectrophotocolorimeter (Colorflex, Hunterlab). Before starting the analysis, the instrument was calibrated with a standard white and black plate. The measurements were performed on two preselected locations at the surface of each sea bass piece at room temperature. The L*, a* and b* parameters of the CIELAB scale were measured according to the lab scale established by Commission Internationale de l'Éclairage (CIE, 2001). Results were expressed as average of 10 measurements for each set of samples.

2.4. Statistical analysis

Statistical analysis and data processing were conducted using SigmaPlot software (Systat Software Inc., San Jose, California, USA), version 16.1.15. Statistical significance of the experimental data was verified by using Student's t-test and Analysis of Variance (ANOVA). To establish a relationship between certain parameters, Pearson correlations were calculated. Differences were considered significant at $p < 0.05$. The comparison analysis in ANOVA was performed by the Tukey test.

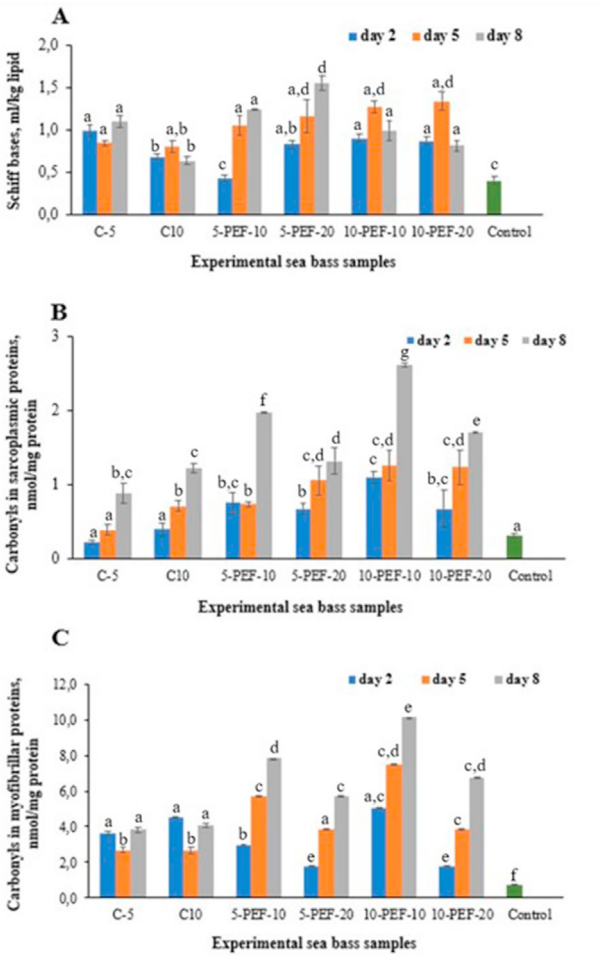

Figure 3. Products of lipid-protein and protein oxidation reactions in experimental sea bass samples: Schiff bases (A) and protein carbonyls in sarcoplasmic (B) and myofibrillar (C) proteins.

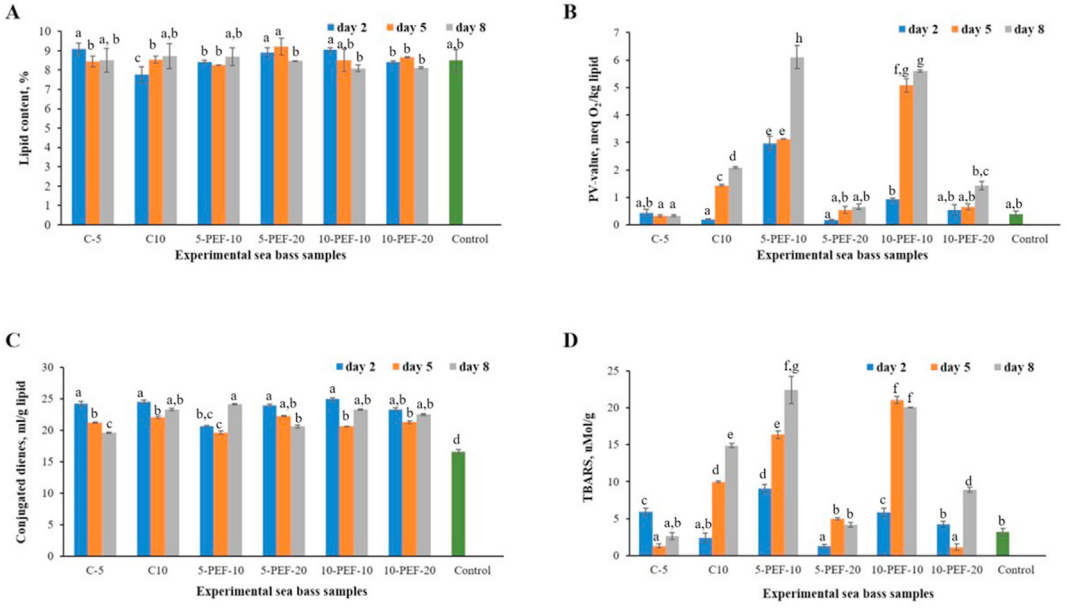

Figure 2. Lipid content (A) and primary and secondary lipid oxidation products: peroxide value (B), conjugated dienes (C) and TBARS data (D) of experimental sea bass samples during brine salting.

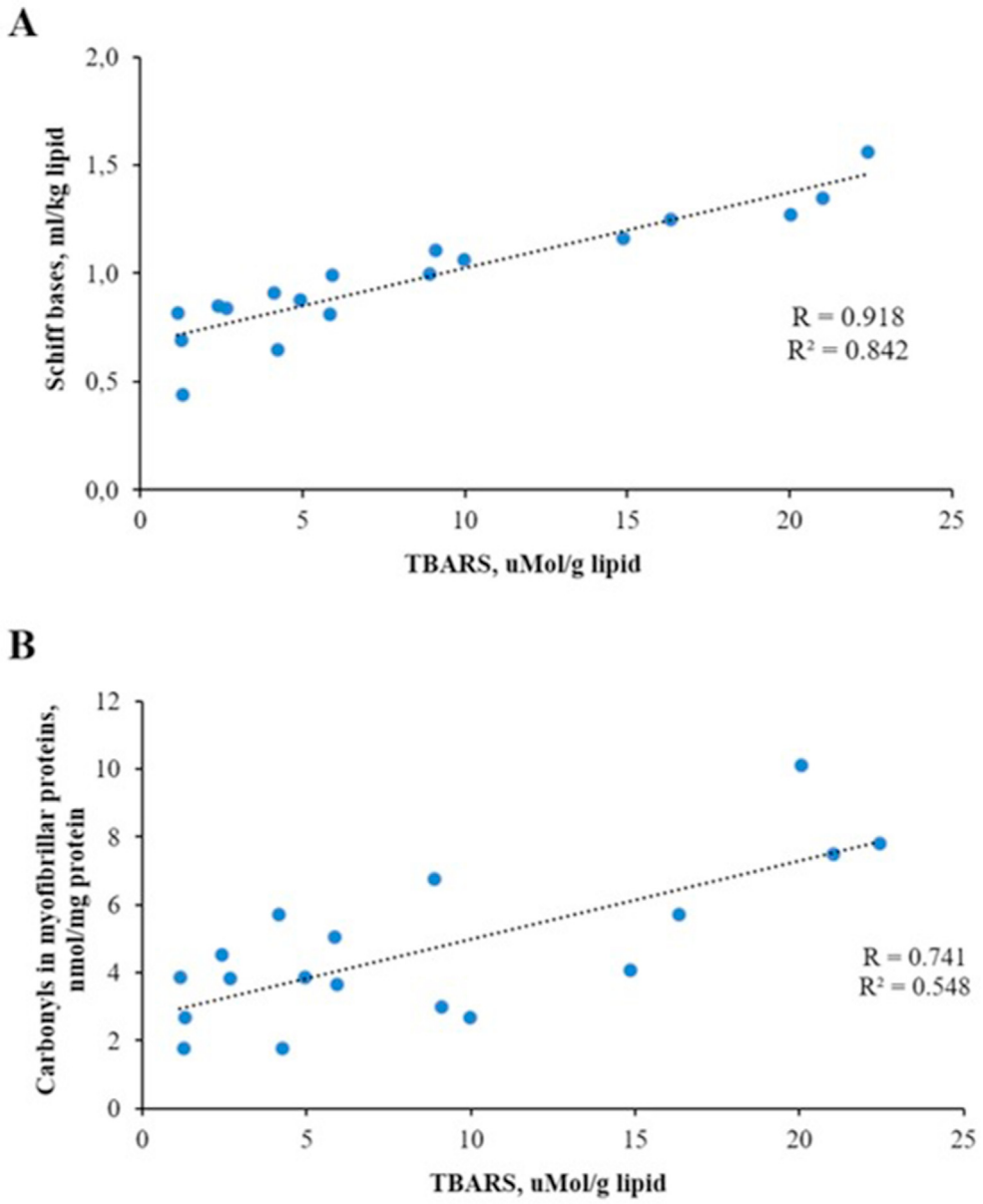

Figure 4. Correlations between lipid and protein oxidation products: Schiff bases and TBARS (A) and carbonyls in myofibrillar proteins and TBARS (B).

Multiple regression analysis was performed to explain the dynamics of the quality modifications in sea bass during salting and to identify the contribution of each of the process parameters to the changes occurring in the product.

3. Results and discussion

3.1. Total lipid content

Total lipid content in brine salted sea bass samples varied from 7.78 ± 0.41 to 9.22 ± 0.43% along the experiment (Figure 2A). At the same time, no significant variation in total lipid content was found between PEF-treated and untreated fish samples over the salting period, as well as between raw (control) and salted sea bass samples.

3.2. Primary and secondary lipid oxidation products

Amount of primary (PV and CD) and secondary lipid oxidation products (TBARS) show that lipid oxidation increased in PEF-treated sea bass samples compared to untreated ones during brine salting (Figure 2).

The PV of sea bass samples subjected to PEF-treatment at 300 V cm^{-1} (corresponding to the current intensity of 10 A) prior to salting, displayed a significant ($p < 0.05$) increase compared to non-treated and PEF-treated fish samples at 600 V cm^{-1} (current intensity of 20 A) during brine salting (Figure 2B). At the same time, no significant variation in CD data was observed between PEF-treated and untreated samples (Figure 2C). However, conjugated dienes displayed a significant ($p < 0.05$) increase in all experimental sea bass samples along the brine salting period compared to CD-values of control samples (Figure 2C). This tendency may be because salt has been reported to enhance lipid oxidation of highly unsaturated lipids (Harris and Tall, 1994). Thus, lipids containing methylene interrupted dienes or polyenes get a shift in the position of the double bond during the oxidation due to isomerization and formation of CDs (Lalas, 2009).

However, only three sea bass samples subjected to electroporation at 10 A (300 V cm^{-1}) exceeded the limit for PV of 5 meq active oxygen/kg lipid established by the Standard for fish oils CODEX STAN 329–2017 (Figure 2B) on day 5 and day 8 of brine salting. The same trend was observed in the variation of TBARS data along the salting experiment (Figure 2D). Thus, fish samples subjected to PEF-treatment at 10 A current intensity (300 V cm^{-1}) had significantly ($p < 0.05$) higher values of

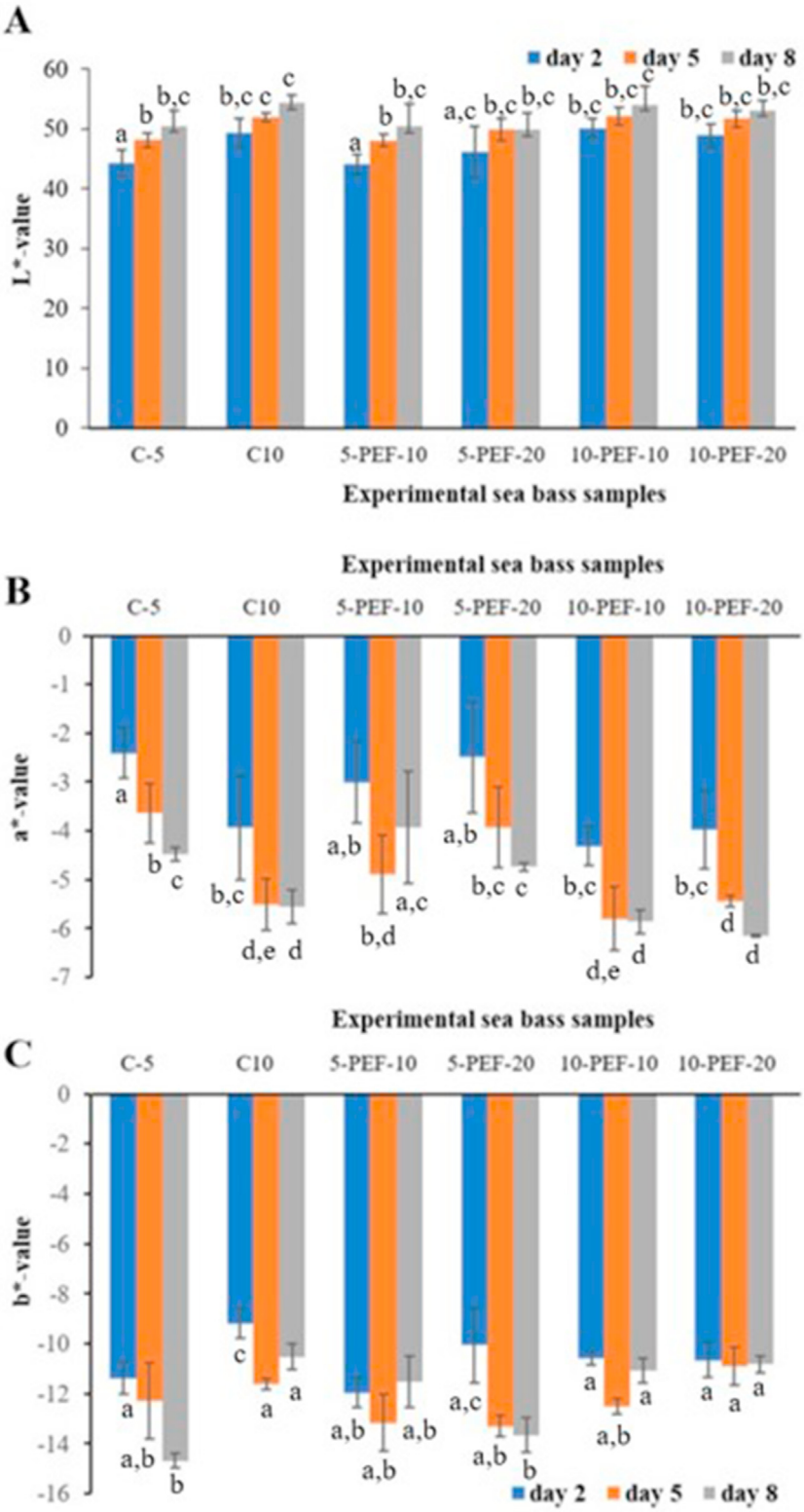

Figure 5. Color characteristics of sea bass samples during brine salting: lightness (L*-value) (A), redness (a*-value) (B), and yellowness (b*-value) (C).

TBARS compared to untreated samples and samples treated at 20 A (600 V cm^{-1}).

PEF treatment prior to salting could result in destabilization of the integrity of the muscle cell membrane due to mechanical damages to the fish muscle structure, thus facilitating the exposure of various pro-oxidants (enzymes, free iron, heme-proteins, etc.) accelerating lipid oxidation. This suggestion is supported by the study of Kantono et al. (2019) who previously investigated the effects of PEF treatment on lipid oxidation in beef muscles. They believed that lipid oxidation in PEF-treated samples is associated with a breakdown of the cell membrane integrity causing a release of pro-oxidants, similar to the effect of freezing.

The phenomenon of lower lipid oxidation in samples treated with a higher field intensity (600 V cm^{-1}) compared to the lower intensity (300

V cm^{-1}) could be explained by an antioxidative mechanism of electroporation inactivating the pro-oxidative endogenous enzymes. A similar behavior was observed in our previous study on high pressure (HP) treatment of fish minces (Cropotova et al., 2020), according to which the lowest lipid oxidation values were obtained after the highest HP-treatment conducted at 300 MPa compared to the lowest treatment at 200 MPa. Thus, two opposite mechanisms were considered to explain the lipid changes observed: *pro-oxidative* – as a result of denaturation of heme-proteins releasing free iron and other pro-oxidants from damaged muscle cells, and *antioxidative* – leading to inactivation of pro-oxidative endogenous enzymes that could increase oxidation (Cropotova et al., 2020). Moreover, PEF inactivation of endogenous enzymes has previously been observed in different food matrixes but at significantly higher voltages (>10 kV cm^{-1}) (Li et al., 2008; Liu et al., 2014; Zhao et al., 2012). However, PEF did not modify the protein profile when applied to *longissimum thoracis* beef muscles at 2–6 kV cm^{-1} (Faridnia et al., 2014) or at 1–1.25 kV cm^{-1} (Chian et al., 2019). Studies comparing the effect on microstructure of fish and meat samples showed that salmon was more sensitive to mild PEF treatment (<2 kV cm^{-1}) compared to chicken. Meat composition in terms of protein and fat content and their distribution in the muscle might be the reason for these differences. However, to our knowledge, there are no studies showing the inactivation of enzymes in fish samples after PEF treatment. On the other side, the use of PEF prior to salting could have enhanced the distribution of salt in the tissue thus promoting protein aggregation. Hence, this behavior should be further investigated.

3.3. Lipid-protein and protein oxidation products

The end products of lipid oxidation can react with proteins, thus affecting the color and flavor of the fish and reducing nutritional value of the proteins. Schiff bases (SB) formation is one of these reactions resulting from a cross-linking between the aldehyde moiety from protein carbonyls and alkaline amino acids in proteins (Estévez, 2011). Assessing the effect of the PEF treatment on formation of SB in salted fish can be a valuable tool to optimize the process and reduce cross-linking reactions which can cause protein polymerization and impaired functionality, including loss of water-holding capacity. In the present study, there was a significant ($p < 0.05$) increase in SB content in all experimental sea bass samples compared to raw fish (Figure 3A).

At the same time, all PEF-treated fish samples showed a significant increase in Schiff bases values on day 5 and day 8 of brine salting compared to non-treated salted sea bass (Figure 3A). Contrarily to results related to fat oxidation, the fish samples salted in 5% brine had significantly higher values of Schiff bases after PEF treatment conducted at higher current intensity (20 A) compared to samples subjected to electroporation at lower current intensity (10 A). The end products of lipid and protein oxidation interact with each other, including a nucleophilic reaction between carbonyl groups and saturated lipid aldehydes producing Schiff bases (Metz et al., 2004). In support to this suggestion, a significant correlation ($p < 0.05$, $R = 0.918$) between TBARS and SB of experimental sea bass samples during brine salting, was revealed (Figure 4A).

To the best of our knowledge no information about the effects of electroporation on protein oxidation in fish is available in the literature. Moreover, the existing information on the influence of PEF-treatment on protein oxidation in various protein-rich food products is scarce and mostly includes studies on eggs and egg products carried out at much higher electric field strength (Wu et al., 2014). Fish is a rich and important source of proteins with bioactive properties, which may be prone to denaturation, aggregation and oxidation when exposed to electroporation, resulting in decline of product quality and nutritional value (Gómez et al., 2019). Therefore, assessment of protein oxidation in the product can be a valuable tool for further optimization of the process parameters (applied electric field intensity, salt concentration, etc.).

Our investigations revealed a significant ($p < 0.05$) increase in total carbonyls in sarcoplasmic and myofibrillar proteins of PEF-treated sea bass samples compared to control and untreated samples on day 5 and 8 of brine salting (Figure 3B-C). This phenomenon can be explained by PEF-induced degradation of cell membranes due to mechanical damages to the fish muscle structure under the applied current of 10 and 20A (300 and 600 V cm^{-1}), leading to the release of various pro-oxidants (enzymes, free iron, heme-proteins, etc.) accelerating protein oxidation reactions (Gómez et al., 2019). Secondary lipid oxidation products can also react with primary amino groups on proteins resulting in protein carbonylation (Hematyar et al., 2019). This is supported by the significant correlation ($p < 0.05$, $R = 0.741$) found between carbonyls in myofibrillar proteins and TBARS, (Figure 4B).

3.4. Color parameters (yellowness)

According to Figure 5A, lightness of experimental sea bass samples varied from 44.2 to 54.2, while increasing gradually over the duration of salting period. Multiple regression analysis revealed that the main factor influencing the changes of L*-value was duration of salting period ($p < 0.05$, $R = 0.974$, $R^2 = 0.951$). However, no significant difference between PEF-treated and untreated sea bass samples was found during brine salting. At the same time, the increase in lightness was accompanied by a simultaneous decrease in redness in sea bass samples during brine salting (Figure 5B). Moreover, there was a significant ($p < 0.05$) decrease in a*-value in PEF-treated samples compared to untreated ones during brine salting, indicating that fish loses its natural flesh pigmentation when subjected to electroporation – a trend similar to cooking discoloration. This phenomenon can be explained by denaturation of some pigments and heme-proteins inside the fish muscle (Gómez et al., 2019). A similar effect was observed in our previous study on high pressure treatment of Atlantic mackerel and haddock minces (Cropotova et al., 2020) suggesting that changes in color characteristics are due to conformational changes in heme-proteins. Based on previous investigations, we hypothesize that the gradual decrease in redness of PEF-treated sea bass samples compared to untreated ones during brine salting is mainly due to PEF-induced denaturation of heme-proteins inside the fish muscle.

Yellowness of experimental sea bass samples displayed a high variation throughout the storage period (Figure 5C). The changes in b*-value of brine salted sea bass are most likely due to accumulation of yellowish-colored compounds generated by decomposition and polymerization of primary products of lipid oxidation, indicated by the positive correlation values (R) of 0.789 ($p < 0.05$) between b*-values and conjugated dienes.

4. Conclusion

The present study investigated the effect of PEF treatment prior to brine salting on lipid and protein oxidation and changes in color characteristics of sea bass. Electroporation negatively influenced the oxidative lipid stability in sea bass samples with regard to primary and secondary lipid oxidation products.

Damage of cell membranes occurring during electroporation resulted in higher oxidation level with respect to primary and secondary oxidation products and protein carbonyls of PEF-treated sea bass samples compared to untreated fish samples during brine salting. However, sea bass samples treated at higher voltage of 600 V cm^{-1} showed significantly lower values of peroxide value and TBARS compared to fish samples treated at 300 V cm^{-1}. At the same time, none of the untreated and PEF-treated samples at the voltage of 600 V cm^{-1} exceeded the acceptable level of 5 meq active oxygen/kg lipids suggesting satisfactory quality of sea bass during brine salting with regard to oxidative lipid stability.

PEF-treated sea bass samples were also characterized as lighter and less reddish compared to untreated fish samples. The yellowness of sea bass samples correlated significantly with conjugated dienes, suggesting the contribution of electrochemically polymerized lipids on further

generation of secondary lipid oxidation products leading to yellowing of the fish flesh.

The present study showed that it is important to consider and select PEF-treatment process parameters prior to brine salting in order to obtain the advantages due to the PEF application on salting kinetics but to avoid extensive lipid and protein oxidation in the fish during the salting process.

Declarations

Author contribution statement

Janna Cropotova: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Silvia Tappi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jessica Genovese: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Pietro Rocculi: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Marco Dalla Rosa: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Turid Rustad: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Paper V

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Study of the influence of pulsed electric field pre-treatment on quality parameters of sea bass during brine salting

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Brine salting
Sea bass
Water distribution
LF-NMR

ABSTRACT

Pulsed electric field (PEF), as an emerging technique, has recently gained increased popularity in food processing and preservation. However, applications in the seafood industry are still scarce. In the present study, sea bass samples were subjected to PEF pre-treatment prior to brine salting to verify the possible acceleration of the brining rate, increasing the salt uptake and ensuring the homogeneous salt distribution in the muscle. The applied intensity of the current was set at 10 and 20 A (corresponding to a field strength of 0.3 and 0.6 kV/cm) prior to sea bass salting in brine with 5 and 10% salt concentration, respectively. The results have shown that PEF pretreatment could effectively shorten the brine salting time compared to control samples (from 5 to 2 days), or increase the salt uptake up to 77%, ensuring at the same time its homogenous distribution in the muscle. However, myofibrillar protein solubility was significantly reduced in PEF pretreated samples. At the same time, no significant differences in water holding capacity and water activity between PEF pre-treated and untreated samples were found during the whole salting period. Freezable water was influenced by PEF application, but the effect was significant only at the lowest salt concentration during the first period of the salting process.

Industrial relevance: PEF-assisted brining appears a promising technology in the fish processing industry due to its efficacy in reducing the salt brining time, increasing the mass transfer and enhancing the diffusion of brine into the muscle to ensure the homogeneous distribution of salt in it. The increased salt uptake of the PEF-treated samples compared to control samples shows future potentiality of using PEF prior to salting in the fish processing industry.

1. Introduction

Fish is a highly perishable raw material where deterioration caused by biochemical phenomena and microorganisms begin soon after slaughtering. Proper handling and preservation practices are therefore needed to prolong the shelf life of the product (Nagarajarao, 2016).

Salting is one of the oldest preservation methods used for long time storage of fish. Salted pelagic fish was well known to the old civilizations including the ancient Greeks and the Romans, the Vikings and other populations that lived on the shores of the Mediterranean Sea and the Atlantic Ocean. Today, a variety of salted pelagic fish products including sardines, anchovies, sea bass, *bacalao*, herring i.e., as well as Scandinavian dried and salted cod called *klippfisk*, literally "cliff-fish", are produced under the common name of "salted fish products" and marketed in many countries of the Mediterranean and the North Sea regions. Due to a fairly good market price and high palatability, these product commodities have become popular and highly appreciated in Europe and the USA. Along with the changes of lifestyle and growing consumer demands towards ready-to-eat, healthy and tasty foods, lightly salted fish products are currently gaining more and more popularity (Fan, Luo, Yin, Bao, & Feng, 2014).

Salting is one of the simplest methods of preserving large quantities of fish from spoilage. Salt is usually used at concentrations high enough to preserve the fish. Salting can be also used as a preliminary operation in smoking, drying and cooking processes helping to improve sensory parameters and increase the shelf-life of the final product (Bras & Costa, 2010). Salt can interact with proteins to increase hydration and water holding capacity of fish muscle thus improving its textural parameters.

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Increasing the water holding capacity of fish muscle helps to decrease cooking loss, thereby enhancing the tenderness and juiciness of the final product. Sodium chloride (NaCl), the common salt, is the main ingredient used in fish salting. It acts as a preservative by dehydration and osmotic pressure inhibiting bacterial growth and deactivating enzymes. Even at low concentrations, NaCl possesses some preservative action (Lupín, Boeri, & Moscidar, 1981). Other substances such as herbs, spices, sugar or antioxidants can also be used in the fish salting process to improve sensory attributes of the product, modify flavor and reduce shrinkage after salting. The conventional fish salting methods include dry-salting and wet-salting. During dry salting, the salt (traditionally sodium chloride) and other ingredients from the curing mixture (sugars and spices) are applied to the fish surface. Wet salting is performed by immersing the product into brine or injecting the brine directly into the fish muscle (Birkeland, Skåra, Bjerkeng, & Rørå, 2003; Hall, 2011). The concentration of salt in the brine affects the weight gain, water holding capacity and commercial quality of the end product (Nguyen, Thorarinsdottir, Gudmundsdottir, Thorkelsson, & Arason, 2010). Weight gain of salted fish products depends on the ability of the myofibril proteins to retain water inside the muscle affected by the salting procedures applied (Thorarinsdottir, Arason, Sigurgisladottir, Valsdottir, & Tornberg, 2011). The brining time usually varies from 2 to 10 days depending on the desired level of salt in the muscle. During immersion brining, fish is covered with brine for a period of time and held at a temperature between 0 and 4 °C. In injection salting, the brine is injected into the fish fillet using a set of needles making this a faster method than immersion brining.

Myofibrillar proteins are of great importance for the functional properties of light-salted fish products, such as water holding capacity (WHC). It is well known that salting of fish alters protein extractability and thermal denaturation and aggregation of many muscle proteins (Nguyen et al., 2010), which in turn affects the WHC. Salting also affects the proteolytic activity responsible for degradation of myofibrils and connective tissue proteins, as well as extra-cellular matrix (Thorarinsdottir et al., 2011). Thus, the influence of salting on the distribution of water within the muscle may be related to direct effects of salt on changes in structural components of the muscle (Larsen & Elvevoll, 2008; Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002). It is also assumed that the main components of fish muscle (proteins, lipids and salts) influence the arrangement of water molecules in a product matrix, thereby having an effect on the product quality and shelf-life (Pacetti et al., 2015). Therefore, it is important to study how the salt content and water distribution within the muscle may affect water holding capacity of the product. Low-field nuclear magnetic resonance (LF-NMR) has been employed in the food industry to study water mobility and distribution within the fish muscle (Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad, 2008; Løje, Green-Petersen, Nielsen, Jørgensen, & Jensen, 2007). This technique has been suggested a tool for rapid and non-destructive analysis of water mobility and identification of intra-myofibrillar or extra-myofibrillar water components (Andersen & Jørgensen, 2004; Jensen, Jørgensen, Nielsen, & Nielsen, 2005; Løje et al., 2007) in the muscle.

The migration of salt from brine to fish matrix is generally quite slow. Different brining methods have previously been tested to accelerate salt transport through the product, for instance high intensity ultrasound brining and marinating (Chemat, Zill-e-Huma, & Khan, 2011; Turhan, Saricaoglu, & Oz, 2013), pulsed vacuum brining (Andres, Rodrigues-Barona, Barat, & Fito, 2002), and vacuum tumbling (Esaiassen et al., 2004; Mathias, Jittinandanana, Kenney, & Kiser, 2003). Pulsed electric field (PEF), as an emerging technology, has great potential to contribute to improved salting of fish products through enhanced diffusion of salt into the fish muscle (Hafsteinsson, Gudmundsson, Arnarson, Jonsson, & Siguroardottir, 2000). However, to our knowledge, no studies have so far been published on PEF applications for salting of fish. Even though the concept of PEF was introduced to the food industry about 50 years ago, this technique can be still considered an emerging technology due to the recent developments related to microbial inactivation applications and improvement of mass transfer through cell disruption (Gómez et al., 2019). In general, PEF technique applies high voltage pulses of short duration to food placed between two electrodes, resulting in specific structural modifications of the tissue including the disruption of cell membrane (Barba et al., 2015). Under the application of the high electric field pulses, the membrane permeability is increasing due to either enlargement of existing pores or generation of new ones (Gómez et al., 2019). This concept was previously applied in the seafood industry with the aim of enhancing water holding capacity of fish and tenderization of shellfish products (Klonowski, Heinz, Toepfl, Gunnarsson, & Þorkelsson, 2006). PEF has also been suggested as a promising technique for accelerating mass transfer which could potentially be used as a pre-treatment in the fish drying process (Gómez et al., 2019).

Therefore, the main aim of the present study was to investigate whether the PEF pre-treatment can be applied to accelerate the brining process and ensure a uniform distribution of salt within the muscle of fish, evaluating mass transfer kinetic and, in parallel, water state and distribution. The study aims also at investigating the effect of PEF pre-treatment on quality parameters of sea bass during salting. It is well known that PEF may affect the extractability and aggregation of proteins, since electroporation within the muscle tissue can result in chemical modifications by the formation of free radicals which can further alter the structure of proteins and the intermolecular forces (Gudmundsson & Hafsteinsson, 2001; Zhao, Sun, & Tiwari, 2019). Therefore, this research also investigated the effect of different PEF pre-treatments on protein functionality by evaluating water holding capacity and protein solubility.

2. Materials and methods

2.1. Materials

Sea bass (*Dicentrarchus labrax*) were supplied by Tagliapietra e Figli s.r.l. (Venice, Italy) in May 2019. The day after catch, the fish were delivered to Economia del Mare (Cesenatico, Italy) where they were gutted, filleted and de-skinned. The sea bass fillets were placed on ice in Styrofoam boxes and transported to the CIRI-Agrifood laboratory in Cesena (Italy), where the experiment was carried out in the same day. Commercial salt 'Sale alimentare di Sicilia' from Italkali s.r.l. (NaCl ~98%) was used for brines preparation.

2.2. PEF pre-treatment and brine salting

Sea bass fillets were cut into small pieces (8.3 ± 0.2 g each) with the dimensions of length 2.3 ± 0.2 cm, width 3.1 ± 0.4 cm and height 1.3 ± 0.5 cm.

Prior to salting, the obtained sea bass pieces were subjected to PEF pre-treatment, performed using a lab scale PEF unit Mod. S-P7500 delivering a maximum output current and voltage of 60A and 8 kV, respectively (Alintel, Bologna, Italy). The generator provides monopolar rectangular-shape pulses and adjustable pulse duration (5–20 μs), pulse frequency (50–500 Hz) and total treatment time (1–600 s). The treatment chamber (50 mm length x 50 mm width x 50 mm height) consisted of two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. Output voltage and current were monitored using a PC-oscilloscope (Picoscope 2204a, Pico Technology, UK). Sea bass pieces were treated at room temperature in tap water delivering $n = 1000$ pulses at fixed pulse width (10 ± 1 μs), frequency (100 Hz), repetition time (10 ± 1 ms) and selecting two different current intensities, 10A and 20A, corresponding to values of electric field strengths of 0.3 and 0.6 kV/cm and specific energy input of 0.25 ± 0.01 and 1.01 ± 0.03 kJ/kg, respectively. The process parameters were chosen on the basis of preliminary experimental trials.

The sea bass pieces were randomly distributed into the three experimental groups (two PEF-treated and one control samples) and

Table 1
Samples code and parameters.

Sample code	NaCl concentration (% *w*/w)	Electric field intensity (kV/cm)	Current intensity (A)
C-5	5	0	0
5-PEF-10	5	0.3	10
5-PEF-20	5	0.6	20
C-10	10	0	0
10-PEF-10	10	0.3	10
10-PEF-20	10	0.6	20

salted by immersion into a brine with two different salt (NaCl) concentrations in tap water (5% and 10% (*w*/w)) and in closed plastic containers (500 ml) each containing a ratio of 4 to 1 w/w brine/fish. Five independent replicates were considered for each sample type and for each sampling time. The salting process was carried out in a cold room at 0–4 °C for 2, 5 and 8 days according to the experimental plan displayed in Table 1.

At each sampling day, sea bass samples were randomly collected and analyzed. Changes in weight yield, water-holding capacity, water activity, freezable water by differential scanning calorimetry and water behavior and distribution inside the muscle by LF-NMR as affected by different PEF pre-treatment and salting parameters, were studied directly after each sampling day at the laboratories of the University of Bologna (Cesena, Italy). The remaining experimental samples from each treatment were frozen at −80 °C and transported to Norwegian University of Science and Technology (Trondheim, Norway) for determination of water and salt content, pH and protein solubility.

Analyses were performed in 3–6 replicates for each sample as described in detail in the following section.

2.3. Physico-chemical analyses

2.3.1. Mass transfer parameters

2.3.1.1. Weight yield. The fish samples were weighed raw and after each sampling day. The weight yield was determined with respect to the weight of the raw fillets as described by Thorarinsdottir, Arason, Bogason, and Kristbergsson (2004).

2.3.1.2. Water content. Water content was determined by drying a sample of 2 g at 105 °C for 24 h to a constant weight, according to the official method (AOAC, 2005). Finely chopped fish obtained from 5 individual pieces was mixed and analyzed in triplicate.

2.3.1.3. Salt (NaCl) content. Salt content in all sea bass samples was determined by titration according to AOAC 976.18 (1995). Briefly, the fish obtained by 5 different pieces was minced with a kitchen blender (Bosch 600 W, Gerlingen, Germany), and 2 g of the resulting mince was weighed in a 150 ml glass beaker, filled with 80 ml warm distilled water (60 °C) and mixed for 5 min until a homogeneous mixture was obtained. Then, 1 ml of 1 M HNO_3 was added to the mixture, the electrode type AgCl 32 and burette tip was placed in the solution, and the titration was performed with an automatic titrator (mod. TitroLine 7800, Xylem Analytics, Mainz, Germany). The analysis was performed in three replicates and the results were expressed in % salt as a mean value ± SD.

The total water and NaCl weight changes ($\Delta M^{0}{}_{t}$, $\Delta M^{w}{}_{t}$ and $\Delta M^{NaCl}{}_{t}$, respectively) of salted samples were determined with Eqs. (1), (2) and (3) as follow:

$$\Delta M_t^{\circ} = \frac{(M_t^{\circ} - M_0^{\circ})}{M_0^{\circ}} \quad (1)$$

$$\Delta M_t^{w} = \frac{(M_t^{\circ} \cdot x_t^{w} - M_0^{o} \cdot x_0^{w})}{M_0^{\circ}} \quad (2)$$

$$\Delta M_t^{NaCl} = \frac{(M_t^{\circ} \cdot x_t^{NaCl} - M_0^{\circ} \cdot x_0^{NaCl})}{M_0^{\circ}} \quad (3)$$

where $M^{\circ}{}_{t}$ and $M^{\circ}{}_{0}$ are the sea bass weights, $x^{w}{}_{t}$ and $x^{w}{}_{0}$ are the water weight fractions, and $x^{NaCl}{}_{t}$ and $x^{NaCl}{}_{0}$ are the NaCl weight fractions, at sampling time *t* and before the salting process *0*, respectively.

2.3.2. Water state and mobility

2.3.2.1. Water activity. Water activity was measured with a Water Activity Meter mod. AQUALAB, (Decagon Devices, US). Briefly, the fish samples were cut into small pieces (0.2 × 0.2 cm) and introduced into sample holders prior to the analysis. Between measurements, the samples were covered with lids and protected with parafilm. For each of the experimental groups, four measurements were performed and the mean value ± SD was calculated.

2.3.2.2. Differential scanning calorimetry (DSC). A differential scanning calorimeter (DSC) mod. Q20 (TA Instrument, Germany), equipped with a low- temperature cooling unit (TA-Refrigetated Cooling System90).) was used to assess freezable water content (FW, g/g of water) and to evaluate the effect of processing on protein denaturation. Temperature and melting enthalpy calibrations were performed with ion exchanged distilled water (mp 0.0 °C) and indium (mp 156.60 °C), while heat flow was calibrated using the heat of fusion of indium (ΔH = 28.71 J/g). For the calibration, the same heating rate and dry nitrogen gas flux of 50 ml/min used for the analysis were applied. Each sample was weighed (about 15 mg) into a 50-μL aluminum pan, sealed hermetically and frozen at −40 °C. Frozen samples were then loaded into the DSC instrument. The heating rate of DSC scans was 5 °C/min over a range of −40 to 90 °C. Empty aluminum pans were used as reference and for baseline corrections. Eight replications for each sample were performed and results were elaborated through PeakFit Software (SeaSolve Software Inc. Framingham, MA, USA).

The FW was determined as follows:

$$FW = \frac{\Delta H_m}{\Delta H_w} \quad (4)$$

where ΔH_w (325 J/g) is the latent heat of melting per gram of pure water at 0 °C, and ΔH_m (J/g) is the measured latent heat of melting of water per gram of sample obtained by the integration of the melting endothermic peak. FW was further related to the water content and expressed as grams per gram of water content (FW^{w}).

PeakFit Software (SeaSolve Software Inc. Framingham, MA, USA) was used to analyse thermal data and obtain deconvoluted peaks and calculate relative melting enthalpy.

2.3.2.3. LF-NMR. A 10 mm deep slice was cut from each sample, then cylinders (6 mm diameter) of about 400 mg were obtained with a cork borer. Signals weighted by T2 were registered with the CPMG pulse sequence (Meiboom & Gill, 1958), using a Bruker mod. Minispec PC/20 spectrometer operating at 20 MHz. Each measurement consisted in 30 K points, spaced 0.080 ms. Subsequent scans were separated by a recycle delay of 3.5 s. The specified interpulse spacing avoided sample overheat but allowed the observation of the protons with T2 higher than a few milliseconds. UPEN software (Borgia, Brown, & Fantazzini, 1998) allowed to obtain an overview of the protons T2 distributions (the relaxograms) by inverting the T2-weighted signals towards a semi-continuous distribution of exponential curves, according to Eq. (5):

$$I(2\tau n) = \sum_{i=1}^{M} I_0(T_{2,i}) exp(-2\tau n / T_{2,i}) \quad (5)$$

where 2τ is the CPMG interpulse spacing, n is the index of each CPMG point while I0 is the intensities of each T2 component extrapolated at *t* = 0, sampled logarithmically. As some components resulted as partially

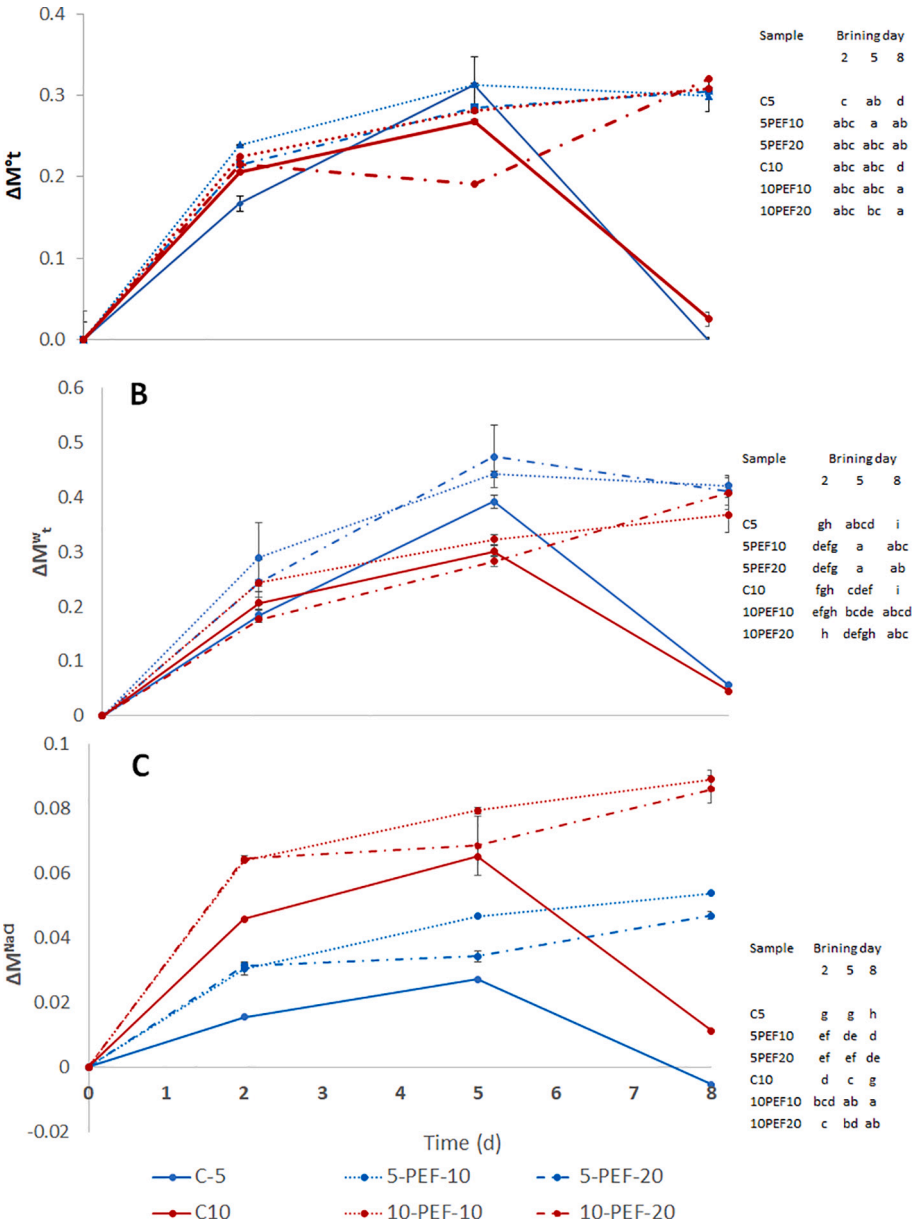


Fig. 1. Total weight change (ΔM^{O}_{t}) (A), water uptake (ΔM^{w}_{t}) (B) and NaCl uptake (ΔM^{NaCl}_{t}) (C) of control and PEF treated sea bass samples during the brining process at 5% and 10% salt concentrations. Results are expressed as means ± standard deviations (error bars) of $n = 5$. Values with different letters in the auxiliary tables differ significantly ($p < 0.05$).

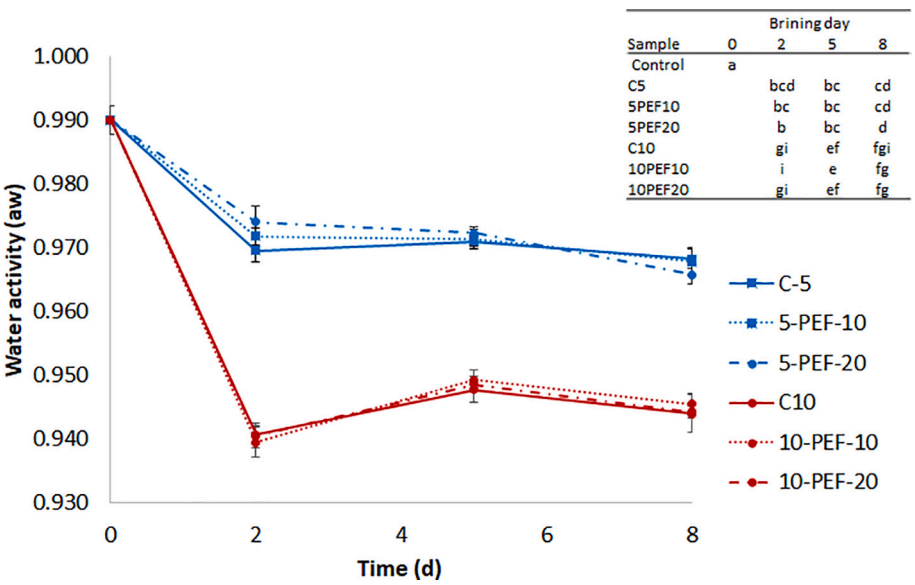
overlapped in the relaxograms from several samples, we observed them separately by fitting the T2-weighted signals to the sum of an increasing number of exponential curves. An F-test showed that the optimum ratio between fitting ability and complexity of the model was reached for most samples with three exponentials. Six measurements were performed for each of the experimental sets.

2.3.3. Protein functionality

2.3.3.1. pH. pH was measured at room temperature by inserting electrode directly into the sea bass mince (mod. MP-220 pH-meter, Mettler-Toledo, Hong Kong) according to Thorarinsdottir et al. (2004). Prior to pH measurements, the pH meter was calibrated with standard buffer solutions. The measurements were performed at least in triplicate, and the mean value ± SD was calculated.

2.3.3.2. Protein solubility. Water and salt soluble proteins were determined in white muscle extracts according to a modification of the methods of Licciardello et al. (1982), as previously described by Hultmann and Rustad (2002). The amount of proteins in the extracts was determined with BioRad protein assay after centrifugation at 8000 *g* and 4 °C for 20 min, using gamma globulin as a standard. The analyses were run in triplicate and the mean value ± SD was calculated.

2.3.3.3. Water Holding Capacity (WHC). WHC of sea bass samples was measured according to the method described by Thorarinsdottir et al. (2004), as follows. The minced samples were placed in centrifuge tubes and centrifuged at 200 *g* for 10 min (0–4 °C). The weight (g) of the fish pieces before and after the centrifugation was determined. WHC was expressed as the amount of released water divided by the original weight (g) of the sample before centrifugation. Four replicates were performed for each treatment group.



Sample	Brining day 0	2	5	8
Control	a			
C5		bcd	bc	cd
5PEF10		bc	bc	cd
5PEF20		b	bc	d
C10		gi	ef	fgi
10PEF10		i	e	fg
10PEF20		gi	ef	fg

Fig. 2. Water activity of control and PEF treated sea bass samples during the brining process at 5% and 10% salt concentrations. Results are expressed as means ± standard deviations (error bars) of $n = 4$. Values with different letters in the auxiliary table differ significantly ($p < 0.05$).

2.4. Statistical analysis

The data sets from the experiment were analyzed by Statistica 8.0 software (StatSoft, Tulsa, USA) The effect of the parameters of PEF treatment (PEF), NaCl concentration (Salt) and brining time (Time) and their interaction on dependent variables was evaluated through the factorial Analysis of Variance (ANOVA). Statistical significance of the experimental data was verified using Tukey as post-hoc ($p < 0.05$). To establish a relationship between certain parameters, Pearson correlations were calculated. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Mass transfer parameters

Fig. 1 reports the total weight change (A), water (B) and salt uptake (C) mass fraction of control and PEF (0.3 and 0.6 kV/cm) treated sea bass samples during the brining process at 5% and 10% salt concentrations.

In control samples, weight increased between 24 and 26% during the first 5 days of brining. However, on the last day of brine salting, the weight yield of control samples was reduced up to −0.13% and 2.56% for 5% and 10% salt concentration in the brine, respectively. The lowest weight yield in the control group on day eight may possibly be explained by an inhomogeneous salt distribution within the inner and outer parts of the fish muscle at the beginning of brining, leading to disintegration of the fish muscle pieces in the last part of the experiment, as previously showed by Thorarinsdottir et al. (2004). Differently PEF treated samples showed a constant increase of weight during the entire brining period. While no significant differences were observed compared to the control until the 5th day of salting, on the 8th day all PEF treated samples (0.3 and 0.6 kV/cm) reached a weight gain of 28–32%.

The total water content in the sea bass samples varied from 73.9 to 88.7% (*w*/w) during brine salting. In all samples, water uptake (Fig. 1B) was observed until the 5th day, when samples immersed in the 5% salt brine showed significantly higher values compared to samples in the 10% one. However, no differences were observed among the control and the PEF treated samples in each of the 2 groups (0.3 and 0.6 kV/cm). At the 8th day, the water uptake showed a drastic drop for both the control samples, as already observed with the total weight change. PEF treated samples in the 5% brine, did not show a further water uptake, while samples in the 10% brine showed a further increase. All PEF treated samples showed similar water fraction values at the end of the brining period.

Initial salt content of sea bass fillets was 0.01 g/100 g. Salt weight fraction changes are reported in Fig. 1C. In control samples, an increase of salt content was observed until the 5th day, reaching values of 0.03 and 0.07 that corresponded to 2.7 and 5.9% of net salt content for the 5 and 10% brining respectively. Hence, as expected, the salt uptake was driven by concentration gradients between the muscle and brine, similarly to previous studies (Nguyen et al., 2010). However, as observed for the weight and water uptake, on the last day of brining, the salt fraction decreased to values corresponding to 0.46 and 2.05% for the 5 and 10% brining respectively.

Following PEF pre-treatment, there was a general increase of the salt uptake in all samples at the end of the salting process. After two days, both 10 and 20A PEF (0.3 and 0.6 kV/cm) treated samples were significantly higher compared to their respective controls, while after 5 days, only the 10A sample and the 20A sample in the 5% brine. Salt concentration in PEF treated sea bass fillets increased slightly between the 5th and the 8th day, but, although samples treated at 10A (0.3 kV/cm) showed and increasing trend, differences were not statistically significant. The higher salt weight fractions reached corresponded to a salt content in the samples of 4.47 and 6.84 g/100 g for the 5 and 10% brining respectively, showing an increase of 77 and 35% compared to the highest salt content obtained in control samples at day five.

Applying PEF pretreatment allowed to reach a similar salt uptake after 2 days of brining, instead of 5 days in the control samples, thus reducing the time necessary for the process.

PEF has previously been shown to increase mass transfer in other animal and vegetable foods, such as ham, cured and salted meat, potato crisps, dried fruits etc. (Gómez et al., 2019). Electroporation is one of the several complex mechanisms attributed to this phenomenon. It was previously assumed that a greater number of pores in the muscle emerges with increasing the electric field intensity, which is why generally a mass transfer increases is obtained (Gómez et al., 2019). Electroporation has been shown to cause increased inter-myofibrillar spacing in fish and meat products (Gómez et al., 2019) which could aid mass transfer, thus increasing the salt uptake by the muscle. Therefore, we suggest that in the present study electroporation facilitated the salt uptake by the fish through increasing the extra-cellular

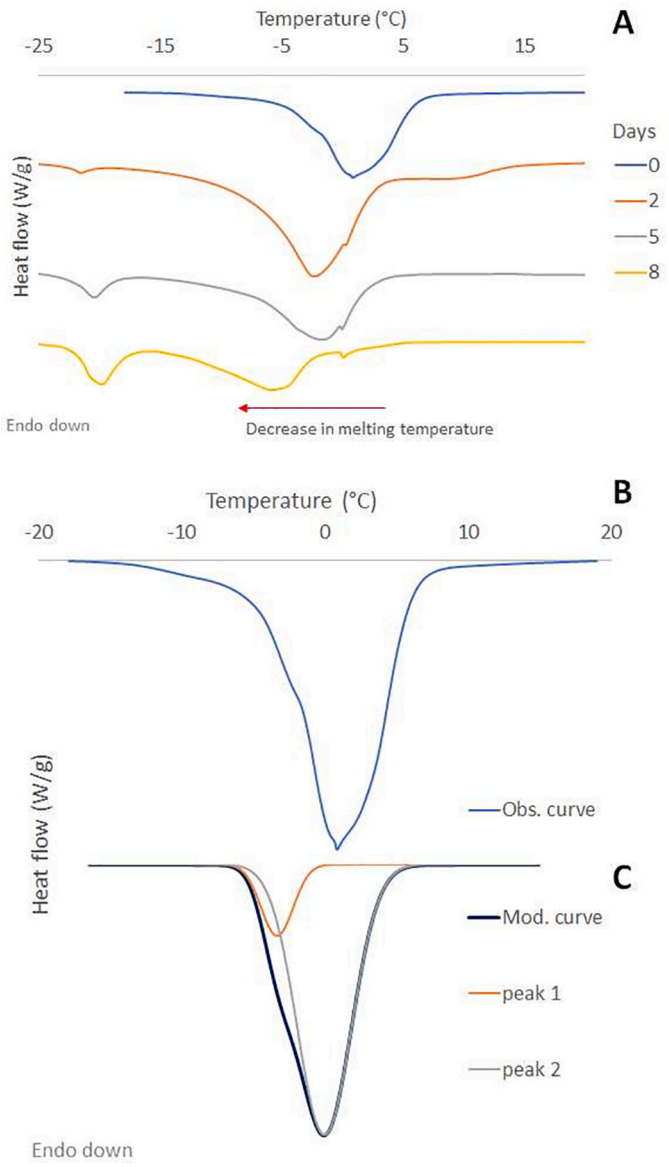


Fig. 3. Example of (A) the obtained thermograms for sample C10 at different brining times (0 to 8 days), (B) of a raw thermogram and (C) of a deconvoluted thermogram related to freezable water (FW^w).

spaces in the muscle serving as additional channels for diffusion of brine. Moreover, Klonowski et al. (2006) found a more porous structure in cod fillets pre-treated by PEF, that might have aided the diffusion of salt. Even though this effect was observed with the application of a higher electric field strength (2 kV/cm) compared to the ones applied in this present research (0.3–0.6 kV/cm), it is possible that a change on the flesh structure might have happened.

The increase of salt concentration in the tissue results, especially at the level of myofibrils, in greater water absorption and swelling under certain conditions (Krasnow, Loss, Ahrens, & Fiore III, 2013). This phenomenon is linked to the action of Cl^- chloride anions, which tend to associate with the positively charged groups of proteins. Positive charges are neutralized and therefore the repulsive force of negative charges increases. The intra-myofibrillary space expands due to the repulsive forces and a greater water retention capacity is determined. However, brines with a saline concentration above 10–15% can lead to an opposite effect, worsening the water retention capacity. In this case the salting-out phenomenon may occur: the ions in excess of Cl-, not being able to interact with the positive charges of the proteins already occupied by the other ions, interfere with them for the interaction with the water molecules, sequestering the solvation water and causing the loss of solubility and the precipitation of proteins (Aberoumand & Nejad, 2015; Kalra, Tugcu, Cramer, & Garde, 2001; Offer & Trinick, 1983). This phenomenon, however, was not observed in PEF treated samples by Klonowski et al. (2006), although the final salt concentration was higher.

We hypothesize that, contrarily to control samples, PEF treatment in the range of 0.3 and 0.6 kV/cm promoted a more homogeneous distribution of NaCl within inner and outer parts on the fish muscle due to formation of small pores in the muscle, facilitating the mass transfer and leading to enhanced diffusion of salt from the brine to the muscle.

3.2. Water state and distribution

The water activity (a_w) of untreated sea bass samples was 0.990 ± 0.002. As shown in Fig. 2, fish tissue brining resulted in a significant decrease of water activity, explained by the bonding of residual fluid from the fish muscle by salt through ionic interactions. These interactions reduce the amount of free water contained in the fish muscle, thus lowering water activity of the product (Lupín et al., 1981). Statistical analysis showed that only the NaCl concentration in the brine had a significant ($p < 0.05$) influence on water activity of sea bass samples during salting, leading to values in the range of 0.966 to 0.972 and 0.941 to 0.949, during the salting period for the 5 and 10% concentration respectively. Neither PEF intensity (0.3 and 0.6 kV/cm) nor duration of brine salting did affect water activity of the fish samples.

According to different authors (da Silva Carneiro et al., 2016; Mudalal, Petracci, Tappi, Rocculi, & Cavani, 2014), there are three different water populations in muscle tissues, the first one (below 5%) exists as true hydration water that is strictly bound to proteins by macromolecular of multimolecular adsorption, the second is water located inside organized protein structures (intra-myofibrillar), and the third one, which is the major one (>70%), is the extra-myofibrillar water, easily mobilizable. The first one is not free; it has an ice-like structure (liquid crystal), it is unfreezable, unaffected by charges on the muscle protein (pH), and it is unavailable to participate in reactions. From a calorimetric point of view, freezable water (FW) is usually associated to the second two fractions, representing the water affected during processing. FW assessment by DSC has been used to determine the gross phase changes of water in polymeric networks (Capitani, Mensitieri, Porro, Proietti, & Segre, 2003) and in food systems, such as meat (Mudalal et al., 2014; Petracci et al., 2012; Venturi et al., 2007).

Fig. 3A reports, as an example, the obtained thermograms of sample C10 at different brining times (zero to height days). As it is possible to observe, the FW peak was actually composed by two superimposed peaks, melting at slightly different temperatures. While in the fresh sample, this difference was small, with the first melting at around −3 °C and the second melting at around 0 °C being almost indistinguishable, as the brining time increased, the first peak appeared at lower temperatures, until reaching −6 °C after 8 days. In order to better understand the phenomena, the total melting enthalpy of FW were calculated and the relative amount of the two peaks were plotted, as shown in Fig. 3B (example of raw thermogram) and **3C** (example of deconvoluted thermogram) respectively.

Fig. 4 shows the total FW^w content, (**4A**), the fraction of peak 1 (**4B**) and the melting temperature of the first peak (**4C**). In the fresh sample, total FW^w content was 0.69 g/g water. In control samples immersed in the 5% NaCl brine, this value increased slightly after two days. However, the increase of salt concentration led to a decrease of the FW^w to the initial values. The first raise was probably due to a fast water uptake that increased the general mobility of the water. However, the simultaneous increase of salt concentration probably counterbalanced this effect. However, differences were not significant. In PEF treated samples, no differences were observed compared to initial value at all brining days.

For samples in the 10% NaCl brine, the total FW^w water content

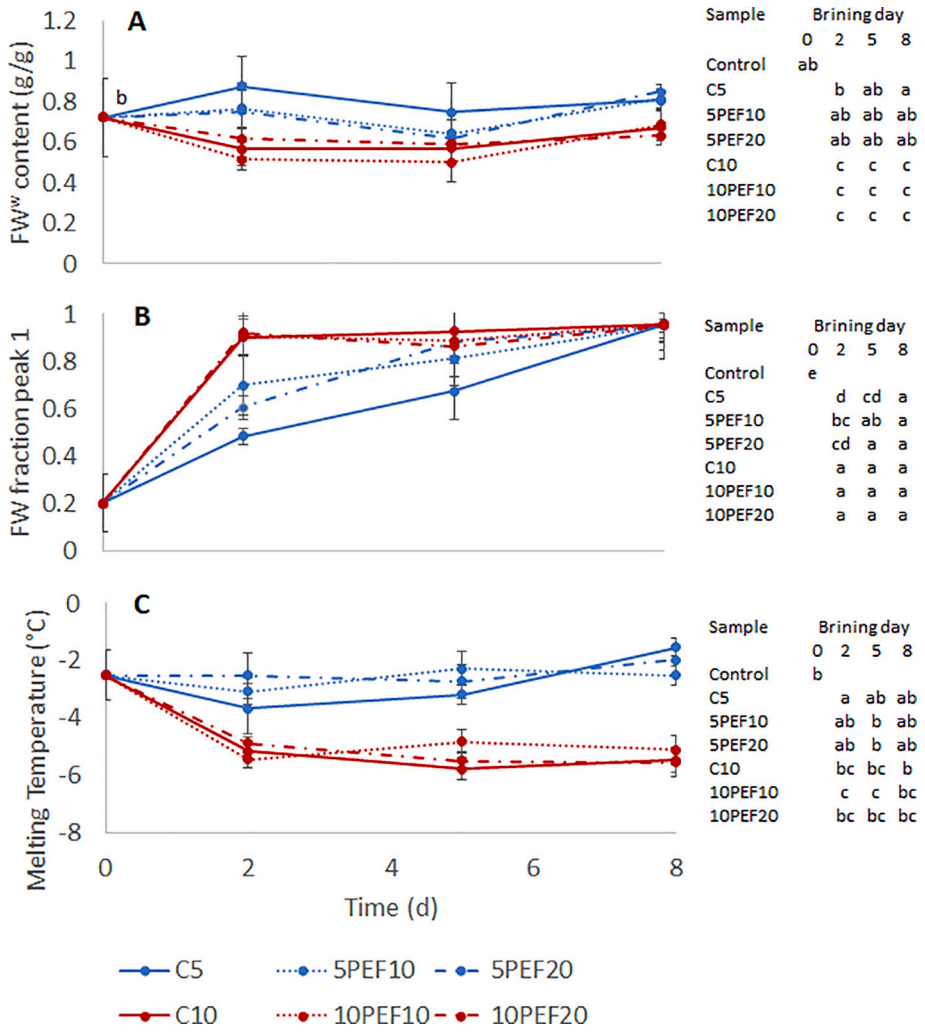


Fig. 4. DSC data of (A) freezable water (FW^w) content, (B) fraction of the first peak composing FW and (C) melting temperature of water of control and PEF treated sea bass samples during the brining process at 5% and 10% salt concentrations. Results are expressed as means ± standard deviations (error bars) of $n = 8$. Values with different letters in the auxiliary tables differ significantly ($p < 0.05$).

showed a slight decrease that was maintained during all brining time, but without significant differences among the samples. The water uptake, as shown in Fig. 1A was similar for the two salt concentrations (Fig. 1B**).** However, samples in the 10% solution showed, as expected, a higher salt diffusion during brining (Fig. 1C), this is the reason for the lowering of FW^w.

Hence, it is possible to observe that the total FW^w was fairly constant in all samples; however, if we take into account the two different peaks, it is possible to observed that, while initially the majority of the water was melting at 0 °C (about 80%), as brining proceeded, the fraction (peak 1) melting at lower temperature increased progressively. In samples in the 10% solution, the increase occurred after the first two days and then values remained similar (between 0.88 and 0.95), while for the 5% samples, the transition was more progressive. The decrease in FW^w and melting temperature depends on the balance between the water uptake and the salt concentration in the tissue. Although at the end of the eighth day values were similar for all samples, control samples (C5) showed higher values for peak 1 after two and five days, showing a slower decrease of the melting temperature transition. As shown by Fig. 1C, in PEF treated samples, salt concentration increased more compared to the control, corroborating the hypothesis of the observed differences.

Moreover, in Fig. 4C the melting temperature related to peak 1 was evaluated for all samples during brining. In the 5% samples the temperature did not change, while for the 10% samples a significant decrease was observed already after two days. Hence, DSC data were able to discriminate samples according to the concentration of salt in the brine showing a proportional reduction of freezable water and a decrease of the melting temperature due to the increasing salt content. However, few significant differences were observed among samples. This was not expected since a higher amount of salt found in PEF treated samples compared to control at different brining times for both 5% and 10% samples. Moreover, the effect of 'salting out' observed in the control samples, was not reflected in the FW measurements. This might be due to a different distribution of salt in the tissue as hypothesized earlier. Indeed, sampling procedure is pivotal for DSC analysis, since the small sample size (about 15 mg). Hence, although we took extra care in collecting representative samples, this could be one of the reasons for the observed unexpected behavior. However, considering that, to our knowledge, there are no reports of FW^w measure by DSC in fish samples during brining, so it is not possible to compare results giving a more exhaustive explanation of the obtained results.

Low-resolution NMR has been successfully used in many previous studies to investigate water mobility and distribution in fish and meat samples subjected to salting (da Silva Carneiro et al., 2016; Gudjónsdóttir, Arason, & Rustad, 2011; Aursand et al., 2008; Wu et al., 2006). As in previous studies, in the present research it was possible to reveal the presence of 3 water populations (displayed in Fig. 5),

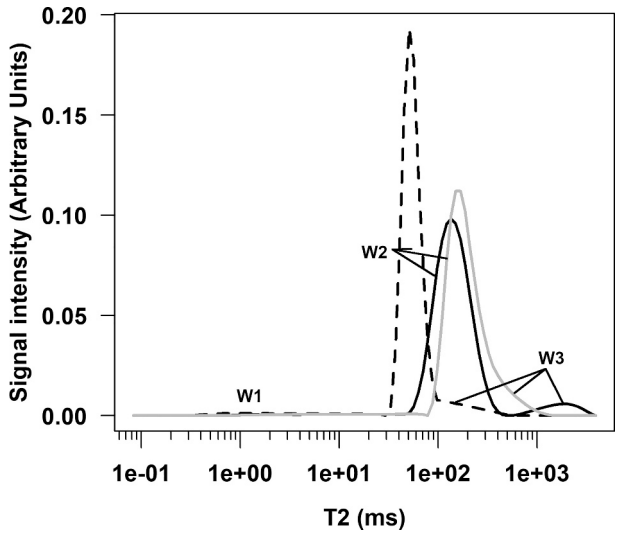


Fig. 5. Three typical transverse relaxation time relaxograms (T_2) obtained on a control sample at day 0 (dashed black line) and at day 8 (solid black line) and on sample salted in 10% brine and treated at 10 A (solid gray line). To allow for a direct comparison among them, the intensities are scaled so that the total area equals one arbitrary unit.

characterized by short, medium and long proton relaxation times. W_B ($T_2 = 1–3$ ms) relates to water bound by secondary bonds to the proteins, W_1 ($T_2 = 40–80$ ms) describes capillary water found in the myofibrillar network, while W_2 ($T_2 = 100–190$ ms) is mechanically immobilized water or extra-myofibrillar which can be further released as drip loss. Table 2 reports the relative intensities expressed as arbitrary units (AU) and the T_2 of the three water populations for all the analyzed seabass samples. According to Aursand et al. (2008) populations W_1 and W_2 represent more than 90% of the total water in the muscle.

In the present study, an evident migration of water from pools W_B and W_1 towards pool W_2, with longer relaxation times was observed from the untreated raw sample to all brined samples. This indicates a migration of water from the myofibrillar network towards extra-myofibrillar pools. Indeed, NaCl not only has a preservation effect, but it also acts as a structures-breaker, allowing the muscle fibers to expand and entrap water. This occurs due to electrostatic repulsion within the myofibrils, exposing protein sidechains to water binding (Strasburg, Xiong, & Chiang, 2007). Similar results were found in the study of Aursand et al. (2008) investigating water distribution and behavior in brine salted cod and salmon by low-field NMR technique. However, in the present research, apart from a few exceptions, no significant differences were observed among samples, neither according to NaCl concentration, nor according to the treatment. The only variable that showed consistently a significant effect on water distribution parameters was brining time ($p < 0.001$).

With regard to relaxation times (Table 2), Wu et al. (2006) found a decrease for the bound water (T_{2B}) and an increase related to T_{21} and T_{22} populations during salting of pork meat. In the present research T_{2B} showed a decrease but the difference was not significant. Instead, salting in 5% and 10% NaCl brine, led to a shift towards longer relaxation times for the other two water populations. T_{21} (intra-myofibrillar water) shifted from about 45 ms to 65–85 ms, while T_{22} (extra-myofibrillar water) from about 106 ms to 130–190 ms, directly reflecting the increased amount of water, which was also observed in other studies conducted on brine salting of fish (Aursand et al., 2008). However, also for this parameter, few significant differences were observed. Specifically, while in T_{22} a significant increase was found during brining time, no differences were observed among samples according to the PEF treatment (0.3 and 0.6 kV/cm). A significant effect was found only for brining time and for NaCl concentration for T_{21} and T_{22}.

3.3. Protein functionality

The pH values of sea bass samples after PEF-treatment and salting performed for 2, 5 and 8 days are shown in Table 3. Untreated sample showed an initial value of 6.7 that decreased progressively during brining, but the only significant differences was observed for C10 after 8 days (pH = 6.18). The results of PEF treated samples (0.3 and 0.6 kV/cm) have shown significantly lower pH values compared to control samples on day 2 and 5 of brining. This could be due to a release of ions from PEF-disrupted cells or structural changes of proteins allowing release of acidic groups (Zhao et al., 2019). Values, however, did not change during brining but apart from the initial decrease, remained stable. Nevertheless, result of multifactorial ANOVA showed that this parameters is influences significantly by all considered variables and their interaction.

WHC of sea bass samples (Table 3) showed very small variations remaining in the range of 97.7 to 98.99%. In some samples, a slight but not always significant increase of WHC appeared. This may have been due to the increased salt concentration as observed by Thorarinsdottir et al. (2004) and Aursand et al. (2008). However, no significant effect of PEF pre-treatment (0.3 and 0.6 kV/cm) or of salt concentration on WHC during salting period was observed in the present study. The only variable affecting WHC was indeed brining time and its interaction with other variables.

The solubility of sarcoplasmic and myofibrillar proteins in sea bass samples during brine salting is reported in Fig. 6 **A** and **B**.

Solubility of water soluble (sarcoplasmic) protein was strongly and significantly reduced during brining in all samples. In seabass brined in the 10% NaCl solution, PEF treated samples showed always significantly lower values compared to the control, but with no differences according to the intensity of the electric field applied, 0.3 or 0.6 kV/cm. For samples in the 5% brine solution, differences were not always significant.

Solubility of salt-soluble (myofibrillar) proteins showed a very different behavior. In control samples, it did not change compared to the initial untreated sample for all brining times. Instead, PEF treated samples reported a remarkable decrease already after 2 days for both 0.3 and 0.6 kV/cm treated samples. However, there were no differences in the values found between salt concentration and during brining.

3.4. Correlation results

In order to get a better understanding on the observed phenomena and of their relation, correlations among the parameters of mass transfer, water mobility and distribution, and protein functionality measured in the sea bass samples were evaluated through the Pearson's correlation. Results are shown in Table 4.

ΔM^{o}_{t} is positively correlated to both ΔM^{w}_{t} and ΔM^{NaCl}_{t}, as they showed similar behavior during brining, but it was also negatively correlated to W_B and to the solubility of both water- and salt-soluble proteins. No significant correlation was observed with any of the other parameters, that, as observed before, did not reflect the effect of salting out.

Water activity and total FW were positively correlated (0.64), however, the evolution of peak 1 of FW (water fraction freezing at a lower temperature) was actually correlated to all the other water state and mobility parameters, measured by LF-NMR and solubility of water-soluble proteins.

Specifically, the solubility of myofibrillar proteins positively correlated with W_B-water pool expressing water bound by secondary bonds to the proteins in PEF-treated samples, while the solubility of sarcoplasmic proteins negatively correlated with W_2-water pool representing mechanically immobilized water. This suggests that the water pool W_B diffused to the extra-myofibrillar spaces of the fish muscle (W_2-water pool) as a result of the PEF-induced increased solvation. Supported by previous investigations (Nguyen et al., 2010), this could be caused by

Table 2
Proton population intensity (AU) and relaxation times T_2 (ms) of the three water populations of the sea bass samples and Fisher (F) values obtained by multifactorial ANOVA.

		Day 2	Day 5	Day 8
Intensity (AU)				
W_B				
Raw	2.64 ± 0.24 ^{ab}			
C-5		2.05 ± 0.21 ^{a}	1.56 ± 0.27 ^{abc}	1.47 ± 0.42 ^{abc}
5-PEF-10		1.90 ± 0.36 ^{ab}	1.78 ± 0.78 ^{abc}	1.47 ± 0.32 ^{bc}
5-PEF-20		2.10 ± 0.33 ^{a}	1.60 ± 0.54 ^{abc}	1.40 ± 0.37 ^{bc}
C10		1.84 ± 0.21 ^{ab}	1.37 ± 0.58 ^{bc}	1.33 ± 0.35 ^{bc}
10-PEF-10		1.94 ± 0.44 ^{ab}	1.40 ± 0.27 ^{bc}	1.14 ± 0.17 ^{c}
10-PEF-20		1.87 ± 0.38 ^{ab}	1.47 ± 0.50 ^{abc}	1.12 ± 0.26 ^{c}
W_1				
Raw	78.78 ± 4.39 ^{a}			
C-5		24.27 ± 15.38 ^{bc}	24.90 ± 28.16 ^{bc}	11.95 ± 4.86 ^{c}
5-PEF-10		16.42 ± 6.44 ^{bc}	28.69 ± 22.56 ^{bc}	20.25 ± 12.07 ^{bc}
5-PEF-20		31.83 ± 14.58 ^{bc}	21.41 ± 13.27 ^{bc}	30.99 ± 21.29 ^{bc}
C10		38.52 ± 17.99 ^{b}	22.56 ± 13.57 ^{bc}	24.86 ± 12.00 ^{bc}
10-PEF-10		28.01 ± 11.99 ^{bc}	28.89 ± 15.18 ^{bc}	28.76 ± 14.42 ^{bc}
10-PEF-20		27.59 ± 8.67 ^{bc}	27.02 ± 12.07 ^{bc}	22.23 ± 14.18 ^{bc}
W_2				
Raw	18.57 ± 4.52 ^{c}			
C-5		73.68 ± 15.45 ^{ab}	73.54 ± 28.10 ^{ab}	86.57 ± 4.89 ^{a}
5-PEF-10		81.68 ± 6.45 ^{ab}	69.54 ± 23.03 ^{ab}	78.28 ± 12.08 ^{ab}
5-PEF-20		66.06 ± 14.62 ^{ab}	76.99 ± 13.54 ^{ab}	67.60 ± 21.23 ^{ab}
C10		59.64 ± 17.96 ^{b}	76.07 ± 13.46 ^{ab}	73.81 ± 12.12 ^{ab}
10-PEF-10		70.05 ± 12.28 ^{ab}	69.71 ± 15.36 ^{ab}	70.10 ± 14.40 ^{ab}
10-PEF-20		70.54 ± 8.71 ^{ab}	71.50 ± 11.90 ^{ab}	76.65 ± 14.13 ^{ab}
Relaxation time (T_2) (ms)				
T_{2B}				
Raw	2.55 ± 0.62			
C-5		1.67 ± 0.57	1.94 ± 0.80	1.70 ± 0.63
5-PEF-10		1.84 ± 0.45	1.74 ± 1.24	2.34 ± 1.16
5-PEF-20		1.73 ± 0.53	1.69 ± 0.71	2.19 ± 1.06
C10		1.85 ± 0.84	1.72 ± 0.75	1.82 ± 0.76
10-PEF-10		1.68 ± 0.36	1.8 ± 0.48	2.11 ± 0.87
10-PEF-20		1.97 ± 0.79	2.01 ± 0.98	2.86 ± 1.36
T_{21}				
Raw	44.96 ± 2.35 ^{c}			
C-5		66.56 ± 8.50 ^{ab}	70.43 ± 12.27 ^{ab}	73.25 ± 12.27 ^{ab}
5-PEF-10		64.76 ± 8.05 ^{b}	71.10 ± 15.12 ^{ab}	72.14 ± 13.82 ^{ab}
5-PEF-20		64.21 ± 4.95 ^{b}	74.62 ± 12.64 ^{ab}	86.51 ± 22.83 ^{ab}
C10		65.92 ± 6.31 ^{ab}	73.75 ± 8.99 ^{ab}	81.25 ± 11.33 ^{ab}
10-PEF-10		65.91 ± 9.47 ^{ab}	79.44 ± 13.20 ^{ab}	81.56 ± 15.05 ^{ab}
10-PEF-20		65.09 ± 8.23 ^{b}	77.45 ± 11.84 ^{ab}	83.92 ± 16.33 ^{a}
T_{22}				
Raw	106.24 ± 24.68 ^{f}			
C-5		132.65 ± 10.26 ^{ef}	170.27 ± 24.53 ^{abc}	168.16 ± 13.97 ^{abc}
5-PEF-10		134.75 ± 10.11 ^{de}	164.32 ± 13.16 ^{abcd}	155.51 ± 15.98 ^{bcde}
5-PEF-20		135.26 ± 13.58 ^{de}	162.82 ± 15.04 ^{abcd}	179.68 ± 20.53 ^{ab}
C10		138.64 ± 20.91 ^{cde}	188.77 ± 15.81 ^{a}	187.88 ± 21.78 ^{a}
10-PEF-10		139.81 ± 19.18 ^{cde}	168.14 ± 16.25 ^{abc}	190.14 ± 21.82 ^{a}
10-PEF-20		128.50 ± 13.33 ^{ef}	163.65 ± 24.99 ^{abcd}	190.61 ± 25.08 ^{a}

	F value					
	W_1	W_2	W_3	T_{2B}	T_{21}	T_{22}
PEF	0.01 ns	0.39 ns	0.39 ns	1.65 ns	0.91 ns	0.90 ns
Salt	11.23***	3.58 ns	3.20 ns	0.68 ns	5.01*	8.50**
Time	170.77***	282.11***	290.82***	13.70***	147.21***	169.08***
PEF*Salt	0.04 ns	2.29 ns	2.27 ns	1.39 ns	0.57 ns	1.53 ns
PEF*Time	0.50 ns	1.80 ns	1.78 ns	1.16 ns	0.79 ns	1.49 ns
Salt*Time	1.60 ns	0.96 ns	0.93 ns	0.16 ns	1.51 ns	3.58*
PEF*Salt*Time	0.61 ns	1.69 ns	1.70 ns	0.46 ns	0.25 ns	0.60 ns

Different letters indicate significant differences at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p \leq 0.001$; ns: not significant.

Table 3
pH and Water Holding Capacity (WHC) (%) of sea bass samples and Fisher (F) values obtained by multifactorial ANOVA.

Sample		day 2	day 5	day 8
pH				
Fresh	6.7 ± 0.02 ^{a}			
C-5		6.70 ± 0.03 ^{a}	6.67 ± 0.02 ^{ab}	6.51 ± 0.01 ^{ab}
5-PEF-10		6.35 ± 0.02 ^{b}	6.33 ± 0.01 ^{b}	6.33 ± 0.02 ^{b}
5-PEF-20		6.32 ± 0.01 ^{b}	6.34 ± 0.01 ^{b}	6.46 ± 0.01 ^{ab}
C10		6.58 ± 0.05 ^{ab}	6.52 ± 0.01 ^{ab}	6.18 ± 0.02 ^{b}
10-PEF-10		6.36 ± 0.01 ^{b}	6.38 ± 0.01 ^{b}	6.32 ± 0.01 ^{b}
10-PEF-20		6.33 ± 0.01 ^{b}	6.43 ± 0.01 ^{ab}	6.23 ± 0.02 ^{b}
WHC				
Fresh	98.07 ± 0.47 ^{abcd}			
C-5		98.95 ± 0.37 ^{ab}	98.57 ± 0.16 ^{abc}	97.73 ± 0.31 ^{cd}
5-PEF-10		98.75 ± 0.45 ^{abc}	97.26 ± 0.76 ^{d}	98.17 ± 0.42 ^{abcd}
5-PEF-20		97.87 ± 0.16 ^{bcd}	98.58 ± 0.19 ^{abc}	98.43 ± 0.07 ^{abc}
C10		98.61 ± 0.27 ^{abc}	98.42 ± 0.22 ^{abc}	98.28 ± 0.51 ^{abcd}
10-PEF-10		98.94 ± 0.74 ^{ab}	97.95 ± 0.49 ^{abcd}	97.94 ± 0.59 ^{abcd}
10-PEF-20		98.99 ± 0.26 ^{a}	97.75 ± 0.33 ^{cd}	97.76 ± 0.45 ^{cd}

	F value	
	pH	WHC
PEF	265.80***	1.44 ns
Salt	107.25***	3.34 ns
Time	770.20***	13.3***
PEF*Salt	75.71***	0.56 ns
PEF*Time	85.19***	3.0*
Salt*Time	68.38***	2.9*
PEF*Salt*Time	19.69***	5.18***

Different letters indicate significant differences at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p \leq 0.001$; ns: not significant.

the reduced hydration due to the increased solvation capacity of salt ions that reduced the hydrodynamic radius of proteins, increasing substantially protein-protein interactions compared to protein-water interactions. The weaker associations between the water molecules bound to proteins resulted in their increased mobility and penetration into extra-myofibrillar spaces of the muscle. At the same time, polar and hydrophobic interactions between proteins became stronger, contributing to their increased hydrophobicity and aggregation (Lin & Park,

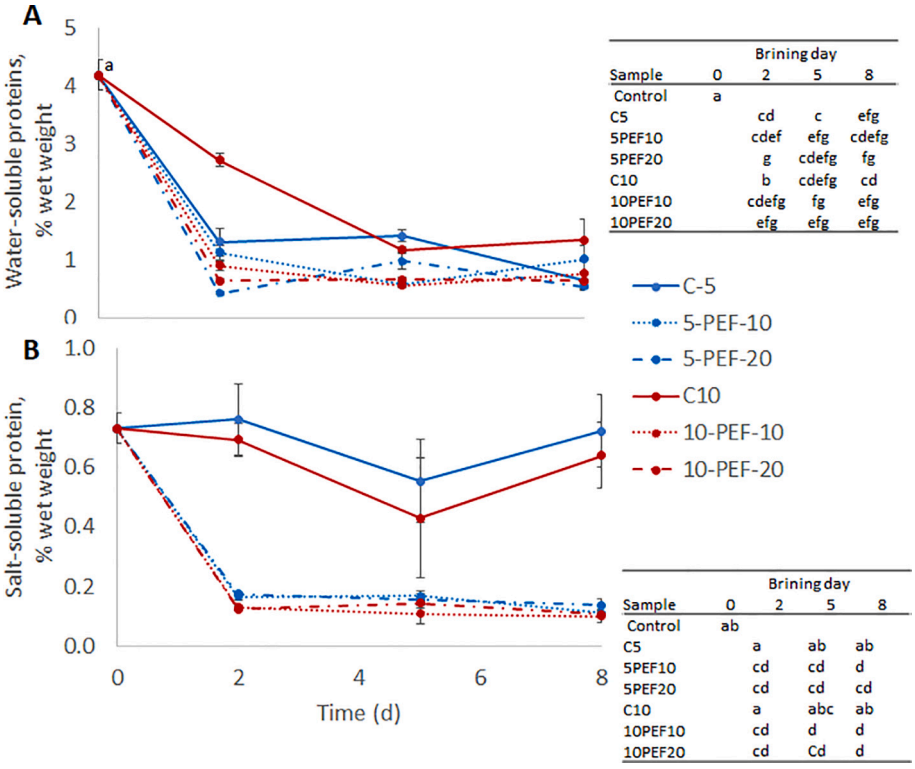


Fig. 6. Content of (A) water- and (B) salt-soluble proteins (% net weight) of control and PEF treated sea bass samples during the brining process at 5% and 10% salt concentrations. Results are expressed as means ± standard deviations (error bars) of $n = 3$. Values with different letters in the auxiliary tables differ significantly ($p < 0.05$).

Table 4
Pearson's correlations among parameters of mass transfer, water state and mobility and protein functionality.

	ΔM	ΔM^W	ΔM^{NaCl}	a_w	FW tot	FW-peak 1	T_{2B}	T_{21}	T_{22}	W_B	W_1	W_2	pH	water soluble proteins	salt soluble proteins	WHC
ΔM	-															
ΔM^W	0.525	-														
ΔM^{NaCl}	0.721	0.420	-													
a_w	-0.181	-0.375	-0.663	-												
FW tot	-0.178	-0.335	-0.569	0.648	-											
FW-peak 1	0.364	0.425	0.541	-0.696	-0.339	-										
T_{2B}	0.098	-0.133	0.249	0.044	0.134	-0.096	-									
T_{21}	0.432	0.666	0.485	-0.472	-0.074	0.781	0.052	-								
T_{22}	0.353	0.711	0.396	-0.390	-0.123	0.692	0.082	0.898	-							
W_B	-0.467	-0.474	-0.509	0.369	0.041	-0.741	-0.140	-0.884	-0.877	-						
W_1	-0.398	-0.395	-0.264	0.328	-0.070	-0.664	0.376	-0.658	-0.543	0.641	-					
W_2	0.404	0.401	0.273	-0.332	0.067	0.672	-0.366	0.670	0.557	-0.657	-0.999	-				
pH	-0.257	-0.307	-0.467	0.439	0.307	-0.610	-0.084	-0.513	-0.402	0.596	0.323	-0.334	-			
water soluble proteins	-0.486	-0.402	-0.443	0.302	0.014	-0.636	0.266	-0.691	-0.516	0.723	0.847	-0.852	0.597	-		
salt soluble proteins	-0.705	-0.295	-0.741	0.239	0.288	-0.442	-0.162	-0.396	-0.224	0.472	0.274	-0.282	0.682	0.641	-	
WHC	0.014	-0.010	-0.106	-0.166	0.037	-0.082	-0.266	-0.255	-0.376	0.476	0.105	-0.116	0.275	0.177	0.160	-

Values in red are significant at $p < 0.05$.

1998; Stefansson & Hultin, 1994).

4. Conclusions

The results of this study have shown that PEF treatment at 0.3–0.6 kV/cm allowed to significantly increase the salt uptake during sea bass brining, that may be due to a more homogeneous distribution of salt in the fish muscle. The study of water state and distribution however did not show many differences among samples that were generally discriminated according to the concentration of salt in the brining solution but not to the PEF treatment applied. On the other side, a remarkable reduction of myofibrillar protein solubility was observed, as a consequence of the application of the electric field.

To sum up, the obtained results suggest that PEF pre-treatment allowed to obtain a significant reduction of the duration of salt brining (more than 50%) or an increase of salt uptake (up to 77%) compared to conventional brining process. However, aspects related to the effect on protein structure and functionality should be further clarified, and different parameters of this innovative processing deeply investigated.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Paper VI

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Application of PEF- and OD-assisted drying for kiwifruit waste valorization

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Abstract
The production of dried snacks with high nutritional functionality and sustainability represents a valid alternative to use the kiwifruit waste as undersize fruits, with a positive economic impact on the entire production chain. Therefore, this work aimed to evaluate the effect of pulsed electric field - PEF (200 V/cm) and/or osmotic dehydration – OD pre-treatments on drying kinetics (50, 60, 70°C), texture, colour and sensorial properties of yellow kiwifruit snacks. The drying kinetics were significantly influenced both by applied treatment and drying temperature. The firmness of the kiwifruit snacks was improved by the combination of PEF/OD pre-treatments. In general, drying temperature of 70°C and the use of combined pre-treatments seem to be a good compromise to reduce drying time and obtain products with high quality in terms of colour, firmness and overall acceptability.

Keywords: Sustainability, kiwifruit waste, emerging processing, fruit snack

Introduction

Food waste affects food systems' sustainability and economic development since it has an important footprint on food quality and safety, natural resources, and environmental protection. Therefore, the management of food waste including food waste valorisation is an important issue and challenge for the food industries (Otles & Kartal, 2018). Concerning kiwifruit, agriculture and industrial food processing generate a large amount of waste and kiwifruit by-products, including culled fruit, pomace, peels, leaves, seeds and pruning residues (Sanz et al., 2021). Moreover, kiwifruits with a weight lower than 65 g are considered waste and poorly paid as they are used in the production of fruit juices and/or in the energy supply chain (The Publications Office of the European Union (EC) No 1673/2004, 2004). Nevertheless, they are rich in vitamin C and other bioactive compounds, which contributes to their high antioxidant activity (Lintas et al., 1991; Mannozzi et al., 2020), helping to fight against heart, vascular and central nervous system diseases, cancer and diabetes (Tyagi et al., 2015).
A recent study indicates that consumers prefer food with high nutritional properties and, at the same time, with elevated convenience and shelf-stability (Ramírez-Jiménez et al., 2018). In alternative to the high-calory snacks available on the market, dried fruits are considered a healthier substitute and are included in the dietary guidelines of many countries (Morais et al., 2018). Fruit snack products are highly appreciated when prepared by optimizing the used resources, minimally affecting or even enhancing their nutritional and beneficial characteristics and providing unique sensorial properties (Ciurzyńska et al., 2019; Jeszka-Skowron et al., 2017; Mannozzi et al., 2020; Villalobos et al., 2018).
Fruit snacks are usually prepared by drying the fruit slices, and one of the most available and employed commercial drying methods is hot air drying. Hot air drying (HAD) consists of the transfer of heat from the hot air to the product by convection, similarly, the evaporated water is transported to the air also by convection (Antal, 2015; Lewicki, 1998). However, the drying processes consume an appreciable part of the total energy used in the food industry, and it is very important to develop new hybrid drying technologies for energy saving and food quality preservation (Chou & Chua, 2001). Some pre-treatments could be used before the drying process such as osmotic dehydration (OD) and pulsed electric field (PEF) among others to accelerate the drying time and create attractive snack products (Mannozzi et al., 2020; Tylewicz et al., 2020; Witrowa-Rajchert et al., 2014). OD causes partial dewatering of the product at room temperature, due to the concentration gradient between the product and osmotic hypertonic solution, giving, therefore,

the possibility to reduce the drying time (Bialik et al., 2020; Dermesonlouoglou, Chalkia, Dimopoulos, et al., 2018) and to preserve the quality of the final product by making them more appreciable to the consumers, especially when a sour or underripe raw material is used (Nowacka et al., 2018; Panarese, Tylewicz, et al., 2012). Concerning the PEF application, those with high and moderate electric field strengths have been proposed for the enhancement of the drying process, allowing to decrease processing time, temperature, and energy consumption (Lammerskitten et al., 2020; Lebovka et al., 2007). The application of PEF pre-treatment at 10 kV/cm and 50 pulses provoked a decrease of drying time of up to 12 % on apples (Wiktor et al., 2013). Moreover, when these two mentioned treatments are combined further beneficial effects, in terms of drying time reduction, better preservation of the colour and bioactive compounds, were observed in carrots (Amami et al., 2008), apple (Amami et al., 2005), red bell pepper (Ade-Omowaye et al., 2003), kiwifruit (Mannozzi et al., 2020), goji berry (Dermesonlouoglou, Chalkia, & Taoukis, 2018) and cranberries (Nowacka et al., 2019).
Therefore, this work aimed to evaluate the effect of PEF and/or OD pre-treatments, as well as their application sequence, on drying kinetics (50, 60, 70°C) and on physicochemical parameters (firmness and colour) of yellow kiwifruit snacks.

2. Materials and Methods

2.1. Raw material handling

Yellow kiwifruits *Actinidia chinensis* (cv. Jintao) with size below 65 g were provided by Jingold Consortium (Cesena, Italy). The fruits were washed, hand peeled and cut into slices of 3±1 mm and subjected to the PEF treatment alone or in combination with OD before the subsequent drying process.

2.2. Pulsed electric field (PEF) treatment

Seven kiwifruit slices, of each sample, were placed into a rectangular treatment chamber (5 x 5 x 5 cm) and subjected to PEF treatment applying 1000 rectangular pulses with an electric field strength of 200 V/cm and a fixed pulse width of 10 µs. Tap water with a conductivity of 421 µS/cm, determined by EC-Meter basic 30+ conductivity meter (Crison Instruments, s.a., Barcelona, Spain), was used as a conductivity medium inside the treatment chamber. The PEF treatments were applied using a pulse generator S-P7500 60A 8kV (Alintel SRL., Bologna). The total energy input was 1.92 kJ/kg.

2.3. Osmotic dehydration (OD) treatment

The OD treatment was carried out by immersing the kiwifruit in 40% (w/w) trehalose (EXACTA + OPTECH Labcenter S.p.A., Italy) solution for 150 min at 35°C, with the product: solution ratio of 1:4, as reported by Mannozzi et al., (2020).

2.4. Hot air drying

Untreated and differently pre-treated kiwifruit slices were subjected to hot air drying by using a hot air cabinet dryer (POL-EKO-APRATURA SP.J., PL). Three different drying temperatures were used 50, 60 and 70°C. The air velocity was 2 m/s, and an air renewal fee of 50% was used.
For each combination of treatments, the amount of 21 kiwifruit slices was used. All obtained samples with related abbreviations are shown in table 1.

Table 1. Samples abbreviations and description of the pre-treatments applied for kiwifruit slices at each drying temperature (50, 60 and 70 °C)

Sample code	Description
C	Non-treated samples (control)
OD	OD treated samples
PEF	PEF treated samples
OD/PEF	OD treated samples followed by PEF treatment
PEF/OD	PEF treated samples followed by OD treatment

2.4. Analytical determinations

2.4.1. Moisture content

Moisture content was determined gravimetrically by drying the samples at 70 °C until a constant weight was achieved (AOAC, 1996).
The analyses were carried out in five repetitions from each sample at each drying temperature.

2.4.2. Modelling of drying kinetics

Three different mathematical models were applied to drying kinetics to evaluate the effect of different pre-treatments on the velocity of the drying process (Table 2).

Table 2: Selected mathematical models used to fit the drying kinetics

Model Name	Model equation	Reference
Newton (Lewis)	$MR = e^{(-k.t)}$	(Sarimeseli, 2011)
Page	$MR = e^{(-k.t^n)}$	(Sarimeseli, 2011)
Weibull	$MR = e^{-(\frac{t}{\alpha})^{\beta}}$	(Corzo et al., 2008)

Drying curves were plotted as a function of dimensionless moisture ratio (MR) during drying. The moisture ratio (MR, dimensionless) was calculated as the gradient of the sample moisture content at any time of drying (M_t, kg water/kg dry matter) to both initial moisture content (M_p, kg water/kg dry matter) and equilibrium moisture content (M_e, kg water/kg dry matter), according to the equation 1.

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad \text{(eq. 1)}$$

Regression analysis was performed using the Curve Fitting app from Matlab. In order to explain the goodness of fit of each model, the correlation coefficient (R^2), root mean square error (RMSE) and sum squared errors (SSE) were calculated. The higher R^2 values (near 1), the lower RMSE and SSE indicate that the model fits better to experimental data.

2.4.3. Texture

The texture analysis was performed using a Texture Analyser mod. TA-HDi500 (Stable Micro Systems, Surrey, Godalming, UK), equipped with a 5 N load cell. A stainless-steel sharp blade was used for the cutting test. Force vs distance curves were obtained using a test speed of 1.0 mm/s and the results are expressed in firmness or hardness (N).
The analyses were carried out in ten repetitions from each sample at each drying temperature.

2.4.4. Colour

The colour parameters were investigated using the CIE $L^*a^*b^*$ scale in a spectro-photocolorimeter mod. Colorflex (Hunterlab, USA) using the D65 illuminant and the 10° standard observer. The instrument was calibrated with a black and white tile (L^* 93.47, a^* 0.83, b^* 1.33) before the measurements. Results were expressed as total colour difference (ΔE)

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

where:
ΔL*, Δa*, Δb* are the differences of mean L*, a* and b* parameters, respectively, between non-treated and treated kiwifruit samples (Radojčin et al., 2015).

The analyses were carried out in ten repetitions from each sample at each drying temperature.

2.4.5. Sensory analysis

Untreated and differently pre-treated samples were subjected to sensory evaluation by a descriptive quantitative analysis (QDA) with a panel test of 12 trained panellists.
A sensory evaluation was done using the hedonic sensory scale (where 9 – like extremely and 1 – dislike extremely). The acceptability threshold value was set to 5 on the scale, according to the preliminary training. The attributes included integrity of the samples, colour, odour, taste intensity, sweetness, acidity, hardness and overall acceptability.

2.4.6. Statistical analysis

The data related to firmness were evaluated and discerned by using an analysis of variance (ANOVA) followed by Tukey's HSD post hoc test to compare the means at the level of confidence of 95% ($p < 0.05$). The analysis was performed using the software STATISTICA 6.0 (Statsoft Inc., Tulsa, UK).

3. Results and discussion

3.1. Modelling of drying kinetics

As expected, the different pre-treatments and drying temperatures affected the drying kinetics. The moisture ratio (MR) after 60 min of drying is presented in table 3. For the samples dried at 50ºC, it can be seen that the control sample has the highest MR, on the other hand, the lowest MR corresponds to the sample treated with PEF followed by OD. For the samples dried at 60ºC, the behaviour seems to be more or less the same, showing the highest values for the control sample, and the lowest for the PEF and PEF/OD samples. Finally, at 70ºC the trend is likely the same, and the lowest MR correspond to the PEF and PEF/OD while the highest to the control sample.

Table 3. Moisture rate of samples dried at different temperatures (after 60 min)

Sample	50ºC	60ºC	70ºC
C	0.4611	0.3262	0.0483

OD	0.1330	0.1824	0.0274
PEF	0.3230	0.1216	0.0210
OD/PEF	0.2698	0.2397	0.0241
PEF/OD	0.0942	0.1255	0.0249

Mathematical modelling is important regarding the scale-shift of the process, from the laboratory to the industrial scale. The model that best fits the experimental data can be used to predict the processing time sufficient to dry the product to particular water content (Wiktor et al., 2013). In table 4 and 5 the regression results of the three models evaluated are presented. All mathematical models presented a good fit of the experimental data; R^2 values were between 0.785 and 0.981; RMSE and SSE were in the range of 0.011-0.101 and 0.002-0.237 respectively.

Table 4. Statistical analysis of Newton, Page and Weibull model

Sample	Temp.	Newton			Page			Weibull		
		RMSE	SSE	R^2	RMSE	SSE	R^2	RMSE	SSE	R^2
C	50ºC	0.066	0.106	0.932	0.068	0.106	0.932	0.065	0.131	0.894
OD	50ºC	0.064	0.132	0.894	0.065	0.131	0.894	0.068	0.106	0.932
PEF	50ºC	0.101	0.237	0.785	0.071	0.111	0.899	0.026	0.016	0.983
OD/PEF	50ºC	0.032	0.020	0.984	0.032	0.019	0.985	0.071	0.111	0.899
PEF/OD	50ºC	0.037	0.027	0.972	0.031	0.019	0.981	0.031	0.019	0.981
C	60ºC	0.024	0.011	0.991	0.024	0.011	0.992	0.031	0.015	0.984
OD	60ºC	0.050	0.043	0.953	0.028	0.012	0.987	0.028	0.016	0.986
PEF	60ºC	0.020	0.009	0.992	0.011	0.003	0.997	0.025	0.008	0.990
OD/PEF	60ºC	0.064	0.103	0.897	0.019	0.009	0.991	0.032	0.019	0.985
PEF/OD	60ºC	0.057	0.078	0.915	0.026	0.016	0.983	0.030	0.018	0.987
C	70ºC	0.045	0.043	0.962	0.028	0.016	0.986	0.019	0.009	0.991
OD	70ºC	0.011	0.002	0.998	0.011	0.002	0.998	0.018	0.005	0.995
PEF	70ºC	0.020	0.006	0.994	0.012	0.002	0.998	0.011	0.002	0.998
OD/PEF	70ºC	0.032	0.018	0.983	0.018	0.005	0.995	0.024	0.011	0.992
PEF/OD	70ºC	0.044	0.028	0.967	0.025	0.008	0.990	0.012	0.002	0.998

Table 5: Constant values for each model

Sample	Temp.	Newton	Page		Weibull	
		k	k	n	α	β
C	50ºC	0.982	0.983	0.998	1.018	0.998
OD	50ºC	1.977	1.934	0.943	0.497	0.943
PEF	50ºC	1.314	1.399	0.450	0.474	0.450
OD/PEF	50ºC	1.323	1.322	0.925	0.740	0.925
PEF/OD	50ºC	2.486	2.121	0.637	0.307	0.637
C	60ºC	1.230	1.225	0.953	0.808	0.953
OD	60ºC	2.154	1.750	0.505	0.330	0.505
PEF	60ºC	1.897	1.740	0.755	0.480	0.755
OD/PEF	60ºC	1.596	1.440	0.475	0.464	0.475
PEF/OD	60ºC	2.197	1.724	0.379	0.238	0.379
C	70ºC	1.993	3.291	2.017	0.554	2.017
OD	70ºC	3.217	3.438	1.109	0.329	1.110

PEF	70ºC	2.793	3.971	1.593	0.421	1.594
OD/PEF	70ºC	2.401	3.824	1.841	0.483	1.841
PEF/OD	70ºC	3.510	2.406	0.261	0.035	0.261

Each model provides different information about the drying process. The simplest model is the Newton model that only consider a kinetic constant (k). The higher the drying velocity, the higher is the constant k. As far as the Page model is concerned, it considers the kinetic constant (k) and introduces an empirical exponent (n) to overcome the shortcomings of the Newton model (also known as the exponential model) (Simal et al., 2005). Finally, the Weibull model considers the scale parameter (α) and the shape parameter (β). The scale parameter is the kinetic constant of the model and represents the time needed to accomplish approximately 63% of the drying. The reciprocal of α could be compared to the effective diffusion coefficient of the diffusion model since those two parameters are the kinetic constants for each model (García-Pascual et al., 2006). On the other hand, the shape parameter is related to the velocity of the mass transfer at the beginning of the drying (the lower is β, the faster is the drying rate at the beginning).

Although the models could fit the relationship between average moisture content and drying time, they do not take into account the fundamentals of the drying process and their parameters have no physical meaning (Simal et al., 2005). Therefore, they cannot give a clear and accurate overview of the important processes and phenomena occurring during drying. Despite this considerations, the knowledge of the drying kinetics and subsequently the selection of an appropriate drying model can be used to understand and predict drying times and thus optimize the drying process for greater efficiency (Olanipekun et al., 2015).

In general, in Table 5 it can be seen that the lowest kinetic constant corresponds to the control sample, while when a pre-treatment like OD or PEF is applied, the drying process is accelerated. For almost all the models, at every temperature, the PEF/OD sample was the one that has the highest drying rate. In order to evaluate the relationship between the kinetic parameter and air-drying temperature, the kinetic constants were plotted against temperature and a linear regression was calculated (Figure 1; only the best two models are shown).

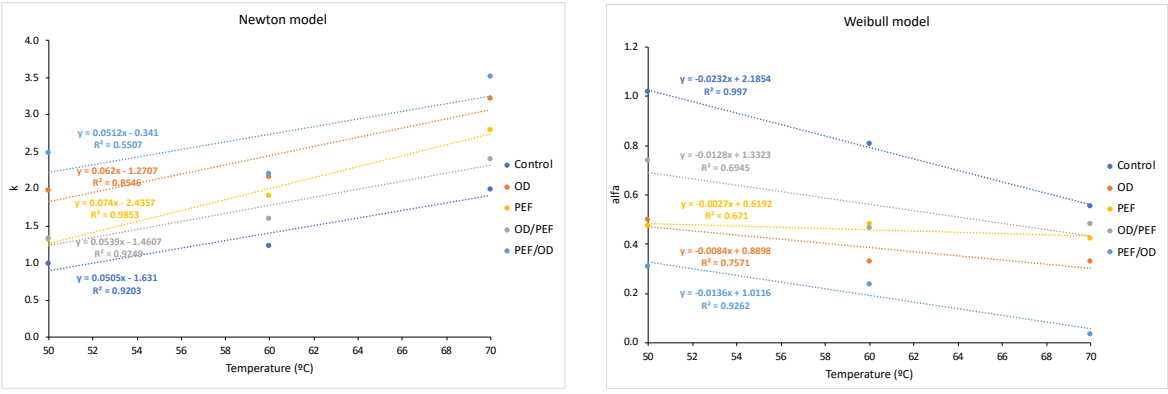

Figure 1. Kinetic parameter of Newton and Weibull models and air-drying temperature relationship.

As expected, the kinetic parameter has a linear correlation with temperature. These linear regressions could be used to predict the drying rate at other temperatures between 50-70ºC. Taking into account the correlation coefficient, the Weibull model explained better the relationship between temperature and drying rate for the control and PEF/OD sample while the Newton model fitted better the changes related to the temperature for OD, PEF and OD/PEF. Besides, the Weibull plot showed a different rate of change for the *alfa* parameter as the temperature increases. From the slope of each sample, it is possible to see that the greatest change on the kinetic parameter as the temperature change has been observed for the control sample, whereas for the pre-treated samples the temperature had a lower effect on the drying rate. This finding was also reported by Mannozzi et al., (2020), as they showed that the pre-treatments caused a higher reduction in drying time at 50ºC but increasing temperature not allowed an increased reduction in the drying time. This trend could be related to the fact that the different pre-treatments change the initial solute/water content by osmotic dehydration and/or could enhance the mass transfer rate by PEF. As a consequence, the resulting drying response no longer depends only on temperature but even on the combined effect of temperature and applied pre-

treatment.

3.2. Texture

Figure 2 shows the results of firmness obtained on differently treated kiwifruit slices after the drying process. Fresh kiwifruit samples had a firmness value of 6.42±1.09 N. Pre-treatment with OD slightly decrease the kiwifruit firmness to values of 5.53±1.26 N; however, this decrease was not statistically significant. Samples treated with PEF instead presented a significant decrease of firmness (1.59±0.21 N), which was even more pronounced in samples treated by the combined treatments OD/PEF and PEF/OD with the values of 1.26±0.08 and 1.43±0.12 N respectively.

As expected, after drying the firmness of all the kiwifruit samples increased due to the loss of water (Lewicki & Jakubczyk, 2004; Tylewicz et al., 2019). The relation between the increase of the firmness and stiffness with the decrease of the water activity was studied by Castagnini et al., (2020). They explained that this increase is due to the non-uniform distribution of the water molecules in the fruit matrix but rearranged within the structure. This anti-plasticizing effect of water is reflected in the reduction of the volume existing between the different cell structures, making more difficult the collapse of the structure. Moreover, the increase of hardness and crispness values could be related to the decrease of the samples T_g, due to the slight increase in the soluble solid phase (Zou et al., 2013). Kiwifruit pre-treated with both OD and PEF alone presented a lower firmness in comparison to the untreated dried samples. In general, the application of OD causes vacuole shrinkage, loss of cell turgor pressure and consequently softening of tissue, due to the structural changes such as distortion and decrease in size of cell walls, cell wall breakdown, increase of intercellular spaces, solubilizing of chelator-soluble pectin of the middle lamella etc. (Fernandes et al., 2008; Panarese, Laghi, et al., 2012). PEF treatment can also affect the plant tissue softening, due to the permeabilization of the cell membrane, which promotes the alteration of the membrane permeability (Tylewicz et al., 2017, 2019; Wiktor et al., 2016). In the present work, the combination of OD followed by PEF further reduced the firmness parameter, showing the lowest values, when the low temperature of drying (50 and 60°C) was used, while the inverted sequence (PEF followed by OD treatment) resulted in the highest firmness (apart for the samples dried at 60°C), compared to other pre-treated samples. Dermesonlouoglou et al., (2016) also observed that combined treatment with PEF and OD resulted in a higher firmness of semi-dried kiwifruits, relating this phenomenon with the humidification of the tissue by the cellular juice coming from the electroporated cells. Probably this thin layer of cellular juice formed on the kiwifruit tissue was sufficient to protect the cell from softening during OD. When PEF was applied on partially dewatered tissue (OD/PEF) probably, the cell disintegration was higher, promoting at the same time the lowering of the texture parameter.

In general, the highest temperature of drying (70°C) promoted a significant increase of the firmness of all the considered samples, followed by the samples dried at 50°C, while samples dried at 60°C showed the lowest firmness. Indeed, Lewicki & Jakubczyk, (2004) observed that the drying temperature could strongly influence the mechanical properties of the final products, however, they noticed this relationship only when the drying temperature increased from 70 to 80°C.

In the untreated samples, the increase in firmness was proportional to the increasing temperature. Similar results related to the crispness were observed by Cortellino et al., (2011) in pineapple samples, even if they tested the air-drying temperature increase from 70 to 80°C.

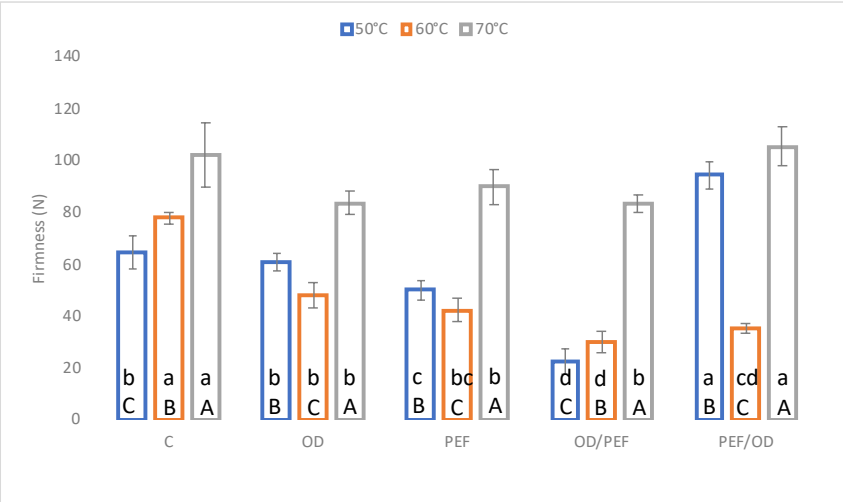

Figure 2. Firmness of untreated and differently pre-treated kiwifruit snacks dried at the temperatures of 50, 60 and 70 °C. Different lowercase letters indicate significant differences ($p < 0.05$) between all considered samples at each drying temperature, while capital letters indicate significant differences ($p < 0.05$) between each sample at the three drying temperatures.

3.3. Colour

Figure 3 shows the total colour difference (ΔE), obtained on differently treated kiwifruit slices after the drying process. This parameter is used to describe the overall changes in samples colour in reference to the untreated fresh sample.

The visible changes are defined by the ΔE threshold, which usually depends on the initial optical properties of the product, and they are in the range from 2 for products with low colour intensity like blood oranges Choi et al., (2002) to 6-7 for products with higher colour intensity like blueberries (Stojanovic & Silva, 2007).

All the pre-treated samples presented lower colour differences in comparison to the untreated ones. This was particularly true for the samples dehydrated at low temperature (50 and 60°C). The lowest colour differences were observed in samples treated with OD/PEF and dried at 50°C. OD treated dried samples showed ΔE values of 5.78 - 6.74. Similar values were observed by Nowacka et al., (2017) and Tylewicz et al., (2020) for kiwifruit subjected to the osmotic dehydration treatment.

When PEF treatment was applied alone or in combination with OD it was possible to observe that the highest drying temperature (70°C) promoted higher changes in the colour. The negative effect on kiwifruit colour related to the combination of PEF pre-treatment and high drying temperature could be due to the electroporation of the cell membrane, which caused both the increased release of enzymes and their substrates for the enzymatic browning reactions (Mannozzi et al., 2020) and pigments oxidation by thermal decomposition (Engin, 2020).

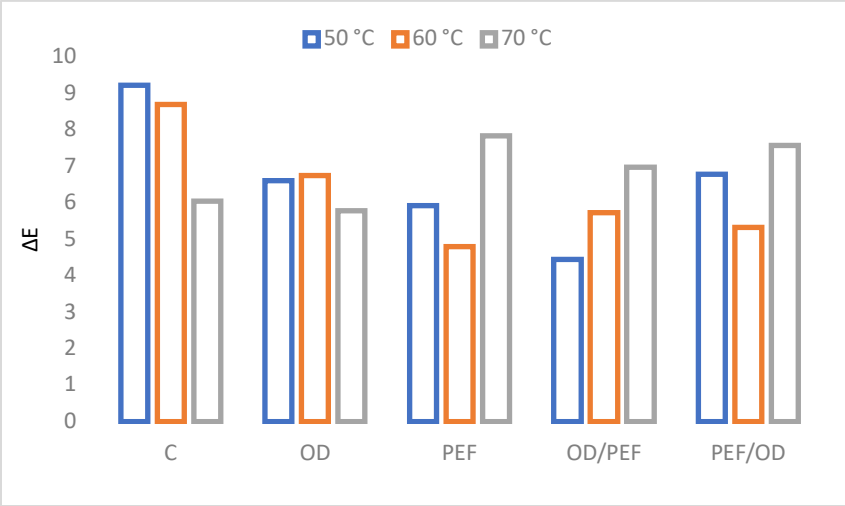

Figure 3. Total colour difference (ΔE) of untreated and differently pre-treated kiwifruit snacks dried at the temperatures of 50, 60 and 70 °C.

3.4. Sensory analysis

Figure 4 a, b, c shows the results of the sensory analysis carried out on differently treated kiwifruit slices after the drying process at the temperature of 50, 60 and 70°C, respectively. The samples treated with OD/PEF and then dried at 70°C was the one with the highest score for overall acceptability, while untreated control sample dried at 50°C showed the lowest acceptability level, under the acceptability threshold, which was fixed to 5 according to preliminary training. In general, with increasing the treatment temperature a increase in the overall acceptability of the samples was observed, regardless of the treatment used; while for samples dried at a lower temperature only the samples pre-treated with OD alone or in combination with PEF presented an acceptable value of this parameter, probably thanks to the increased sweetness of the samples.

Concerning the singular sensory parameters, the integrity of the slices was high for all the samples, suggesting that the preliminary operations did not affect too much the cell structure. The untreated samples were those, which presented the highest acidity, regardless of the drying temperature used, and a high level of parameters such as colour, odour and taste intensity. PEF and OD pre-treated samples alone also presented a good level of colour, odour and taste intensity. Kiwifruit slices

pre-treated with OD followed by PEF, when dried at a lower temperature (50 and 60°C) showed an intermediate value of all parameters, while when dried at 70°C, in addition to having the highest score for the overall acceptability, showed also the highest texture and a good balance between the sweetness and acidity level. Finally, samples treated first with PEF and then with OD presented the highest sweetness and the lowest acidity and therefore were always upper the overall acceptability threshold value.

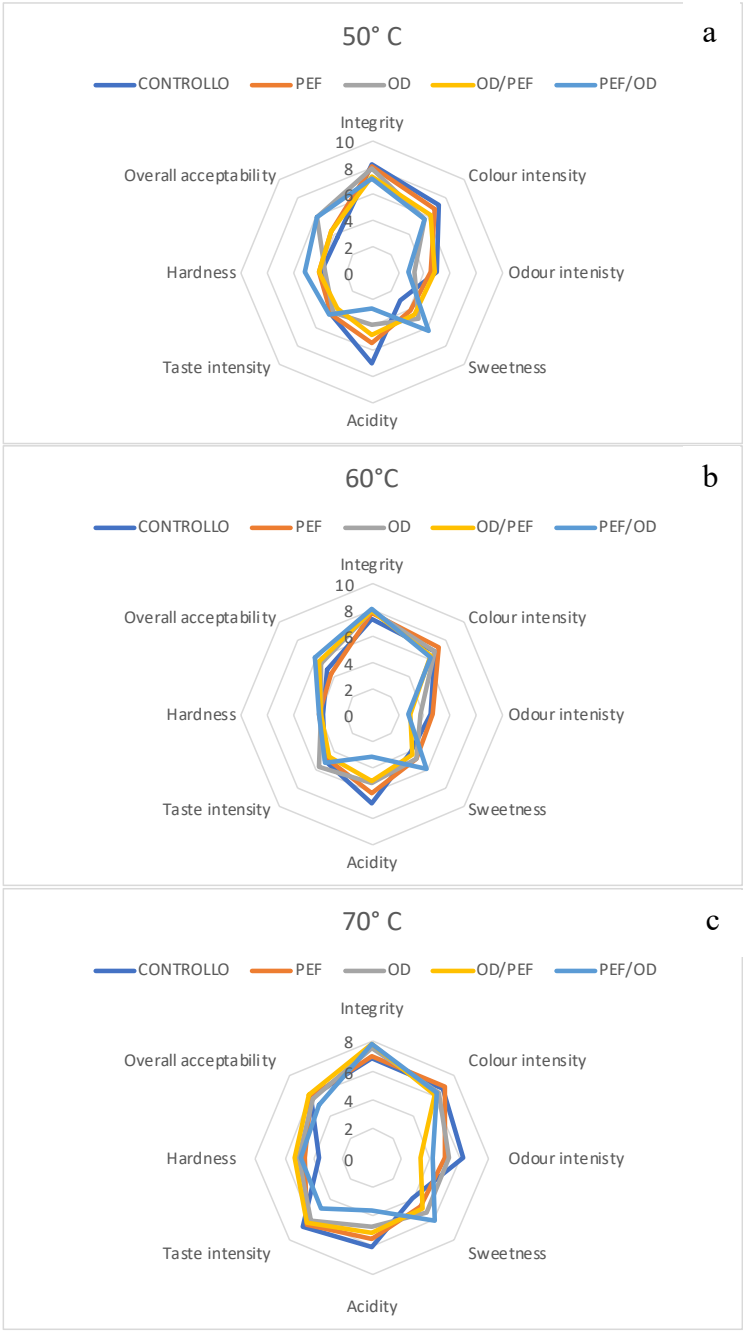

Figure 4. Sensory analysis of untreated and differently pre-treated kiwifruit snacks dried at the temperatures of 50 (a), 60 (b) and 70 (c) °C.

4. Conclusions

The drying kinetics of kiwifruit snacks samples were significantly influenced both by the applied treatments and the drying temperature. Among the three different models (Lewis, Page and Weibull) used, the Lewis and Weibull models presented the best goodness of fit. At every temperature, the PEF/OD sample showed the highest drying rate. Moreover, this kiwifruit snacks also presented the highest firmness and good overall quality acceptability evaluated by the sensory analysis, while the lowest impact on colour was observed in samples treated by PEF alone or applied after OD.

In conclusion, the combination of PEF/OD could represent an alternative sustainable pre-treatment step, in terms of energy consumption and costs, to the drying process guaranteeing the nutritional features and the tasty flavour of obtained fruit snack products. Further studies are in due course in our lab, in order to evaluate the overall sustainability increase of the purposed approach, through life cycle assessment (LCA) analysis.

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Paper VII

Genovese, J., Tylewicz, U., Mannozzi, C., Castagnini, J.M., Romani, S., Rocculi, P., Dalla Rosa, M. (2021). Kiwifruit waste valorization through innovative snack development. *Acta Horticulturae*, *X International Symposium on Kiwifruit* 1332, pp. 407-414. 2021.

Kiwifruit waste valorisation through innovative snack development

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Abstract

Currently, in the case of kiwifruits, those fruit with a weight lower than 65 g are considered waste. The production of dried snacks with high nutritional functionality could be a valid alternative to use the kiwifruit waste, with positive economic impact on the entire production chain. Therefore, the aim of this work was to evaluate the effect of pulsed electric field – PEF (200 V cm^{-1}) and/or osmotic dehydration – OD (trehalose at 40%) pre-drying treatments on drying kinetics at 50, 60, and 70°C, and on colour and nutritional properties (vitamin C and antioxidant compounds) of 'Jintao' (yellow-fleshed) kiwifruit snacks. At every temperature, the PEF treated snacks showed the highest drying rate. Moreover, PEF treatment appeared to be a valid innovative alternative for the production of fruit snacks with high nutritional quality. A better retention of vitamin C and antioxidant compounds was obtained in dried yellow kiwifruit subjected to PEF treatment.

Keywords: *Actinidia chinensis*, pulsed electric field, osmotic dehydration, hot-air drying

INTRODUCTION

Kiwifruits (*Actinidia* spp.) that are undersized (<65 g) and that do not meet the requirements in accordance with Commission Regulation (EC) No 1673/2004 are considered not adequate for sale and distribution, therefore are considered waste. In general, undersized kiwifruits are utilized in industrial food processing for the production of fruit juices and/or for biorefineries. Kiwifruit production generates large quantities of waste and by-products, including peels, seeds and pruning residues. Hence, an important challenge for food industries is to manage this waste and, when possible, its valorisation (Galanakis et al., 2016). The production of dried snacks with high nutritional functionality could be a valid alternative for the valorisation of waste kiwifruits, with a positive economic impact on the entire production chain. Kiwifruit are a good source of bioactive compounds (i.e., vitamin C, polyphenols) that contribute to their high antioxidant capacity (Ma et al., 2017). Besides the high nutritional value of this type of fruit, it has to be considered that food processing steps could damage the biological tissues and consequently change the functionality of the nutritional components, for example inducing oxidative reactions (Aguilera et al., 2003). The production of fruit snacks usually requires the use of high drying temperatures (>60°C), with a high energy consumption and which may negatively affect the overall quality of the product (i.e., sensory, nutritional and functional attributes). Among conventional drying methods, hot-air drying (Tylewicz et al., 2019) and osmotic dehydration (Luchese et al., 2015) are commonly used in post-harvest production. However, these drying techniques are usually time and energy demanding, resulting in the production of poor quality dried fruits (Onwude et al., 2016a, b). Innovative technologies can be applied as pre-drying treatments in combination with conventional ones, for the production of dried snacks. The innovation could, in this way, be useful in reducing the drying times, the drying temperature and, therefore, lead to the production of high-quality food products by means of a sustainable process approach.

Among the innovative technologies, pulsed electric fields (PEF) is gaining a lot of

attention as a treatment applied before the drying process. The modification of cell membrane permeability that PEF causes could increase the velocity of mass transfers and, thus, affect the drying kinetics. Moreover, PEF treatment can be combined with osmotic dehydration (OD) for the modulation of specific plant structural features, for example, the retention of colour and inhibition of enzymatic browning (Khan, 2012).

The aim of our work was to assess an innovative pre-drying process (applying PEF and OD treatments, alone or in combination) for the valorisation of kiwifruit waste by the production of functional kiwifruit snacks.

MATERIALS AND METHODS

Raw materials

Undersized yellow kiwifruits *Actinidia chinensis* 'Jintao' with an average weight of 63±1.6 g, a total soluble solids content of 13±1 °Brix, and with a firmness of 66.5±13.5 N, were provided by Jingold Consortium (Cesena, Italy). Before trials, the fruits were stored in refrigerated conditions (4±1°C) for a period not longer than one week. Before processing, the fruits were manually peeled and cut into discs 3 mm thick and then subjected to OD and/or PEF treatments before the subsequent drying process.

Treatments

1. Pulsed electric fields (PEF).

PEF treatment was performed using a lab-scale pulse generator (Alintel srl., Bologna, Italy) connected to a cubic treatment chamber (5×5×5 cm) holding the sample and the parallel-plate electrodes. The PEF protocol consisted of an electric field strength of 200 V cm^{-1} (calculated as the ratio between the applied voltage and the distance between the electrodes), 1000 rectangular-shape pulses with a fixed width of 10 μs, with a repetition frequency of 100 Hz. The total energy input was of 1.92 kJ kg^{-1}. Tap water with a conductivity of 421 μS cm^{-1} (conductivity meter, Crison Instruments, Barcelona, Spain) was used as the conductive medium in the treatment chamber.

2. Osmotic dehydration (OD).

The OD treatment was carried out by immersing the disc-shape pieces of kiwifruit in 40% (*w*/*w*) trehalose (Exacta + Optech Labcenter spa., Modena, Italy) solution at 35±1°C for 150 min (1:4 product/solution ratio).

3. Drying process for snack preparation.

Each of the four samples (untreated (C), PEF treated (PEF), OD treated (OD), and PEF and OD combined (PEF-OD)) was subjected to hot air drying at 50, 60 and 70°C in a convective dryer (Pol-Eko-Aparatura SP.J., Wodzisław Śląski, Poland). The air velocity was set to 2 m s^{-1}, and an average of 21 discs were used for the preparation of each sample.

Physical analyses of snacks

1. Water activity.

Water activity (a_w) was measured using a dew-point water activity meter (Aqualab, Decagon Devices, WA, USA) at 25°C. Measurements were conducted in triplicate (i.e., 3 slices).

2. Drying kinetics.

The dimensionless moisture ratio (MR) was calculated as the gradient of the sample moisture content, following Equation 1. The moisture content was determined gravimetrically by drying the samples at 70°C until the achievement of a constant weight (AOAC, 1996). Measurements were conducted in triplicate (i.e., 3 slices).

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (1)$$

where M_0, M_t and M_e are the moisture content (kg water kg^{-1} dry matter) at the beginning, at any time of drying and at the equilibrium (i.e., constant weight), respectively. The drying times were plotted as a function of MR.

3. Kiwifruit colour.

A spectrophotocolourimeter (Hurterlab, Virginia, USA) was used to investigate the snacks' colour following the different processing treatments applied. For each sample, the instrumental parameters L*, a* and b* from CIE L* a* b* scale were measured. Measurements were carried out in 10 slices for each drying temperature, for each sample.

Chemical analyses of snacks

An extraction step was performed before the following chemical analyses. 0.5 g of dried kiwifruits were mixed and vortexed for 2 min with 10 mL of methanol 60% (w/w). Extractions were performed in triplicate (i.e., 3 extractions for each sample). The mixture was centrifuged for 10 min at 3000 ×g and the supernatants were collected.

1. Vitamin C quantification.

The concentration of Vitamin C, measured as ascorbic acid, was determined following a redox titration, using an iodine solution (0.005 mol L^{-1}) and starch as indicator (Ciancaglini et al., 2001). The reaction oxidizes ascorbic acid to dehydroascorbic acid and reduces the iodine into iodine ions. When all the ascorbic acid present is oxidized, the excess of iodine reacts with the starch forming a blue complex indicating the end of the titration. Vitamin C content of kiwifruits snacks was expressed as mg of ascorbic acid 100 g^{-1} of dry matter of kiwifruits. The measurements were carried out in triplicates (i.e., 3 titrations).

2. Antioxidant capacity.

The antioxidant capacity of kiwifruits snacks was evaluated on the basis of scavenging the 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) and 1,1-diphenyl-2-picrylhydrazylm($DPPH^{\cdot}$) radicals, following the methods described by Mannozzi et al. (2020). The $DPPH^{\cdot}$ scavenging activity was determined by mixing 0.1 mL of kiwifruit extract, 0.2 mL of methanol and 0.25 mL of DPPH (Sigma-Aldrich, USA) and by measuring its absorbance at 517 nm. The $ABTS^{\cdot+}$ scavenging activity was determined by mixing 30 µL of the fruit extract with 3 mL of ABTS (Sigma-Aldrich, USA) diluted solution (as described by Re et al., 1999) and by measuring the absorbance at 734 nm every 30 s for a total of 6 min. The results of both essays were expressed as µmol Trolox 100 g^{-1} of dry matter of fruits, and the measurements were carried out in triplicates (i.e., 3 measurements per essay).

Statistical analysis

Significant differences between data were calculated by parametric analysis of variance (ANOVA) and Tukey multiple comparison, with a significance level of 95% ($p<0.05$). The analysis was performed using the software STATISTICA 6.0 (Statsoft Inc., UK).

RESULTS AND DISCUSSION

Drying kinetics

The variations of moisture ratio (MR) as a function of drying times at 50, 60 and 70°C are given in Figure 1. The treatments applied (i.e., PEF, OD, PEF-OD) in this study had an influence on the drying kinetics. In particular, the total drying time at the studied temperatures was affected and it varied as a function of both the pre-drying treatments and the drying temperature. The drying of kiwifruits started with an initial water activity of 0.98 and finished at 0.2-0.3, this water activity is typical of dried snacks. In Table 1 the end of the drying process (time) for each sample at each drying temperature is reported.

Table 1. Total drying time (h) of untreated (C) and osmotic dehydration (OD), pulsed electric field (PEF) and PEF-OD treated 'Jintao' kiwifruit snacks dried at 50, 60 and 70°C.

Drying temperature (°C)	Drying time (h)			
	C	OD	PEF	PEF-OD
50	16	6	6	6
60	8	5	4	6
70	7	4	3	5

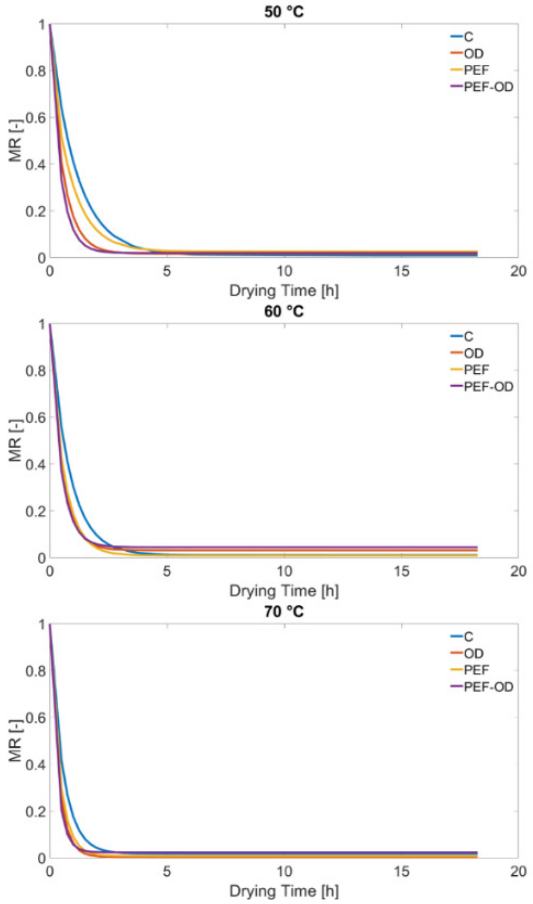

Figure 1. Moisture ratio (MR) of untreated (C) and osmotic dehydration (OD), pulsed electric field (PEF) and PEF-OD treated 'Jintao' kiwifruit snacks dried at 50, 60 and 70°C.

At the beginning of the drying process, it was possible to see the major differences between the samples and, these differences were more pronounced when the lowest (i.e., 50°C) drying temperature was used. For the samples dried at 50°C, the untreated sample (C) presented the highest MR after 1 h of drying time, while the lowest MR corresponded to the kiwifruit treated by PEF followed by OD. The same trend was shown at the drying temperature of 60 and 70°C.

Although after the first hour of drying the drying kinetics showed to be related to both the drying temperature and pre-drying treatment used, the endpoint (i.e., with a_w=0.3) of the drying process was reached faster when the kiwifruit slices were treated by PEF alone (Table 1). This trend could be explained by the mass transfer enhancement due to cell membrane electroporation. Pulsed electric field treatment, in fact, causes an increase in cell membrane permeability and, therefore, mass transfers (i.e., leakage of intracellular fluid into the extracellular space) could be positively affected (Donsì et al., 2010).

Colour

Table 2 shows the instrumental colour values, expressed as L* (lightness), a* (green-red index) and b* (blue-yellow index) of dried kiwifruit snack samples. It can be discerned that, in general, only slight modifications of colour parameters followed the treatments used. The combination of PEF and OD decreased the L* values of the products at all the drying temperatures tested. From the literature it can be seen that different tissues behave in different ways following PEF treatment. Wiktor et al. (2015) observed unchanged L* value or its slight decrease in apple samples, while increased values in carrot samples were observed. Tylewicz et al. (2020a) observed a decreased L* values in strawberry and kiwifruit samples following PEF, OD and PEF-OD treatments. In general, a decrease of the values of the colour parameters could be due to the leakage of pigments into the treatment solutions and due to the higher availability of the substrates for the enzymatic reactions (Wiktor et al., 2016). The PEF treated kiwifruits exhibited a slight increase of the chromatic parameter a* at the highest drying temperature. Nevertheless, the drying temperature of 60°C seems to be the more suitable for the maintenance of the fresh-like colour of all products.

Table 2. Colour parameters (L* – lightness, a* – red index, b* – yellow index) of untreated (C) and osmotic dehydration (OD), pulsed electric field (PEF) and PEF-OD treated 'Jintao' kiwifruit snacks dried at the temperatures of 50, 60 and 70°C. Results are expressed as mean values of *n*=10 with the standard deviation (SD).

Sample	L*		a*		b*	
	Mean	SD	Mean	SD	Mean	SD
			50°C			
C	47.1^{aA}	2.1	5.5^{aA}	0.6	29.2^{aA}	2.7
OD	45.1^{aA}	4.0	4.6^{bA}	0.8	27.3^{aA}	2.8
PEF	43.9^{aA}	2.3	5.4^{aB}	0.8	26.1^{aA}	1.9
PEF-OD	38.7^{bAB}	3.7	5.0^{abA}	0.5	24.3^{aA}	2.1
			60°C			
C	43.5^{aB}	3.5	5.4^{aA}	0.9	28.8^{aA}	1.9
OD	43.2^{aAB}	4.8	5.3^{aA}	0.9	27.1^{aA}	3
PEF	43.1^{aA}	2.7	5.1^{aB}	0.5	24.8^{aA}	1.9
PEF-OD	40.6^{aA}	2.5	5.1^{aA}	0.8	24.1^{aA}	2.2
			70°C			
C	43.3^{aB}	5.8	5.1^{aA}	0.9	26.0^{aA}	2.9
OD	42.5^{aB}	2.2	5.0^{aA}	1.0	26.2^{aA}	1.6
PEF	42.7^{aA}	2.3	6.1^{bA}	0.9	27.8^{aA}	1.7
PEF-OD	36.9^{bB}	1.8	4.8^{aA}	0.8	22.3^{bA}	1.1

Different lowercase letters indicate significant differences ($p<0.05$) between all considered samples at each drying temperature.
Different uppercase letters indicate significant differences ($p<0.05$) of each sample at the three drying temperatures.

Vitamin C

The content of vitamin C in the untreated and treated kiwifruit snacks dried at 50, 60 and 70°C is shown in Figure 2. The untreated sample showed the highest amount of ascorbic acid at all the drying temperatures tested. However, among the treated samples, the PEF treated showed the best retention of vitamin C when dried at 50 and 60°C, while at the highest drying temperature tested (70°C) the OD sample showed a better vitamin C retention.

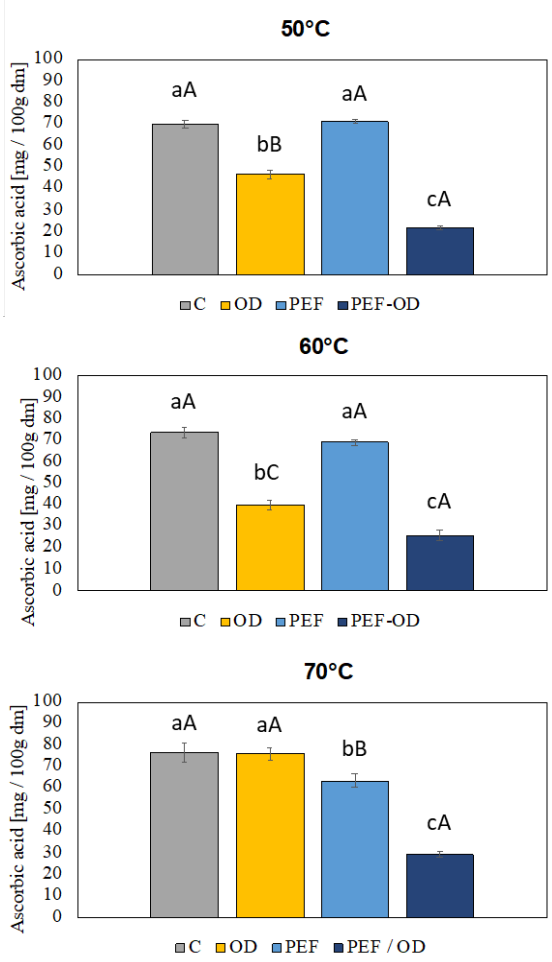

Figure 2. Vitamin C content, expressed as mg of ascorbic acid 100 g^{-1} of dry matter, of untreated (C) and osmotic dehydration (OD), pulsed electric field (PEF) and PEF-OD treated 'Jintao' kiwifruit snacks dried at the temperatures of 50, 60 and 70°C. Results are expressed as mean values of *n*=3 with standard deviations (error bars). Different lowercase letters indicate significant differences ($p<0.05$) between all considered samples at each drying temperature. Different uppercase letters indicate significant differences ($p<0.05$) of each sample at the three drying temperatures.

Kiwifruits are, in general, rich in vitamin C, which represents the main nutritional compound (Huang et al., 2002), and for this reason could be used as a good indicator of the overall quality of processed kiwifruits. In our study, it is clearly shown a decrease of ascorbic acid content with the increase of pre-drying steps. Nevertheless, the PEF treated kiwifruits contained a similar amount of vitamin C to the untreated fruits, especially when 50 and 60°C were used. This behaviour could be explained as a consequence of the exposure to high temperatures for a shorter period of time for PEF treated samples (as shown in Table 1). In fact, it is well known that the exposure of various types of fruit to increasing high temperature and time could cause a dramatic decrease of vitamin C content (Piga et al., 2003).

Antioxidant capacity

The antioxidant capacity quantified by $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ assays was strongly affected by both the pre-drying treatment applied and the drying temperature. The highest retention of bioactive compounds was in the untreated kiwifruit snacks dried at 60°C and evaluated with the $DPPH^{\cdot}$ method. However, the $ABTS^{\cdot+}$ scavenging activity increased for the PEF treated

kiwifruit snacks at all the drying temperatures, probably because of the shorter time necessary to complete the drying process (Table 3).

Table 3. Antioxidant activity by $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ assays of untreated (C) and osmotic dehydration (OD), pulsed electric field (PEF) and PEF-OD treated 'Jintao' kiwifruit snacks dried at the temperature of 50, 60 and 70°C. Results are expressed as µmol Trolox 100 g^{-1} dry matter, and as mean values of *n*=3.

Sample	50°C		60°C		70°C	
	Mean	RSD	Mean	RSD	Mean	RSD
			$ABTS^{+}$ (µmol Trolox 100 g^{-1} dm)			
C	2075.6^{bB}	8.9	2833.5^{bA}	16.1	1207.8^{bC}	14.2
OD	1209.3^{dA}	4.4	966.6^{dA}	22	1309.8^{bA}	13.6
PEF	4500.2^{aA}	6	4284.1^{aA}	1.9	4726.8^{aA}	6.7
PEF-OD	1739.7^{cA}	8.9	1659.5^{cA}	13.3	1690.2^{bA}	9.8
			$DPPH^{\cdot}$ (µmol Trolox 100 g^{-1} dm)			
C	2429.3^{aC}	4.5	6175.1^{aA}	2.8	2750.6^{aB}	8.7
OD	789.4^{bC}	3.9	3774.5^{bA}	5.7	2852.5^{aB}	8.3
PEF	2284.6^{aB}	6.6	2150.9^{cB}	4.4	2749.9^{aA}	13.4
PEF-OD	606.9^{cB}	8.7	916.9^{dA}	7.5	588.6^{bB}	8.7

RSD = % relative standard deviation.
Different lowercase letters indicate significant differences ($p<0.05$) between all considered samples at each drying temperature.
Different uppercase letters indicate significant differences ($p<0.05$) of each sample at the three drying temperatures.

The discrepancies between the two essays in the resulted antioxidant capacity are related to the different capability of the assays used to detect certain bioactive compounds. It is generally reported that the $ABTS^{+}$ method is more suitable for the determination of ascorbic acid scavenging activity, while the $DPPH^{\cdot}$ assay seems to be more appropriate for the identification of flavonoids (Del Caro et al., 2004). The same trend was previously described by (Tylewicz et al., 2020b), where strawberries treated by PEF at a specific energy input of 1.92 kJ kg^{-1} displayed a better retention of antioxidant compounds than strawberries subjected to lower energies. The increased antioxidant capacity in PEF treated snacks could be due to changes of the cell membrane that could lead to a greater release of bound bioactive compounds. The combination of PEF treatment with trehalose OD reduced the overall antioxidant capacity of kiwifruits, as reported previously by Tylewicz et al. (2020b).

CONCLUSIONS

The application of PEF and/or OD treatments influenced the drying kinetics of kiwifruit snacks. Both pre-drying treatments contributed to the reduction of the total drying time, an important aspect for energy saving in any future industrial applications. Moreover, the shortest drying times required for the OD and PEF treated samples could be beneficial for the production of fruit snacks with high functionality (i.e., higher concentration of bioactive compounds) and better overall quality. A further study would be of value to determine the costs for a possible industrial scale-up.

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Paper VIII

Genovese, J., Tappi, S., Tylewicz, U., D'Elia, F., de Aguiar Saldanha Pinheiro, A.C., Rocculi, P. (2021). Dry-salted cod (*Gadus morhua*) rehydration assisted by pulsed electric fields: modelling of mass transfer kinetics. *Journal of the Science of Food and Agriculture* (under review).

Dry-salted cod (*Gadus morhua*) rehydration assisted by pulsed electric fields: modelling of mass transfer kinetics

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Abstract

Dry-salted cod (*G. morhua*), prior to consumption, must be rehydrated, and this step could last up to five days. The industrial-scale cod desalting presents many problems, mainly linked to long processing times and the quality of the final product. For this reason, many researchers have focused on finding new desalting methods to improve the mass transfer processes. The application of pulsed electric fields (PEF) has been proposed as an alternative method to enhance the mass transfer phenomena in many food processes. However, there is no previous literature on the use of PEF to improve the desalting of animal tissues of food interest. Therefore, the aim of this work was to evaluate the possibility of applying PEF pre-treatment in salted cod rehydration process and to determine any influence in the mass transport kinetics. Results indicated that the use PEF technology enhanced the rate of the rehydration process of dry-salted cod and, moreover, it affected the redistribution of salt.

Keywords: Pulsed Electric Fields; Rehydration kinetics; Mass transfer; Peleg model; Dry-salted cod

1. Introduction

Dry-salted cod (*Gadus morhua*) is a highly appreciated product, traditionally imported by Mediterranean countries and commercialised with different moisture content depending on the extension of the dehydration process. The traditional production of salted cod is carried out alternating layers of product and dry salt crystals in stacked piles, where the product is kept for about 2-8 weeks, depending on the desired level of dehydration in the resulting product [1]. Generally, the final product reaches a salt concentration of 2-3 % and a decrease of moisture content of 0.80-0.65 (w/w). Moreover, the decreasing of water activity by the removal of water and the increase of salt content in the tissue results in the inhibition of deteriorative processes, increasing the shelf-life and the fish stability [2]. Prior to consumption, the fish must undergo a rehydration/desalting process, in order to reduce the salt concentration to levels suitable for eating (in the range of 2-3%). This final step is usually performed by the consumer, and it is carried out by immersing the product in stagnant water at room temperature or refrigerated conditions [3]. At this stage, a two-ways mass transfer takes place, resulting in the leaching out of sodium chloride ions from the tissue matrix and, contrarily, in the water uptake, observing a tendency of cod weight increase as well as volume, and in the re-solubilization of salt and proteins.

Considering that market trends evolve towards ready-to-use products, the cod industry is adapting its procedures to consumer requirements, and nowadays the desalting step is often implemented and included among the industrial operations. However, the industrial-scale cod desalting presents many problems mainly linked to long processing times (generally about 2-3 days, depending on the thickness of the fish pieces) and the quality of the final product. Large-scale traditional rehydration is similar to the desalting procedure performed by consumers at home [3,4]. It employs handling of large amount of water, as several water renewals are necessary to improve the process efficiency (also in terms of process yields). Therefore, the industrial desalting step presents criticisms related also to the wastewater management. The latter should be taken into consideration as a process parameter for the optimization of the desalting operation [5]. In fact, the residual brine is a polluting effluent characterised by dissolved and suspended solids (mainly Na^+ and Cl^- ions, and, in smaller portion, dissolved proteins), and it must be treated before its spill in the municipal sewage system [6]. For this reason, the industrial cod desalting step should be optimized yielding to a commercial product with higher process yield (high desalted cod/initial salted cod weight ratio) and limited waste. In the last years, many researchers have focused on finding new methods to improve the kinetics of desalting/rehydration processes, such as the use of vacuum pulses [7], high pressures [8] or high-intensity ultrasounds [9].

The application of pulsed electric fields (PEF) has been proposed as an alternative method to enhance the mass transfer phenomena in many food processes. However, to the best of our knowledge, there is no previous literature in the use of PEF treatments to improve the desalting of foods. PEF technology consists in an electrical treatment of short time (from several nanoseconds to several milliseconds) with electric field

strengths from 100-300 V cm^{-1} to 20-80 kV cm^{-1} [10]. High electric fields (> 20 kV cm^{-1}) are generally used to inactivate alterative and pathogenic microorganisms and quality related enzymes [11]; while lower electric field strengths (0.5 – 3 kV cm^{-1}) are used alone or in combination with other food process unit operations and they could lead to the loss, temporary or permanent, of cell membrane semi-permeability [12]. Cell membranes could be considered as a physical barrier to the diffusion processes, hence their permeabilization degree could influence mass transfer phenomena.

For this reason, our study focused on the study of mass transfer kinetics, PEF pre-treating the studied food materials prior to the desalting/rehydration process.

1. Material and Methods

1.1 Raw Materials

Dry-salted cod (*G. morhua*) fillets were supplied by a local importer and, prior to desalting experiments, they were manually cut in cubic-shape pieces (2 x 2 x 2 cm), obtained from the upper part of the fillet. They had an average weight of 13.8 ± 1.1 g, and they were kept refrigerated at 4 ± 1 °C before trials.

1.2 Pulsed Electric Fields (PEF) treatments

PEF treatments were performed using a lab-scale PEF unit delivering a maximum output voltage and current of 8000 V and 60 A, respectively (Mod. S-P7500, Alintel, Italy). The generator provides monopolar near-rectangular pulses and adjustable pulse duration (5-20 µs), pulse frequency (50-500 Hz) and total treatment time (1-600 s). The treatment chamber (50mm length x 50mm width x 50mm height) consisted of two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. Output voltage and current were monitored using a PC-oscilloscope (Picoscope 2204a, Pico Technology, UK). Samples were treated at room temperature in tap water, with an initial electrical conductivity of 396 ± 5 µS cm^{-1} at 25 °C (EC-meter Mod. Basic 30, Crison, Spain). Trials were conducted filling the treatment chamber with a product-to-water ratio of around 1:5 (w/w) and delivering n=1000 pulses at fixed amplitude (10 ± 1 µs), frequency (100 Hz) and repetition time (10 ± 1 ms). Treatments were performed applying 2 different electric field strengths / specific energy inputs (500 V cm^{-1} and 1000 V cm^{-1}, 6.8 ± 1.3 kJ kg^{-1} and 23.1 ± 2.4 kJ kg^{-1}, respectively) chosen on the basis of preliminary experimental trials. Temperature changes due to PEF treatments were negligible. The cod samples were named control (untreated), and PEF (1) and PEF (2) (pre-treated).

1.3 Rehydration experiments

The rehydration process was carried out in static conditions immersing dry-salted cod samples in cold tap water (5 ± 0.5 °C) and using a cod-to-water ratio of 1:10 (w/w). For the study of rehydration kinetics, total weight gain, moisture content, salt (NaCl) content and water activity were determined at 0, 4, 6, 24, 48, 72, 96, 120 and 144 h of the rehydration process.

1.4 Rehydration kinetics

The rehydration of dry-salted cod implies two mass transports, as the sample both gain water and lose salt. In this study, the rehydration kinetics were studied considering the global evolution of the net sample weight taken at regular time intervals. For each sampling point five cod samples were drained from surface water with absorbent paper and weighted. Therefore, relative mass changes (ΔM_t^o), water uptake (ΔM_t^w), solutes loss (ΔM_t^s), and salt loss (ΔM_t^{NaCl}) were estimated according to the following equations:

$$\Delta M_t^o = (M_t^o - M_0^o)/M_0^o \quad (1)$$

$$\Delta M_t^w = (M_t^o \cdot x_t^w - M_0^o \cdot x_0^w)/M_0^o \quad (2)$$

$$\Delta M_t^S = (M_t^o \cdot x_t^S - M_0^o \cdot x_0^S)/M_0^o \quad (3)$$

$$\Delta M_t^{NaCl} = (M_t^o \cdot x_t^{NaCl} - M_0^o \cdot x_0^{NaCl})/M_0^o \quad (4)$$

where M_t^o and M_0^o are the cod weight (g) at the sampling time t and 0. x_t^w and x_0^w the cod water weight fractions. x_t^s and x_0^s the cod solutes weight fractions. x_t^{NaCl} and x_0^{NaCl} the cod NaCl weight fractions at time t and 0, respectively.

1.5 Kinetic Model

To describe the rehydration kinetics of dry-salted cod, Peleg's empirical model (Peleg, 1988) was considered:

$$M_t^w - M_0^w = \frac{1}{k_1^w + k_2^w \cdot \mathrm{t}} \quad (5)$$

$$M_t^S - M_0^S = -\frac{1}{k_1^S + k_2^S \cdot \mathrm{t}} \quad (6)$$

$$M_t^{NaCl} - M_0^{NaCl} = -\frac{1}{k_1^{NaCl} + k_2^{NaCl} \cdot t} \quad (7)$$

where k_1 is a kinetic parameter (Peleg rate constant), k_2 is related to equilibrium moisture content (Peleg capacity constant).

In our study, the same equation, rewritten as:

$$M_t^o - M_0^o = \frac{1}{k_1^o + k_2^o \cdot \mathrm{t}} \quad (8)$$

was also used in order to model total mass change kinetics, according to [14].

This kinetic model offers the advantage that calculating $\frac{1}{k1}$ and $\frac{1}{k2}$ it is possible to obtain the initial rate value of mass transfer parameters and the one at the equilibrium condition.

1.6 Analytical determinations

The cod moisture content was determined gravimetrically by oven drying at 105 ± 1 °C until a constant weight was achieved. Sodium chloride was determined after sample homogenization in distilled water using an Ultraturrax at 10000 rpm for 1 min and centrifugation to remove debris left in the sample. The chloride ion concentration was determined by titration of the centrifuged sample with standard $AgNO_3$ solution (0.1 mol L^{-1}) and K_2CrO_4 as indicator (Mohr's method) [15]. Water activity (a_w) was measured at 25 °C using a dew point water activity meter with ± 0.003 a_w accuracy (Aqua Lab. Decagon Devices). For each analytical determination five cod samples, randomly distributed among two experimental groups (Control and PEF-treated), were used at each sampling time.

1.7 Statistical analysis

Significant differences between results were calculated by parametric analysis of variance (ANOVA) and Tukey multiple comparison. with a significance level of 95% ($p<0.05$). If Shapiro-Wilk test for normality and Levene's test for homoscedasticity of data resulted statistically significant ($p<0.05$). non-parametric multiple range test Kruskal-Wallis and Holm stepwise adjustment were used. with a significance level of 95% ($p<0.05$) (R Foundation for Statistical Computing. Austria). All results were expressed as mean ± standard deviations of replications. In order to estimate the kinetic model constants (k_1, k_2), standard errors (SE) and the coefficient of determination (R^2), non-linear regression was carried out by means of the quasi-Newton calculus algorithm using STATISTICA 6.0.

2. Results

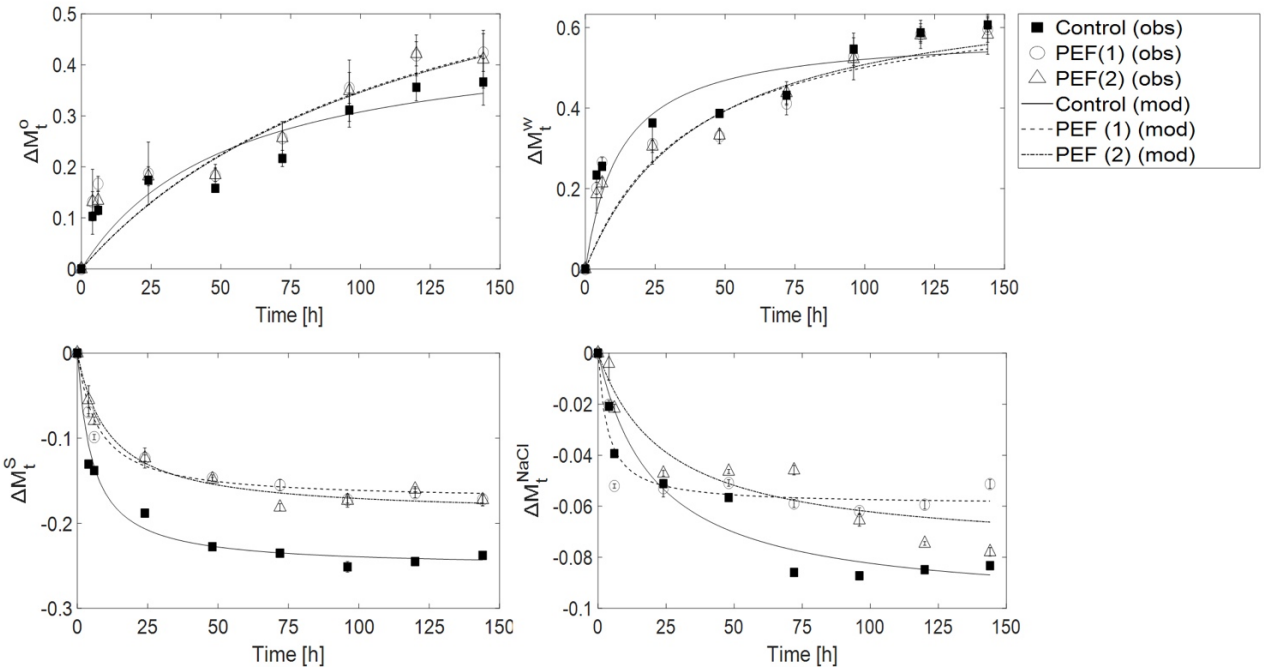

Figure 1. Mass uptake (ΔM_t^o), water uptake (ΔM_t^w), solutes loss (ΔM_t^S), salt loss (ΔM_t^{NaCl}) of untreated and PEF pre-treated dry-salted cod samples as a function of the rehydration time. Experimental data (obs) with the curve fitting (mod) are shown. Error bars correspond to the standard deviation of *n*=5.

The kinetics of mass (ΔM_t^o), water uptake (ΔM_t^w), solutes (ΔM_t^S) and salt loss (ΔM_t^{NaCl}) during the rehydration of dry-salted cod, modelled according to Peleg's equation, are shown in Fig. 1. Constants of Peleg's equation (k_1 and k_2) and their reciprocal values are reported in Table 1. Comparing the observed and calculated values of mass fraction in the figures and considering the R^2 values (between 0.656 and 0.955), the model confirmed to efficiently describe mass transfer phenomena during cod rehydration. All the investigated parameters were influenced by the PEF pre-treatment. Mass uptake (ΔM_t^o) increased following both PEF treatments applied in this study. Although the initial rate of mass uptake was higher in control sample (higher value of $1/k_1$), at the end of the process, the mass uptake at equilibrium was higher in PEF treated samples (higher value of $1/k_2$). Considering only the water fraction, its uptake (ΔM_t^w) was faster in untreated (control) samples at the beginning, while at the end of the rehydration process (i.e. at the 6th day) PEF treated samples presented values of water uptake comparable to the untreated product. The total solutes variations (ΔM_t^S) highlights a more pronounced loss in untreated samples, maintaining higher both initial and final mass transfer rate. In order to better understand the loss of NaCl during the desalting process, the salt content was

monitored during the whole process of rehydration. Salt loss represented about the 35% of total solutes loss in control and PEF (1) treated samples, while in PEF (2) the loss was about the 37%. In Figure 1 it is clearly shown that for both PEF pre-treatments selected, the samples showed a lower salt loss than control samples during the whole rehydration process. The initial rate of salt loss was higher in PEF (2) treated samples, while at the end of the process higher rate of salt loss was observed in control samples.

Table 1. Peleg's kinetic model of mass and water uptake and solutes and salt loss during rehydration of dry-salted cod.

	k_1	SE	k_2	SE	R^2	$1/k_1$	$1/k_2$
			Mass variation				
Control	112.939	14.311	2.121	0.183	0.776	0.009	0.471
PEF (1)	158.749	15.068	1.282	0.178	0.656	0.006	0.780
PEF (2)	160.175	13.840	1.287	0.163	0.714	0.006	0.777
			Water uptake				
Control	21.416	2.489	1.704	0.523	0.841	0.047	0.587
PEF (1)	54.872	5.305	1.447	0.079	0.707	0.018	0.691
PEF (2)	57.505	4.481	1.392	0.065	0.804	0.017	0.718
			Solutes loss				
Control	20.994	1.314	3.966	0.423	0.955	0.048	0.252
PEF (1)	42.286	2.780	5.774	0.066	0.915	0.024	0.173
PEF (2)	59.695	3.646	5.254	0.074	0.925	0.017	0.190
			Salt loss				
Control	209.338	18.746	10.049	0.322	0.901	0.005	0.100
PEF (1)	58.213	7.117	16.841	0.232	0.795	0.017	0.059
PEF (2)	321.131	33.178	12.873	0.544	0.785	0.003	0.078

Table 2. Water activity (a_w) of untreated and PEF treated samples during rehydration of dry-salted cod. Different letters correspond to significantly differences ($p<0.05$) between groups at the same selected time.

Time [h]	CTRL	PEF (1)	PEF (2)
	Mean ± SD	Mean ± SD	Mean ± SD
0	0.752 ± 0.001 $_a$	0.752 ± 0.001 $_a$	0.752 ± 0.001 $_a$
4	0.923 ± 0.004 $_a$	0.937 ± 0.006 $_b$	0.932 ± 0.005 $_c$
6	0.935 ± 0.004 $_a$	0.945 ± 0.005 $_b$	0.946 ± 0.005 $_b$
24	0.986 ± 0.003 $_a$	0.971 ± 0.004 $_b$	0.971 ± 0.005 $_b$
48	0.994 ± 0.002 $_a$	0.989 ± 0.002 $_b$	0.993 ± 0.003 $_a$
72	0.998 ± 0.001 $_a$	0.992 ± 0.003 $_b$	0.993 ± 0.003 $_b$
96	0.997 ± 0.002 $_a$	0.998 ± 0.001 $_a$	0.998 ± 0.002 $_a$
120	0.997 ± 0.002 $_a$	0.998 ± 0.002 $_a$	0.998 ± 0.002 $_a$
144	0.997 ± 0.002 $_a$	0.993 ± 0.003 $_b$	0.997 ± 0.002 $_a$

Water activity (a_w) of untreated and PEF pre-treated samples during the rehydration process of dry-salted cod is shown in Table 2. The initial a_w of cod samples was of 0.752. As expected, during the rehydration process there was a progressive increase of a_w values in all the samples, showing in almost all cases significantly ($p<0.05$) higher values in control samples. At the end of the process control and PEF (1) sample presented a value of a_w of 0.997, while PEF (2) presented a significantly ($p<0.05$) lower value of 0.993.

3. Discussions

The aim of this study was to explore the applicability of PEF technology to increase the velocity of desalting/rehydration of dry-salted cod. Two different PEF pre-treatments were selected, namely PEF (1) and

PEF (2), in order to understand if the increased permeabilization of cell membranes due to the electroporation process, could lead to an increase of mass transfers phenomena that characterise the rehydration of salted cod (i.e. water uptake and leaching out of salt). As expected, during the whole rehydration period considered in our study (six days), the weight and water content increased, while the salt content decreased. From the study of mass transfer kinetics, it was possible to highlight some discrepancies between the untreated (control) and PEF-treated samples. Moreover, the empirical Peleg's model adequately described the cod desalting/rehydration kinetics (coefficients of determination, R^2, are given in Table 1), with the only exception of the PEF (1) mass variation (ΔM_t^o), which reported the lowest R^2 (0.656). The application of PEF technology positively influenced the weight gain along the cod rehydration, showing an increased total mass variation compared to the untreated sample, independent of the treatment specific energy inputs applied (i.e. PEF (1) and PEF (2) reached similar values of mass variation after 144 hours of rehydration). In details, the higher weight of the pre-treated product is caused by the poor solid loss from the food matrix. In fact, both pre-treatments led to a higher retention of solutes than the untreated sample, and, in particular, to a lower leaching of NaCl. Nevertheless, the calculated salt content in both pre-treated sample showed to be within the range of commercial rehydrated cod products, being around 1- 2 % of NaCl (1.01 ± 0.7 % NaCl Control; 2.0 ± 0.6 % NaCl PEF (1); 1.5 ± 0.6 % NaCl PEF (2)) [16]. However, a sensory evaluation of the PEF treated products should be considered to confirm if the processed food material is acceptable for consumption. Although, it is well known that at certain level, higher salt concentrations, could positively influence the water holding capacity (WHC) of the protein matrix, in our study the final water uptake was similar in all sample groups considered. As already highlighted in the introduction, to the best of our knowledge, this is the first study showing how the PEF technology could influence the desalting of food materials. Other non-thermal technologies have been previously studied for the enhancement of cod desalting process, such as the application of high-intensity ultrasound, which improved both the moisture and NaCl diffusivities [9]. As the fish rehydration could be, in a simplified way, considered as the opposite process to salting, the influence of PEF pre-treatment has been demonstrated to affect the brine salting process of fish fillets. As reported by [17], although the PEF pre-treatment of sea bass fillet could positively influence the brine uptake, its application could negatively affect some qualitative characteristics of the food matrix, such as its protein and lipid oxidation stability. In the light of this considerations, further research should be focused on the influence of PEF pre-treatment on the physicochemical properties along the rehydration of cod fish.

4. Conclusions

This explorative study showed the applicability of PEF technology as a pre-treatment of dry-salted cod for the improvement of rehydration kinetics. We showed as selected PEF pre-treatments positively affect the weight gain of the cod throughout the desalting/rehydration process. Moreover, a less pronounced salt loss was observed in the pre-treated fish, reaching, at the end of the desalting process, salt levels comparable to the commercialised product. These results could indicate the advantageous application of the technology in the industrial environment, since its application could lead to higher process yields (higher weight gain) and to the possibility of reducing the water renewals, as less NaCl is lost in the waste brine, yielding to lesser water wastes.

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Paper IX

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PEF-treated plant and animal tissues: Insights by approaching with different electroporation assessment methods

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ABSTRACT

The goal of our investigation was to explore the level of membrane permeabilization in plant and animal food matrices (potato, apple, chicken) as resulting from subjecting these matrices to pulsed electric field treatment whereby we employed different assessment methods (electrical impedance spectroscopy, current-voltage measurements, magnetic resonance imaging). Our study was performed using a number of amplitudes of eight electric pulses of 100 μs in duration, and results were expressed in terms of the change in electrical properties as evaluated by electrical impedance spectroscopy, current-voltage measurements, and changes in the water (re) distribution as evaluated by magnetic resonance imaging and T_2 mapping techniques. The findings of our research provide useful insights and could be in support of an appropriate choice of electroporation assessment methods in relation to the food matrix characteristics, and for the determination and selection of appropriate PEF treatment conditions.

1. Introduction

Electroporation or pulsed electric field (PEF) treatment is a process known to cause an increase of cell membrane permeability and consequently an increase of the cell membrane and tissue conductivity. This phenomenon takes place when a biological tissue is exposed to an externally applied electric field of sufficient strength and is most often explained by the creation of aqueous pathways (i.e. pores) in the lipid domain of the cell membrane (Kotnik, Rems, Tarek, & Miklavcic, 2019). The application of PEF treatment in food processing is gaining momentum, and it is currently under intensive research and development. New electroporation-based treatments are continuously put to the test and are optimized both at the laboratory and industrial scale processes (Mahnič-Kalamiza, Vorobiev, & Miklavčič, 2014). PEF treatment offers increasing benefits in terms of low energy requirements and minimization of food quality deterioration. Following this preamble, it becomes clear that an appropriate choice of methods assessing changes due to electroporation occurring in biological matrices of alimentary interest is crucial. Despite a considerable number of scientific papers accumulated in the field, detailed information regarding the detection and quantification of the effects of electroporation in complex and highly inhomogeneous multicellular systems, such as real food systems, is still limited. Moreover, due to the unique characteristics and properties of the biological tissue processed, a case-by-case PEF treatment optimization protocol is often required (Bhat, Morton, Mason, & Bekhit, 2019; Chalermchat, Malangone, & Dejmek, 2010; Golberg et al., 2016).

In food-related PEF applications, measurements of the dielectric properties of the tissue are often used for the determination of the degree of cell membrane disruption due to electroporation (Lebovka & Vorobiev, 2017). The electrical impedance spectroscopy (EIS) has been suggested as a reliable method to estimate the extent of tissue damage due to PEF treatment. This method relies on the theory that, from an electrical point of view, an individual cell can be represented as an insulating membrane exhibiting relatively high resistance to electric current and considerable capacitance, and intra- and extra-cellular media (electrolytes) that behave as a resistive (ohmic) load up to hundreds of MHz (Grimnes, Martinsen, Grimnes, & Martinsen, 2015). Most animal and vegetable tissues typically display a dispersion of impedance between low- and high-frequency fields, that arise at a spectral band starting from about 50 Hz and ending at about 10 MHz (Castellví,

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Mercadal, & Ivorra, 2017). Multifrequency impedance measurement can therefore be used to assess the degree of membrane permeabilization due to PEF treatment (Angersbach, Heinz, & Knorr, 2002), and its utility has already been demonstrated for various biological systems (Angersbach, Heinz, & Knorr, 1999; Cukjati, Batiuskaite, André, Miklavčič, & Mir, 2007; Mahnič-Kalamiza, Miklavčič, & Vorobiev, 2015). Another possible but infrequently used approach for the evaluation of tissue electrical properties involves the analysis of the voltage and current waveforms recorded during the application of the electric pulses. Electric current signals can be used to detect changes in the dielectric properties of the cell membrane in real-time, and can thus be used to assess the electroporation process. A discrete number of research papers reported the analysis of the current signals during the application of the high-voltage pulse, and it has been demonstrated that the dynamics of current can be used as a key characterization feature of tissue electroporation (Cukjati et al., 2007; Langus, Kranjc, Kos, Šuštar, & Miklavčič, 2016; Lv et al., 2020; Pavlin & Miklavčič, 2008).

Nevertheless, measuring changes in the electrical properties, such as complex impedance for example, presents a clear drawback of being affected by additional (accounted or unaccounted for) phenomena, whose effects may be mixed with (or superimposed to) physical changes of interest that take place after exposure to PEF. Biological tissues present high complexity of structure and spatially dependent properties, and it has been demonstrated that the modification of cell membrane permeabilization due to electroporation is also associated with modification of the structure (i.e. intra- and extracellular volume change), and ionic concentration variation due to leakage of intracellular content (Mahnič-Kalamiza et al., 2015; Pavlin et al., 2005). Hence, other techniques evaluating the effects of tissue electroporation have been suggested. Magnetic resonance imaging (MRI) techniques have been applied to monitor the spatially dependent effect of PEF treatment in vegetable tissues (Dellarosa et al., 2018; Kranjc, Bajd, Serša, de Boevere, & Miklavčič, 2016). Transverse relaxation time T_2 has been employed as an indication of the redistribution of water and solutes in the tissue following the PEF treatment (Hjouj & Rubinsky, 2010). Moreover, MRI and magnetic resonance electrical impedance tomography (MREIT) were proposed as methods to monitor the electric field distribution in the tissue while applying the PEF treatment (Kranjc et al., 2016).

In our study we combined the electrical impedance spectroscopy, the current-voltage measurements, and the magnetic resonance imaging technique to explore the level of membrane permeabilization occurring due to electroporation in raw plants and skeletal muscles of interest for food and/or feed. We examined the variation of electrical properties and water mobility of vegetable and animal tissues subjected to various intensities of PEF treatment in order to investigate the consistency of the PEF assessing methods in relation to the type of food matrix. Experiments were performed in potato tuber and apple tissue since these vegetable matrices are of high interest in industrial PEF applications. Chicken broiler *Pectoralis major* was selected as a reference skeletal muscle, as it is one of the most homogeneous muscles in farmed animals, being almost entirely constituted of IIB fibre type, i.e. fast-twitch glycolytic (Verdiglione & Cassandro, 2013). We performed impedance measurements before and after the application of pulses, as it is an often used method in food-related PEF applications to determine the degree of membrane disruption (Lebovka & Vorobiev, 2017). We also performed and analysed current and voltage measurements to gain additional information about the electroporation of cell membranes, as well as magnetic resonance imaging for the characterization of the tissue's PEF-response by T_2 mapping.

2. Materials and methods

2.1. Raw materials

2.1.1. Plant tissues

Two different plant tissues were used for this study: potato tubers (*Solanum tuberosum*, cv 'Liberta'), and apples (*Malus domestica*, cv 'Golden Delicious'), purchased from the local market. At the time of experiments, the soluble solid content of apples was 11.3 ± 0.3°Brix.

The raw materials, before trials, were manually cut using a sharp cork-borer to obtain cylindrical-shape samples of 26 mm in diameter. The thickness of the disks was 6 mm for electrical impedance spectroscopy, and 30 mm for magnetic resonance imaging.

2.1.2. Skeletal muscle tissues

Boneless and skinless *Pectoralis major* muscles were obtained from the same flock of broiler chickens (Ross 308 hybrid, slaughter age 46 days, average weight 2.9 kg) farmed and processed under commercial conditions and, before trials, stored at 1 °C. Trials were conducted 48 h *post-mortem*. Each fillet was sampled using the cranial portion of the breast and cut along the direction of muscle fibres. From each sample, disks of 26 mm in diameter were manually cut using a sharp cork-borer. The thickness of the disks was 6 mm for electrical impedance spectroscopy, and 30 mm for magnetic resonance imaging.

2.2. Experimental setup

2.2.1. Electrical impedance spectroscopy and current-voltage measurements

Two electrical impedance measurement systems were assembled by connecting: 1) 4-needle electrodes, and 2) a treatment chamber consisting of two stainless-steel parallel plate electrodes, to a precision LCR meter (model E4980A, Keysight Inc., USA). The needle electrodes (copper with nickel plating) were 6 mm long and 1 mm in diameter and were spaced 5 mm (centre-to-centre) from each other along a straight line. All four electrodes were employed to deliver the high-voltage electroporation pulses, with the left-most two electrodes connected (in parallel) to one polarity on the generator, and the right-most two electrodes connected (in parallel) to the opposite polarity. In the case of two-plate electrodes, the treatment chamber was used to hold the sample, while the electrodes, spaced at 6 mm from each other, were employed to both deliver the high-voltage electroporation pulses as well as for impedance measurements. In both systems, switching relays were used to switch the electrical connections of the electrodes between the high-voltage pulse generator and the LCR meter.

Cylindrical samples were treated by delivering 8 rectangular monopolar pulses of 100 μs duration to the tissue with a pulse repetition rate of 1 Hz. For each treatment, different pulse amplitudes were used; from 50 V to 1500 V for plant tissues, and from 250 V to 1500 V for animal tissues.

Multi-frequency parallel capacitance (C_p) and parallel resistance (R_p) measurements of untreated and treated samples (i.e. between 3 and 5 s after the pulse application) were performed in the frequency range of 50 Hz to 1 MHz by applying a 100 mV (for plant tissues) and 500 mV (for animal tissues, due to the higher conductivity compared to plant tissues) peak voltage to the outer electrodes. The outer electrodes (centre-to-centre distance 15 mm) thus served as the current source and sink, while the inner pair (centre-to-centre distance 5 mm) were used to measure the voltage drop, as customarily configured in 4-electrode impedance analysis. Data were acquired using an in-house developed software for the LCR meter control and data capture (based on the Arduino and National Instruments LabVIEW software platforms). The magnitude of impedance was calculated according to Eq. (1).

$$|Z| = \left(\sqrt{\left(\frac{1}{R_p}\right)^2 + \left(\omega C_p\right)^2} \right)^{-1} \quad (1)$$

where R_p and C_p are the measured resistance and capacitance in parallel, respectively, and ω is the angular frequency ($\omega = 2\pi f$) and f is the excitation signal frequency in range of 50 Hz to 1 MHz as set in the impedance analyser.

For each PEF treatment, the delivered voltage and current were

measured and recorded by a high-voltage probe (model HVD3206A, LeCroy USA) and a current probe (model CP031A, LeCroy USA) connected to a sequencing digital storage oscilloscope (model HDO6104A-MS, LeCroy USA). The recorded voltage-current data were analysed using MATLAB 2019b software (MathWorks, Natick, MA, USA).

2.2.2. Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) was performed on plant and animal tissues while applying the PEF treatment. PEF treatment protocol consisted of two sequences of 4 pulses of 100 μs duration, with a pulse repetition frequency of 5 kHz and adjusting the voltage amplitude to obtain sufficient signal-to-noise ratio of electric field in samples. The electric pulses were delivered using a laboratory prototype electric pulse generator (Novickij et al., 2016) connected to 2-needle electrodes with a diameter of 1 mm made of platinum-iridium alloy (Pt/Ir: 90/10%). Electrodes were inserted into the sample tissue at a centre-to-centre distance of 10.4 mm, and the samples were PEF-treated while they were scanned by the MRI. The MRI scanner consisted of a 2.35 T (100 MHz proton nuclear MR frequency) horizontal bore superconducting magnet (Oxford Instruments, Abingdon, UK) connected to an Apollo spectrometer (Tecmag, Houston TX, USA) and equipped with micro-imaging accessories with maximum gradients of 250 mT/m (Bruker, Ettlingen, Germany).

2.2.2.1. Current density imaging (CDI) and magnetic resonance electrical impedance tomography (MREIT). The Current Density Imaging (CDI) method relies on current-induced magnetic field change in the sample that is detected via phase shift registration by magnetic resonance imaging (Joy, Scott, & Henkelman, 1989). Next, Magnetic Resonance Electrical Impedance Tomography (MREIT) J-substitution algorithm, based on iterative solving of the Laplace equation, was employed to calculate the conductivity map and also the electric field by using CDI data along with known sample geometry and potentials on the electrodes as inputs for the algorithm (Khang et al., 2002; Kranjc et al., 2016).

The two-shot RARE CDI pulse sequence (Serša, 2008) was used to image current density in the biological tissues during application of electric pulses. Delivery of electroporation pulses was synchronized with the CDI sequence using TTL pulses from the NMR/MRI spectrometer to trigger the electric pulse generator. The following imaging parameters were used: field of view 30 mm; imaging matrix 64 × 64; inter-echo delay 2.64 ms. In the sequence, block of electroporation pulses was positioned between the excitation RF pulse and the first refocusing RF pulse. MREIT was performed by using the finite element method with the numerical computational environment MATLAB 2019b (MathWorks, Natick, MA, USA) on a desktop personal computer.

2.2.2.2. Multiparametric magnetic resonance imaging (MRI). A multi-spin-echo (MSE) imaging sequence based on the Carr-Purcell-Meiboom-Gill (CPMG) multi-echo train (Carr & Purcell, 1954) was chosen to acquire T_2 weighted MR images. T_2 mapping was performed before and immediately after the PEF treatment (i.e. total imaging time 18 min after the PEF treatment). The calculation of T_2 maps was performed by the MRI Analysis Calculator plug-in of the ImageJ (NIH; US) image-processing software, fitting raw MSME data at variable TE (n = 8 echoes) ($R^2 > 0.9$).

2.3. Statistical analysis

Significant differences among the results were evaluated by paired samples Student's *t*-test, parametric analysis of variance (ANOVA) and Tukey multiple comparison, with a significance level of 95% ($p < 0.05$). If Shapiro-Wilk test for normality and Levene's test for homoscedasticity of data resulted statistically significant ($p < 0.05$), non-parametric multiple range test Kruskal-Wallis and Holm stepwise adjustment were used, with significant level of 95% ($p < 0.05$) (R Foundation for Statistical Computing, Austria). Results are expressed as mean ± standard deviations of replications (n = 4).

3. Results

3.1. Impedance measurements

For various applied voltage amplitudes and different materials analysed, the ratio between the absolute impedance value after the electroporation ($|Z_2|$) and before PEF treatment ($|Z_1|$) is reported. As a result of β dispersion (i.e. the phenomenon associated with the polarization of the cell membrane), the frequency dependence of the impedance can be observed in plant tissues (Figs. 1, 2 A,C). Considering that low-frequency currents cannot penetrate the intact cell membrane and are restricted to pass via the extracellular space; when electroporation occurs, it will cause an increase of cell membrane permeability, resulting in a decrease in the magnitude of electrical impedance (i.e. low-frequency currents can pass through the intracellular space). Hence, for a better visualization of the dependency of the impedance decrease on the magnitude of the applied electric field, the normalized absolute impedance at sampling frequency of 5 kHz is shown (Figs. 1, 2 B,D). The indicated frequency has been selected considering that, in the case of two-electrode impedance measurements, the method suffers from electrode polarization effects. Thus, a high enough frequency must be used to enable comparison of plate to 4-electrode measurements. Moreover, for both electrodes configurations (4-needle and plate electrodes) it has to be taken into account that the impedance value (Z_2) depends on the pulse parameters applied (i.e. 8 pulses of 100 μs in duration).

In the potato tissue, a consistent drop of the normalized impedance from approx. 1 (i.e. no differences in the impedance measured after electroporation compared to the corresponding value before electroporation) to 0.57 ± 0.07, measured with 4-needle electrodes, has been attained after applying 150 V (Fig. 1B). The same critical electroporation value was found when parallel-plate electrodes (i.e. two-electrode measurements) were used (Fig. 1D), showing a significant drop of the impedance magnitude at 150 V of applied voltage amplitude (i.e. electric field strength of 250 V/cm, calculated as the ratio between the applied voltage and the electrodes distance). For both methods used, i.e. needles and plates, applied voltage amplitudes above 500 V did not result in a further impedance decrease, suggesting that maximum cell membrane permeabilization was achieved using this particular experimental protocol (i.e. number of pulses and pulse duration) (Fig. 1 B,D). Given the similar inter-electrode distance between the two electrode configurations (i.e. 6 mm for plates and 5 mm for needles), the same absolute voltages result in comparable voltage-to-distance ratios, thus in comparable electric field strength (although it is important to remember that the field strength is highly inhomogeneous for needle electrodes).

Apple tissue showed a pronounced and significant decrease ($p < 0.05$) in the normalized impedance when 300 V pulses were applied with 4-needle electrodes (Fig. 2B), as well as when applied with parallel-plate electrodes (U = 300 V, i.e. 500 V/cm voltage-to-distance ratio) (Fig. 2D).

We also used the 4-needle electrodes configuration system to evaluate the anisotropy of skeletal muscles at different levels of PEF (i.e. how impedance varies when electric field vector is aligned predominantly along or across muscle fibres). Results of the absolute impedance ratio with both fibre orientations, parallel (along the fibres) and perpendicular (across the fibres), are reported (Fig. 3B). As opposed to the needle electrode configuration, with plate electrodes the electric field was applied only perpendicularly to the muscle fibre orientation. The absolute impedance ratio for chicken breast showed much smaller differences, compared to plant tissues, when plotted on the same scale as in Figs. 1,2. However, on an expanded scale, the ratio decreases with the amplitude of the applied voltage (Fig. 3 B,D – see inserts). The decrease of the normalized impedance started to be significant ($p < 0.05$) after

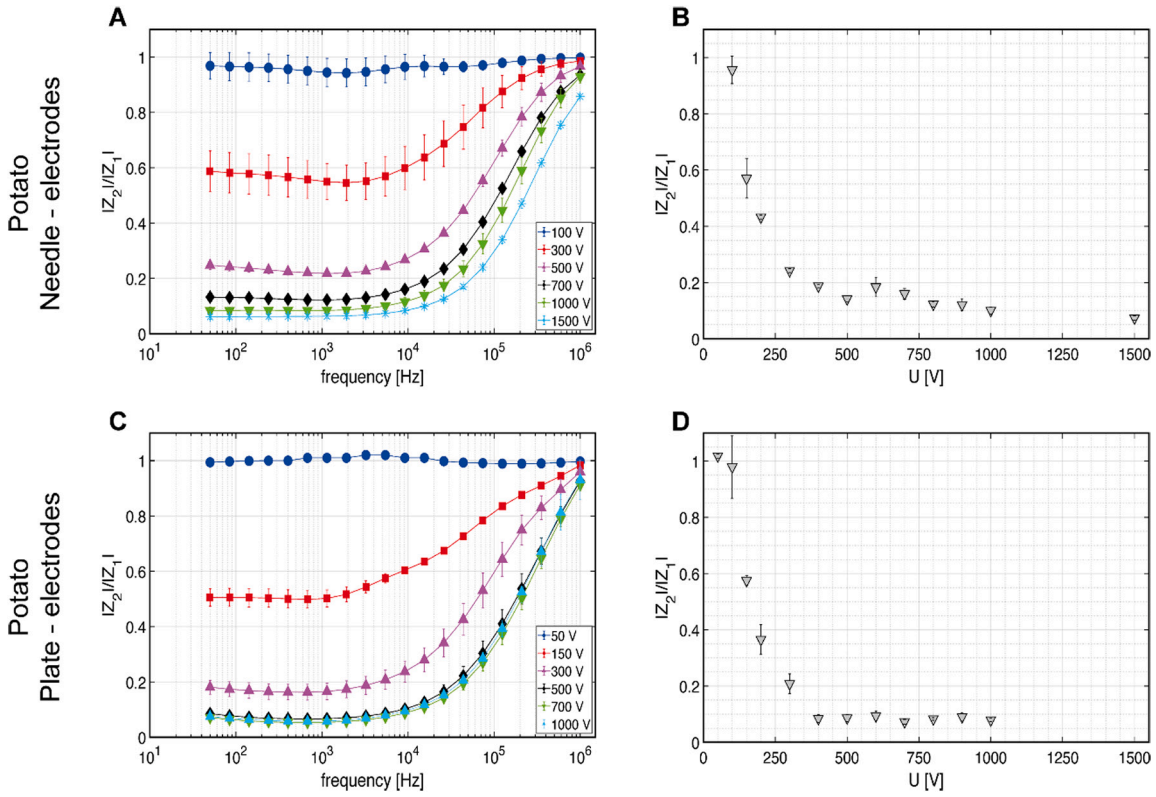

Fig. 1. Potato tuber normalized absolute impedance versus frequency at different applied voltage [U] amplitudes measured with 4-needle-electrodes (A), and plate-electrodes (C) configuration (not all the applied voltages are shown for clarity of presentation). Normalized absolute impedance at sampling frequency of 5 kHz versus the applied voltage [U] measured with 4-needle-electrodes (B), and plate-electrodes (D) configuration. Results are expressed as means ± standard deviations (error bars) of n = 4.

applying a voltage of amplitude of 1200 V, for both needle- and plate-electrodes (Fig. 3 B,D). As shown in Fig. 3B, the electrical impedance spectroscopy did not show any skeletal muscle tissue anisotropy, as the variations of the normalized absolute impedance did not present any statistical difference ($p > 0.05$) between the parallel and the perpendicular orientation of the muscle fibres.

3.2. Voltage-current signal analysis

The changes of the dielectric properties of tissues occurring due to electroporation can also be assessed by analysing the current during the application of electric pulses. Electric current signals can be used to detect changes in the dielectric properties of the cell membrane, and it can thus be used to assess the electroporation process. A typical current signal during PEF application to a tissue consist of a rapid initial increase followed by an exponential decrease (a current spike), which corresponds to the current required for charging of the membrane. When electroporation occurs, the current shows further increase before reaching a constant level at the end of the pulse (schematic presentation in Fig. 4A). This further increase during the pulse is consistent with the cell membrane permeabilization (Cukjati et al., 2007; Pavlin & Miklavčič, 2008). In fact, when cell membrane electroporation / permeabilization increases, the conductivity increases, so the total current increases for constant applied voltage. Fig. 4A depicts the electric current difference between the value of I_{fin} (current reached at the end of the pulse, i.e. 100 μs after initial current increase) and I_{init} (current value at 10 μs after initial current increase, of arbitrary choice) normalized to the current value at the end of the pulse (ΔI_{norm}) for all the investigated specimens. In potato tissue, the permeabilization level was attained at applied voltage of amplitudes of about 100 V for both electrode configurations, needles and plates (Fig. 4 B,C), reaching a maximum permeabilization above 300 V. In apple tissue, when high-voltage pulses were delivered using 4-needle electrodes (Fig. 4B), the maximum increase of current during the pulse has been observed at permeabilizing applied voltage intensities of about 200 V, reaching a saturation level above 500 V. A slightly lower 'critical' electroporation value was found when the PEF treatment was delivered using plate electrodes (Fig. 4C), presenting the highest increase of current at 150 V (i.e. approx. 250 V/cm of the voltage-to-distance ratio). The animal tissue investigated displayed a constant level of current during the pulse at all the applied voltage amplitudes considered.

In addition, measured voltage and current waveforms, when parallel-plate electrode configuration was used, were analysed in terms of conductivity at 100th μs of the pulse (i.e. at the end of the first pulse) (Fig. 5). The conductivity was calculated according to Eq. (2).

$$\sigma = (I/U)(d/S) \quad (2)$$

where I/U is the ratio between current and voltage measured at 100 μs of the pulse, d is the distance between the electrodes, and S the contact surface between the electrode and the sample.

Fig. 5 displays the conductivity change at different applied voltage amplitudes (U). Raw data were fitted following a sigmoid curve (potato $R^2 = 0.90$; apple $R^2 = 0.83$; chicken $R^2 = 0.08$). It can be observed that the conductivity of plant tissues depends on the electric field magnitude. While in the potato specimen the conductivity slowly increases for U between 50 and 500 V, reaching a plateau at $U \geq 600$ V; in apple σ shows an abrupt increase between 100 and 200 V, and saturates at higher voltage amplitudes. Conversely, the conductivity of the chicken sample did not display any increase with increased voltage amplitude. The chicken was subjected to a different range of voltage amplitudes (i.e. between 200 V and 1000 V) than the one used for vegetable tissue (i.e. between 50 V and 1000 V), as the previously reported EIS data (Fig. 3D) clearly showed no changes in impedance for $U = 250$ V. Therefore, we

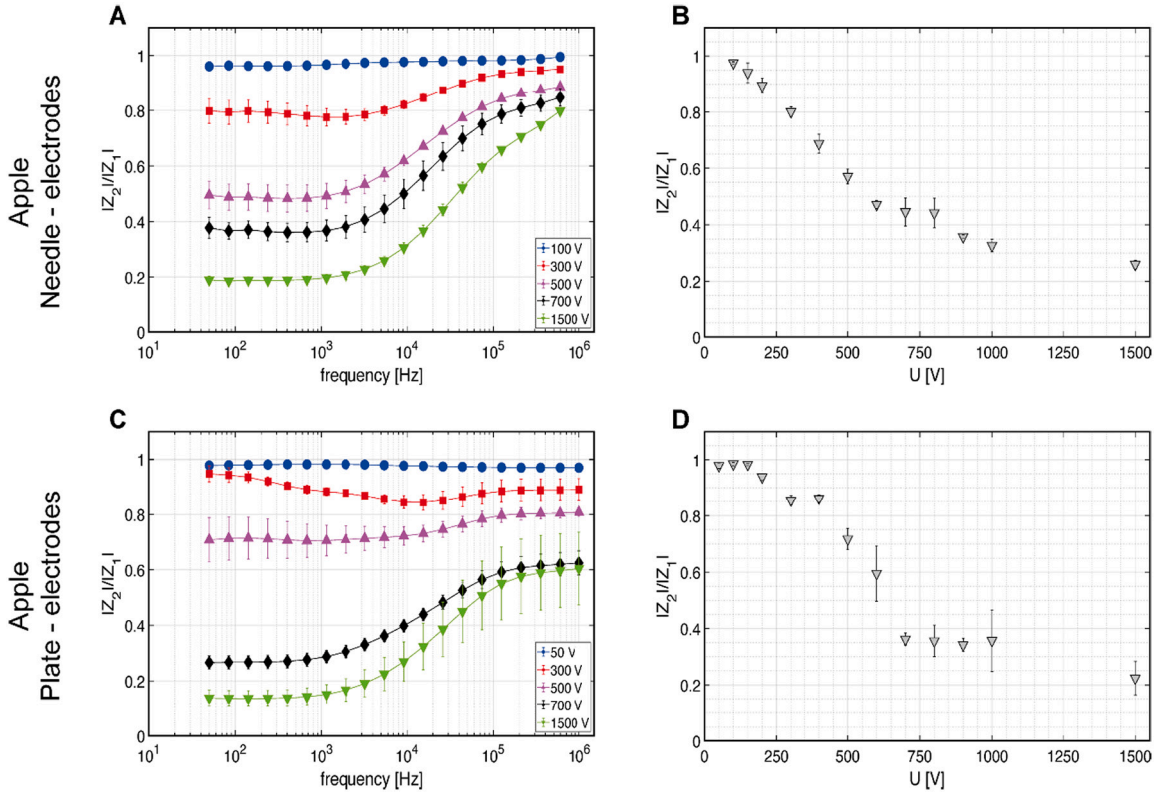

Fig. 2. Apple tissue normalized absolute impedance versus frequency at different applied voltage [U] amplitudes measured with 4-needle-electrodes (A), and plate-electrodes (C) configuration (not all the applied voltages are shown for clarity of presentation). Normalized absolute impedance at sampling frequency of 5 kHz versus the applied voltage [U] measured with 4-needle-electrodes (B), and plate-electrodes (D) configuration. Results are expressed as means ± standard deviations (error bars) of n = 4.

assumed that $U < 250$ V would not affect the tissue with the presented experimental set up.

3.3. *Electric field distributions and T_2 mapping in plant and animal tissues*

By means of MREIT we obtained electric field distributions in the potato, apple, and chicken samples when subjected to high-voltage electric pulses. For each biological sample considered, an optimal voltage amplitude was selected to obtain a good signal-to-noise ratio of MR signals (i.e. potato 750 V; apple 1180 V; chicken 860 V).

In addition to MREIT, T_2 mapping was also performed in the same samples to determine changes of tissue water content that would occur due to the application of electroporation pulses. In fact, T_2 relaxation value is correlated by the proton exchange between water and solutes and by diffusion of water protons through internally generated magnetic field gradients, causing magnetic susceptibility differences in the tissue exposed to the magnetic field, such as interfaces between air and liquid-filled pores. Therefore, T_2 values reflect – to a certain extent – the structure of the sample based on its water content (Defraeye et al., 2013). In our study, the effect of PEF treatment on T_2 relaxation times was evaluated in potato tuber, apple tissue and chicken skeletal muscle (Fig. 6) by measuring T_2 values before and after the application of the electroporation pulses. An area crossing the middle of the samples, with electrodes included, was taken into consideration as this area was exposed to a wide range of electric field values (solid lines in Fig. 6). In case of potato tuber, T_2 values obtained before application of high-voltage pulses (dotted line in Fig. 6A) revealed different water content in the tuber tissue's heterogeneous structure. After application of high-voltage pulses (dashed line in Fig. 6A), T_2 values decreased in areas exposed to the electric field strengths above approx. 250 V/cm, consistent with threshold value determined by EIS and current analysis. Interestingly, structures with a higher water content, i.e. with higher T_2 values, released more water after application of the pulses (measured as a larger decrease of T_2 values) compared to areas with a lower water content. The obtained trend of the T_2 decrease due to the application of high-voltage pulses is in accordance with the results obtained in the only comparable MR study on potato tuber (Kranjc et al., 2016), although that study did not take the pre-existing heterogenous distribution of water content within the tuber into consideration.

Apple exhibited less variation in T_2 values along the plane of observation compared to potato tuber. Slow decrease of T_2 values (dotted line in Fig. 6B) towards the edge of the apple sample suggests a higher water content in the centre of the sample. After the application of high-voltage pulses, we obtained a decrease of T_2 values (dashed line in Fig. 6B) in the middle of the apple and a slight increase of T_2 values towards the edge, presumably as a consequence of alteration of the tonoplast and the plasma membrane, causing a damaged compartmentalization that initiated diffusion of intracellular water towards extracellular spaces (Dellarosa et al., 2018, 2016). Due to limitation of the method, the apple tissue was exposed to higher applied voltage than potato, therefore we were not able to determine the electroporation threshold.

T_2 mapping of untreated and PEF-treated chicken breast fillets was also performed to understand if the applied PEF treatment could contribute to a change in the protein-water interaction and could lead to a loss of water from the sample. In our study, the application of PEF treatment did not induce any considerable change of the T_2 relaxation times (Fig. 6C).

4. Discussion

The aim of our study was to analyse the effect of PEF treatment by

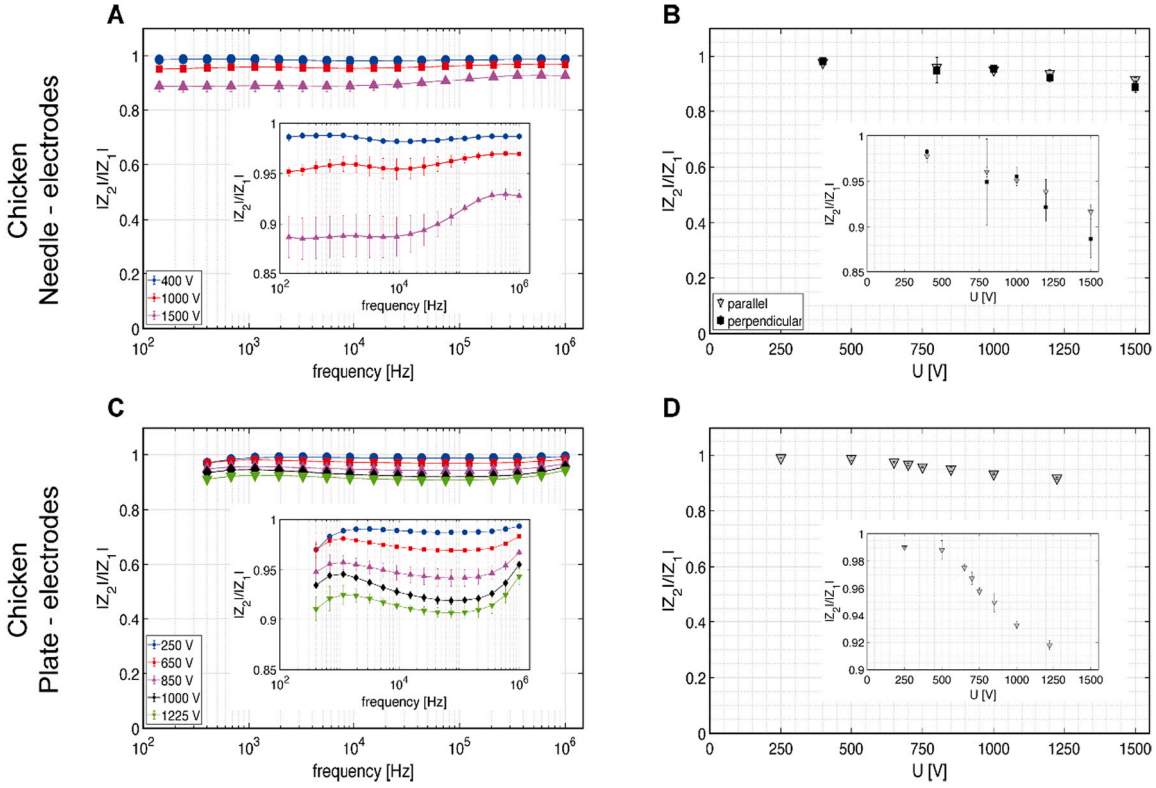

Fig. 3. Chicken breast muscle normalized absolute impedance versus frequency at different applied voltage [U] amplitudes measured with 4-needle-electrodes (A), and plate-electrodes (C) configuration (not all the applied voltages are shown for clarity of presentation). Normalized absolute impedance at sampling frequency of 5 kHz versus the applied voltage [U] measured with 4-needle-electrodes (B), and plate-electrodes (D) configuration. Measurements with 4-needle electrodes were performed applying high-voltage pulses parallelly or perpendicularly to the muscle fibre orientation. Results are expressed as means ± standard deviations (error bars) of n = 4.

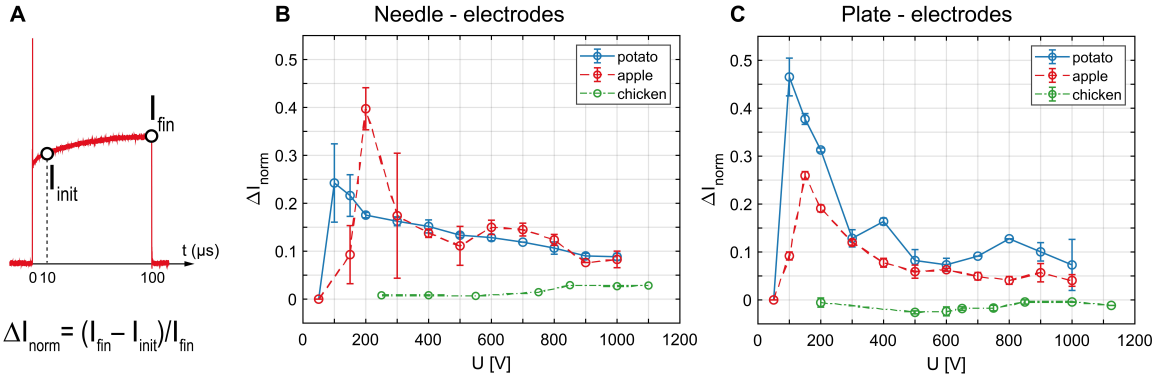

Fig. 4. Normalized electric current difference (ΔI_{norm}) between the final (I_{fin}) and initial (I_{init}) current values of the pulse portion with current increase following a decaying exponential function during the pulse (A). The difference was normalized to the current value at the end of the pulse (I_{fin}). An example of ΔI_{norm} obtained in potato, apple and chicken tissue exposed to electric pulses applied using needle (B) and plate (C) electrodes. U – applied voltage.

measuring electrical properties and water mobility in different raw biological materials of plant and animal origin, and to determine the level of membrane permeabilization due to electroporation.

Appropriate choice of methods used to test the cell membrane integrity in different multicellular systems could be of value in both food industry and research setting for determining and selecting adequate PEF treatment conditions and to monitor the lab- / industrial–scale processes. For successful application of the PEF treatment, it is important to understand that methods used to assess the degree of electroporation depend, among other, on highly inhomogeneous properties and complexity of food materials (Mahnič-Kalamiza et al., 2014), such as electrical properties, porosity, and structure. For this reason, the goal of our investigation was to explore the level of membrane permeabilization in diverse food materials (vegetables and meat), employing different methods used for the assessment of membrane permeabilization (i.e. EIS, current-voltage measurements, MRI, and T_2 mapping).

In general, the impedance of PEF-treated plant tissues (Figs. 1 and 2) shows a strong dependence on the magnitude of the applied voltage / electric field strength. Moreover, it can be observed that the analysed vegetables exhibit a non-linear decrease of their impedance modulus following the PEF treatment, suggesting the thresholding nature of the phenomenon (Figs. 1 and 2 B). The remarkable decrease of impedance

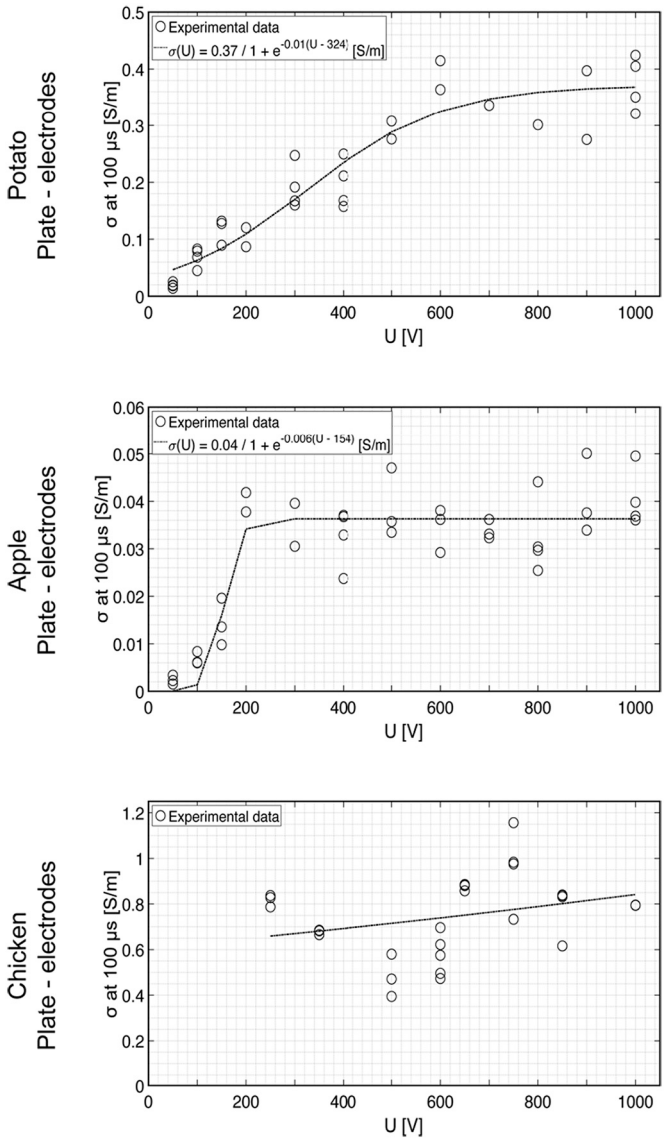

Fig. 5. Conductivity values at 100th µs of the first pulse recorded using plate electrodes configuration versus the amplitude of applied voltage [U], and the sigmoid function used to model the data.

can be explained by permeabilization of the cell membranes. In fact, since the cell membrane exhibits high impedance prior to the treatment, when electroporation occurs, it becomes more permeable, leading to a decrease of the said impedance (Ivorra, 2010). When analysing the impedance variation with different electrodes (needle and plate), the impedance decrease is reached at the same threshold levels. Thresholds, however, were found to be lower in potatoes than apples ($U \sim 150$ V; 250 V/cm for potatoes, $U \sim 300$ V; 500 V/cm for apples). The analysis of the current measured during the delivery of pulses can also be used as a simple method to detect changes in the dielectric properties of the tissue exposed to PEF treatment. When electroporation occurs, the cell membrane conductivity and permeability increase, which is reflected in the increase of the current during the pulse. As for the decrease in impedance, the maximum current increase was found to occur after a certain 'threshold' permeabilization level. The same as found by impedance analysis, the threshold as determined by current analysis was shown to be lower in potato than in apple ($U \sim 100$ V for potatoes, $U \sim 150$ V for apples) (Fig. 4). Observing the trend of measured conductivity at the end of the first pulse in plant tissues, reported in Fig. 5, a simple interpretation of these results would be that potato tissue is, from the water distribution perspective, much more homogeneous, and its conductivity increases almost linearly with the subsequent delivered electroporation pulses as more and more cells are electroporated since this renders a proportionally greater amount of extracellular liquid available for ionic transport (and thus current conduction). In the apple tissue, due to large amount of air in the tissue (i.e. higher porosity than potato) and dis-associated domains of tissue caused by pockets of air, higher field strength is necessary to cause a sufficient release of liquid. In fact, once a sufficiently conductive pathway in tissue is formed by a critical amount of released liquid, a step-like increase of conductivity is observed. Hence, different 'threshold' values of the magnitude of the applied voltage have been determined in relation to the different methods/plant tissues analysed in this study. The obtained results were not expected. For instance, since the apple cell size (≈ 130 µm diameter) is larger than that of potato (≈ 35 µm diameter), a lower electroporation threshold was expected in apple (i.e. the critical transmembrane potential is reached with external electric fields proportionally decreasing with the cell radius (Kotnik, Miklavčič, & Slivnik, 1998)). Similar observations were also previously reported in studies employing EIS for the evaluation of PEF-treatment efficacy in orange and potato tissues, where the latter, constituted of smaller size cells, was more efficiently electroporated when voltage-to-distance ratios (U/d) equal to 400 V/cm and 1000 V/cm were applied, respectively, employing two-plate electrodes (Ben Ammar, Lanoisellé, Lebovka, Van Hecke, & Vorobiev, 2011). The reported discrepancies could be explained by the dissimilar characteristics of the plant materials that could affect the electrical parameters, such as the electrical conductivity change (i.e. the ratio between the electrical conductivity of electroporated tissue and the electrical conductivity of intact tissue) (Ben Ammar et al., 2011); cell packing density; spatial distribution of air; overall tissue moisture; and initial cell turgor pressure. Moreover, plant cells are embedded in a matrix of cell walls, that can also affect passive electrical properties of biological systems. In fact, the charged groups of polysaccharides and other polymers that constitute the porous structure of cell walls contribute to the bulk

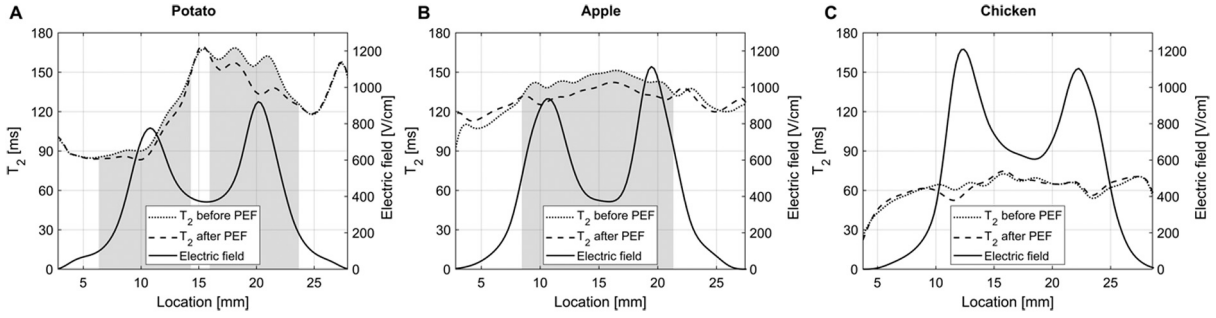

Fig. 6. T_2 – values acquired before (dotted lines) and after PEF treatment (dashed lines) in potato tuber (A), apple tissue (B), and chicken breast (C) along an area crossing the middle of the sample. Electric field (solid lines) was obtained by means of MREIT. Ranges of electric field corresponding to variations of T_2 value are marked by gray color background.

electrical properties of the tissue, and the change of ionic concentration in the extracellular media that may occur after electroporation (i.e. release of the cytoplasmatic fluid) could, in turn, induce changes in the measured electrical characteristics (Markx & Davey, 1999).

When chicken breast was analysed by applying the same train of 8 high-voltage pulses, we did not observe changes in its electrical properties as we did in plant tissue. In fact, only a small (but progressive with the increase of the applied voltage) decrease of the absolute impedance was shown (Fig. 3), which could be ascribed to a slight heating of the tissue subjected to electric pulses. Changes in current/conductivity observed (Figs. 4 and 5) were negligible. Similar ex vivo experiments have previously been conducted on chicken and beef liver (Kranjc, Bajd, Sersa, Woo, & Miklavcic, 2012; Langus et al., 2016) where, contrary to observations in the present study, an exponential increase in current amplitude has been shown when applying the same train of pulses. The discrepancies found, although surprising, can be explained by the different anatomy of chicken breast vs. beef liver, the latter being constituted of hepatocytes that are not affected by *post-mortem* degradation processes typical of muscle fibres (Zaefarian, Abdollahi, Cowieson, & Ravindran, 2019).

The electroporation assessment methodologies we used in this study are largely associated not only with the degree of cell permeabilization, but with other secondary effects. For instance, some of the consequences of membrane permeabilization correspond to structure modifications and intracellular fluid leaking outside the cell (Puértolas, Luengo, Álvarez, & Raso, 2012). The latter could play an important role in affecting the measured electrical properties of plant cells, characterised by high gradient of osmotic pressure between the intra- and extracellular fluids. On the other hand, *post-mortem* skeletal muscle undergoes a series of physical and biochemical modifications related to myofibrillar structure, cytoskeleton, and membranes (Schreurs & Uijttenboogaart, 2000). Soon after the onset of rigour, plasma membrane and the membrane of the sarcoplasmic reticulum degenerate from their normal laminar structure, increasing their degree of porosity (Damez, Clerjon, Abouelkaram, & Lepetit, 2008). *Rigor mortis* onset in poultry is more rapid than in bovine and porcine muscles since, chicken breast in particular, is mainly composed of fast-twitching fibres that are associated with the anaerobic glycolysis (Schreurs & Uijttenboogaart, 2000). As rigour progresses, the space for water to be held in the myofibrils is reduced, and fluid can be forced into the extra-myofibrillar spaces, where it is more easily lost as drip (Huff-Lonergan & Lonergan, 2005). Therefore, it is quite possible that, at the time we performed treatment and analysis (ca. 48 h *post-mortem*), the muscle fibre membranes were already significantly degraded (Schreurs & Uijttenboogaart, 2000), and so no further changes in the electrical properties were detected following the PEF treatment.

To confirm this hypothesis, our experimental design included the characterization of the food tissue response in terms of water mobility to different levels of electric field strength by the MRI technique. By employing T_2 relaxometry measurements, it is possible to obtain information related to the water content and the interaction of water with the surrounding macromolecules. Electroporation pulses can induce structural changes, such as the cell membrane damage, affecting NMR relaxation rates. In fact, the primary effect of PEF treatment is related to cell membrane electroporation and to a consequential release of the intracellular content. We observed a general decrease of T_2 in plant tissues (Fig. 6 A,B) that can be ascribed to the loss of compartmentalization and diffusion of intracellular water and ion leakage through the tonoplast and the plasmalemma membranes, resulting in inner morphology modifications (e.g. overall volume shrinkage) and in different water-solutes interactions, as already reported previously (Dellarosa et al., 2018; Hills & Remigereau, 1997; Kranjc et al., 2016). In our study, we also observed that potato specimens exhibit a larger variation of T_2 relaxation rates compared to apple, which could conceivably be attributed to a more extensive (re)distribution of intracellular fluids. This could explain the lower electroporation 'threshold' we detected in potato tissue (as compared to apple) when measuring the electrical properties. Moreover, the extent of T_2 changes in PEF-treated potato and apple parenchyma we observed are comparable to those already reported in literature (Dellarosa et al., 2018; Kranjc et al., 2016). On the contrary, we were unable to detect changes in bulk water mobility in chicken breast muscles under the present experimental conditions by means of the MR imaging. Similar observations were previously reported in PEF-treated post rigour beef muscle (72 h *post-mortem*, electric field applied 1.9 kV/cm), where no major changes of the T_2 relaxation times were observed, suggesting that the PEF treatment did not induce any modification of the muscle fibre membranes, while a significant weight loss was due to the fluid in the extracellular space (O'Dowd, Arimi, Noci, Cronin, & Lyng, 2013).

5. Conclusions

In our study, the application of PEF treatments to different matrices of food interest (plant tissues and skeletal muscles) was experimentally investigated, and the level of membrane permeabilization due to electroporation was explored by means of various PEF assessing methodologies. Electrical properties measurements were performed comparing two different electrode configurations, a 4-electrode system with needles inserted in the tissues, and a plate-electrode system in contact with the surface of samples. In the plant tissues considered (potatoes and apples) we demonstrated that data recorded before and after the high-voltage pulses are significantly influenced not only by the structural characteristics of biological tissues (e.g. cell size), but that the exchange of fluids between intra- and extra-cellular environments dramatically influences the electrical properties. The same effect was observed analysing the conductivity change by monitoring current waveforms. Nevertheless, skeletal muscle used in this study did not yield similar results, which demonstrates that the aforementioned electroporation assessment methods must be used critically and with caution for PEF treatment optimization. Magnetic resonance electrical impedance tomography for monitoring the electric field distribution and T_2 mapping confirmed that the plant cell membranes' breakdown due to electroporation caused a significant decrease of T_2 relaxation times, most probably due to the release of intracellular content into the extracellular space. On the contrary, the muscle fibres seemed to be unaffected by the PEF treatment. We assume this is a consequence of alteration of sarcoplasmic membranes caused by *post-mortem* biodegradative processes. The findings of this research can be of support in the future selection of meat conditions (i.e. aging time) for the effectiveness of PEF treatment applications. This work provides important insights and calls for critical choice of electroporation assessment methods, also with respect to matrix characteristics (e.g. physiological state) in determination of PEF treatment conditions.

Conflict of interest and authorship conformation

All authors have participated in drafting the article or revising it critically for important intellectual content and have approved the final version. Details on authors' individual contributions are found in the separate Author statement document.

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