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The forgotten method? Pulsed electric field thresholds from the perspective of texture analysis

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ABSTRACT

Pulsed electric field (PEF) technology has found applications in various industrial food sectors, including the potato industry, winemaking, biorefinery, and juice extraction, among others. The practical implementation of PEF technology in the food industry is however still hindered by several challenges. The detection and quantification of PEF effects are complex due to the variable characteristics and properties of raw materials, including cellular composition, structural organization, textural properties, and tissue porosity. Moreover, the PEF treatment parameters (e.g., pulse amplitude, duration, shape, rate), and process parameters (e.g., temperature, pH, medium conductivity) further complicate the optimization of PEF protocols, requiring a case-by-case approach. Knowledge of treated material properties and their functional dependence on PEF is a crucial prerequisite to informed, intelligent design of treatment protocols. We present an experimental study designed to gain insights into the mechanism behind the changes in textural properties induced by PEF in both plant and animal tissues. These changes in texture are then compared with findings from our previous study on electrical impedance, to highlight how different methods of detection of PEF-induced changes in tissue can yield vastly different results based on the method of analysis used depending on tissue properties. Furthermore, texture analysis unveiled the less-explored effects of PEF treatment on electroosmosis phenomena in both plant and animal tissues. We provide a comparative analysis between plant and animal tissues to elucidate the differences in deformation resulting from PEF treatment. We thus demonstrate how important it is, be it in the development phase or for process control during industrial operation, to choose an appropriate method of characterising PEF-induced changes in tissue to avoid under- or overtreatment.

1. Introduction

Pulsed electric field (PEF) technology involves applying short-duration electric pulses, typically ranging from nanoseconds to milliseconds, using electric field strengths ranging from 0.1 to 80 kV cm^{-1} (Vorobiev and Lebovka, 2008). The application of an external electric field of sufficient strength can increase cell membrane permeability and tissue conductivity, which is commonly attributed to the formation of hydrophilic pores in the cell's phospholipid bilayer (Kotnik et al., 2019). PEF technology is gaining importance and popularity in the food industry due to its simplicity, compatibility with industrial parameters, low energy consumption, and ability to minimize food deterioration (Pandiselvam et al., 2022). It can be easily integrated with other food processing steps, such as pressing, thermal treatment, etc. (Barba et al., 2015), or used independently for inactivation of foodborne pathogens and spoilage microorganisms in liquid food (Álvarez et al., 2006).

PEF technology has found applications in various industrial food sectors, including the potato industry (Hill et al., 2022), winemaking (Puértolas et al., 2010), biorefinery (Polikovsky et al., 2016), and juice extraction (Bazhal et al., 2001), among others. However, the practical implementation of PEF technology in the food industry is still hindered by several challenges. The detection and quantification of PEF effects are complex due to the variable and often specific characteristics and properties of raw materials, including cellular composition, structural organization, textural properties, and tissue porosity. Additionally, PEF treatment parameters, encompassing pulse amplitude, duration, shape, pulse delivery rate, and process parameters, such as temperature, pH, and conductivity of the media, further complicate optimization of PEF

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protocols, requiring a case-by-case approach often relying on trial-and-error.

To gain a better understanding of the potential effects of PEF on food processing, comprehensive investigations of structural alterations occurring in plant and animal tissues are crucial. Among the various parameters studied, changes in the textural properties can provide valuable qualitative characterization of PEF-induced changes. Electroporation is well-documented to impact the texture of plant tissues, resulting in softening of the tissue, attributed to cellular breakdown (Grimi et al., 2009; Lebovka et al., 2004). Previous studies have indicated that PEF treatment enhances and facilitates the release of liquid during mechanical compression in plant materials (Bouzrara and Vorobiev, 2003; Lebovka et al., 2003; Mahnič-Kalamiza and Vorobiev, 2014; Vorobiev and Lebovka, 2008, 2006). When PEF treatment is applied to plant tissues, cells experience partial or complete loss of turgor pressure (De Vito et al., 2008; Lebovka et al., 2014), resulting in cell debonding and subsequent tissue deformation. Instrumental texture studies such as texture profile analysis (TPA) (Karki et al., 2023) and Warner-Bratzel shear force measurements (Bolumar et al., 2022), have been used to determine the effects of PEF post-treatment on meat tenderness. However, to the best of our knowledge, limited or no textural studies have been conducted on animal tissues to determine the effects of PEF during the application of the electrical pulses. Additionally, while plant tissues rely on the structural support provided by their rigid cell walls, animal cells lack cell walls, resulting in a more flexible structure.

It should be noted that textural parameters indirectly reflect the changes induced by PEF and can vary depending on the mode of textural analysis. Therefore, it is important to compare the texture analysis results with other PEF assessment methods to accurately interpret PEF data. Changes in the electrical properties of food tissues, particularly increases in conductivity, have been extensively studied as a means of detecting electroporation. The conductivity increases are related to increased permeabilization of cell membranes, leading to the rapid release of ions from the intracellular environment to the extracellular spaces (Angersbach et al., 2002; Vorobiev and Lebovka, 2006). Moreover, the effectiveness of PEF treatment depends on the local distribution of the electric field, prompting previous research on characterizing the electric field distribution and its effects in plant and animal tissues using techniques such as magnetic resonance imaging (MRI) (Genovese et al., 2023; Kranjc et al., 2016) and microscopic imaging techniques (Faridnia et al., 2015).

Building upon our previous studies, (Genovese et al., 2021, 2023), the main objective of this study is to gain insights into the mechanism behind the changes in textural properties induced by PEF in both plant and animal tissues. Specifically, we aim to provide a comparative analysis between plant and animal tissues to elucidate the differences in structural properties resulting from PEF treatment. In plant tissues, the primary factor contributing to changes in textural properties is the reduction of tissue turgidity, while animal tissues generally lack turgor pressure and cell walls, leading to different deformation mechanisms. We conducted experiments using apple parenchyma and potato tuber as representative plant tissues, selected for their distinct levels of tissue complexity, water content, and water distribution. Additionally, chicken broiler *Pectoralis major* was selected as a reference animal tissue. During the application of PEF treatment, compression tests were performed simultaneously, allowing to assess the textural changes in the tissue. The results obtained from compression tests were then compared to the changes in the electrical properties of the tissues analysed through measurements of electrical impedance.

2. Materials and methods

2.1. Raw materials

2.1.1. Plant tissues

The plant tissues used in this study were apples (*Malus domestica*, cv 'Golden Delicious'), and potatoes (*Solanum tuberosum*, cv 'Agata'), purchased at the local market (Ljubljana, Slovenia). Before the experiments, structurally similar sections of each plant type were first selected and then manually cut with a sharp stainless-steel cork-borer into cylindrical samples 0.6 cm high and 2.6 cm in diameter. At the time of the experiments, the soluble solids content of the apples was 11.3 ± 0.3 °Brix, measured with an optical Brix refractometer (model RBO, Optech, Germany). For each sample, the analyses were carried out in triplicate.

2.1.2. Animal tissue

We used boneless and skinless *Pectoralis major* muscles obtained from the same flock of broiler chickens (Ross 308 breed, age 46 weeks, average weight 2.892 kg), which were stored at 1 °C before the experiments. Each sample was obtained from the cranial part of the breast and cylindrical disks of 2.6 cm diameter and 0.6 cm height were manually cut from each sample using a sharp stainless-steel cork-borer. The muscle fibres in each sample were aligned along the wider dimension, i.e., along the axis parallel to the cylinder's diameter. Analyses were performed at room temperature in triplicate from the same batch of chicken breasts.

2.2. Experimental setup

2.2.1. Force-response (texture) analysis

The mechanical properties of apples, potatoes, and chicken samples were studied simultaneously during the application of PEF treatment, using the method proposed by Boussetta et al. (2009). A PEF treatment chamber consisting of two parallel stainless-steel plate electrodes, spaced 6 mm apart, was used to hold the cylindrical samples and apply the high-voltage electroporation pulses and compression simultaneously. For this purpose, a texture analyser (model Inspekt Solo 1kN, Hegenwald & Peschke, Germany) was used, and the treatment chamber was placed under the piston applying a constant force of 5 N, 10 N, 20 N, or 30 N at a steady (maximum) velocity of 20 mm/min. Each sample was pre-compressed with the target force for 30 s to remove air pockets in the tissue and ensure good contact between the electrodes and the sample. The samples were then treated with 8 rectangular monopolar pulses of 100 μs duration and a pulse repetition rate of 1 s^{-1} (schematic presentation in Fig. 3A). Different pulse amplitudes were used for each treatment: from 50 V to 900 V for plants, and from 500 V to 1000 V for the chicken samples (for additional information on PEF treatment parameters refer to Table A.1 in the Appendix). The reason for using a different range of pulse amplitudes for chicken compared to plants is based on previously reported data (Genovese et al., 2021), which demonstrate that voltage amplitudes below 500 V (and same sample geometry, thus comparable voltage-to-distance ratio) had no noticeable effects on chicken breast. For each PEF treatment, the piston displacement during 300 s of compression (after the 30 s pre-compression phase) was recorded, thus recording the deformation of the sample along the axis of pressure application (i.e. perpendicular to the electrodes and parallel to the direction of the electric field strength vector). The deformation of the sample is defined and presented as $\frac{d-d_0}{L}$, where d and d_0 are the piston displacement point after application of the PEF treatment and the initial piston displacement (i.e., before application of the PEF treatment), respectively, and L is the original height of the sample.

2.2.2. Current-voltage and electrical impedance measurements

For each PEF treatment, as described in section 2.2.1, the delivered voltage and current were measured and recorded using a high-voltage

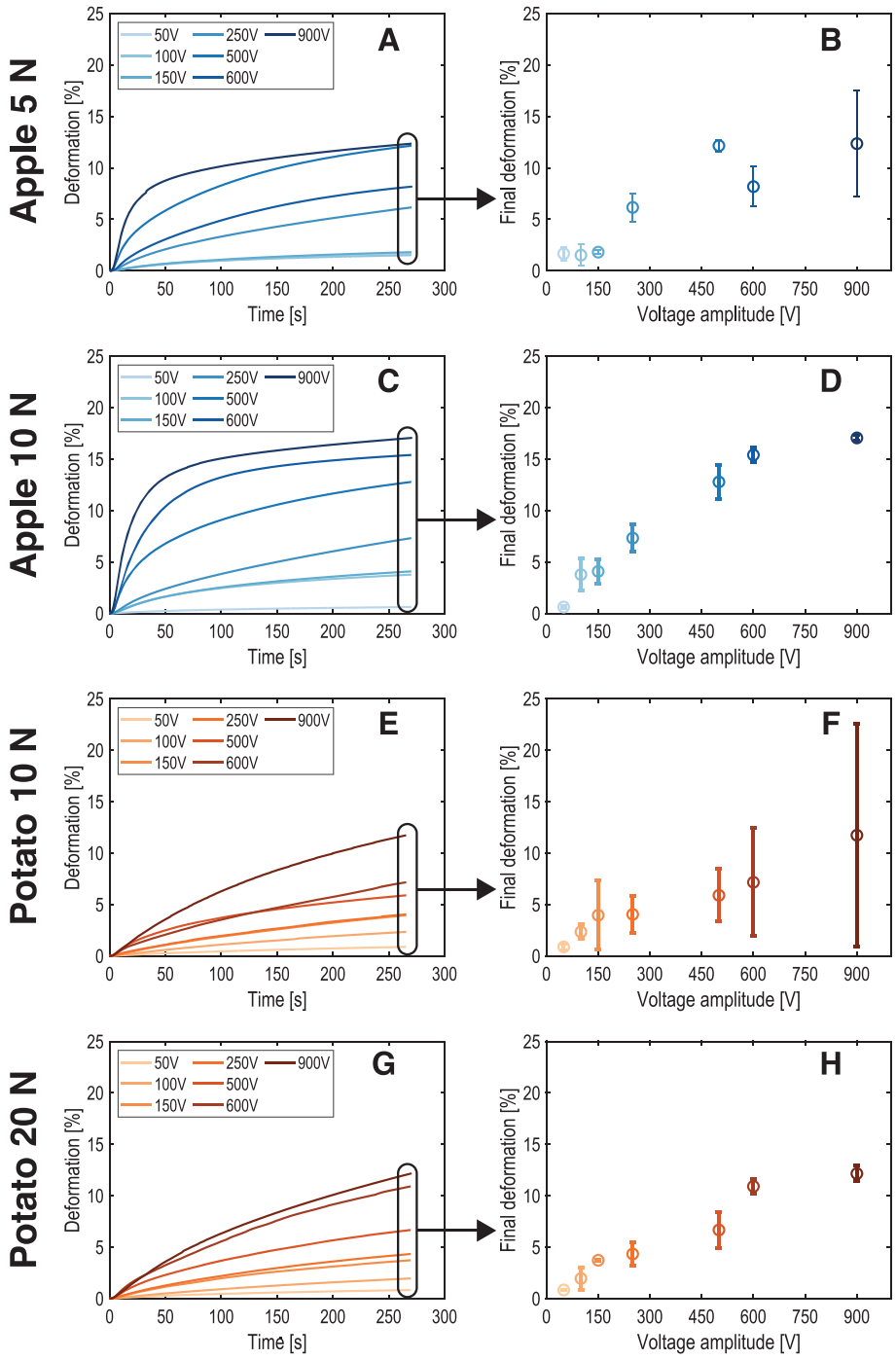

Fig. 1. A, C, E, G: Deformation curves (average results of replicates and presented in % of sample height) from the point of PEF application for apples compressed at constant forces of 5 N and 10 N, and for potatoes compressed at constant forces of 10 N and 20 N subjected to different amplitudes of applied voltage amplitude. **B, D, F, H:** Final deformation (in % of sample height) at the end of the compression tests as a function of the amplitude of the applied voltage amplitude. The results are given as mean values ± standard deviations (error bars) of n = 3.

probe (model HVD3206A, LeCroy, USA) and a current probe (model CP031A, LeCroy, USA) connected to a sequencing DSO (digital storage oscilloscope, model HDO6104A-MS, LeCroy, USA). The recorded voltage and current data were analysed using MATLAB 2019b software (MathWorks, USA). In addition, the electrical impedance of samples that underwent various PEF treatments were quantified using a precision LCR meter (model E4980A, Keysight Inc., USA) while connected to a treatment chamber composed of two parallel plate electrodes made of stainless steel, as previously reported in the study conducted by Genovese et al. (2021). Briefly, parallel capacitance (C_p) and parallel resistance (R_p) at multiple frequencies were measured on samples before the PEF treatment and after the treatment (i.e. between 3 and 5 s after the pulse application) in the frequency range of 50 Hz to 1 MHz by applying a 100 mV (for plant tissues) and 500 mV (for chicken, due to the higher conductivity compared to plant tissues) peak voltage to the electrodes. Data were acquired using an in-house developed software for the LCR meter control and data capture (based on the Arduino and National Instruments LabVIEWsoftware platforms).

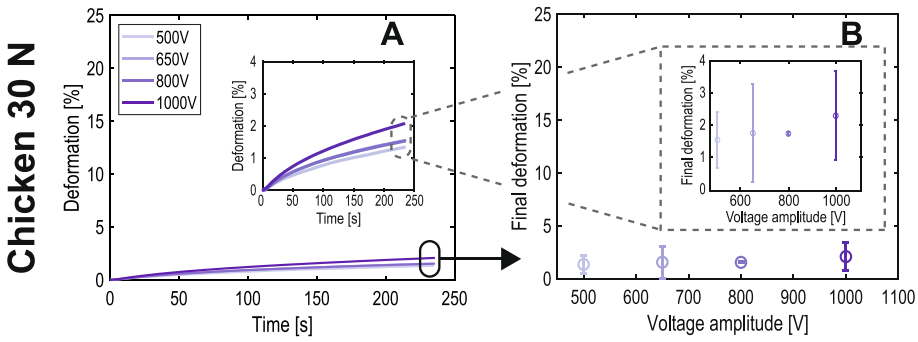

Fig. 2. A: Deformation curves (average results of replicates and presented in % of original sample height) from the point of PEF application for chicken compressed at constant force of 30 N and subjected to different voltage amplitudes. **B:** Final deformation (in % of sample height) at the end of the compression tests as a function of the voltage amplitude. The results are given as mean values ± standard deviations (error bars) of n = 3.

2.2.3. Statistical analysis

Significant differences among the results were evaluated by parametric analysis of variance (ANOVA) and Tukey multiple comparison, with a significance level of 95 % ($p < 0.05$). When the Shapiro-Wilk test for normality and Levene's test for homoscedasticity of the data yielded statistically significant results ($p < 0.05$), the Kruskal-Wallis non-parametric multiple range test and Holm's stepwise adjustment with a significance level of 95 % ($p < 0.05$) were used. Results are expressed by means ± standard deviations of replications ($n = 3$). Statistical analysis was performed in R statistical software (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. PEF-induced changes in plant tissues

The deformation of apple fruit and potato tuber tissue under different compressive forces, as defined in section 2.2.1, following the application of voltages of different magnitudes is shown in Fig. 1 (A, C, E, G). In addition, Fig. 1 (B, D, F, H) shows the correlation between the final deformation (i.e. the deformation at the end of the compression test) of apples and potatoes and the magnitude of the applied voltage for different compression forces (5 N and 10 N for apples; 10 N and 20 N for potatoes). Note that the pre-compression step of 30 s was subtracted from the data (a schematic illustration of analysis steps can be seen in Fig. 3A). The control curves (compression at 0 V) are so close to 0 deformation as to overlap the abscissa and are intentionally omitted from the plots for improved clarity of presentation. The observed trend of increased final deformation in the studied plant tissues as the voltage amplitude is increased suggests a correlation between the two variables. However, it is important to acknowledge that the final deformation of the sample is influenced not only by the voltage amplitude, but also by the compressive force applied during the experiment. Lower compressive forces, such as 5 N for apples and 10 N for potatoes, resulted in less consistent data, particularly at higher applied voltages and established electric fields (as indicated by the large error bars denoting the standard deviation in Fig. 1B, F). This suggests it is necessary to use a compressive force that is sufficiently strong to detect uniform deformation of the target tissue without causing any mechanical damage. In our study, a compressive force of 10 N for apples and 20 N for potatoes was chosen (as these were the forces that resulted in the most consistent data) to evaluate the effect of PEF treatment on the textural properties of plant cells. The data presented in Fig. 1 (C, G) explain the relationship between PEF treatment (under the conditions used in this study) and the deformation of plant tissues with different levels of complexity of structure (potato having more complex structure than apple (Genovese et al., 2023)), and specific biomechanical properties.

It is generally accepted that the application of an electric field of sufficient magnitude and duration (pulse length and number) to plant tissues can cause a non-thermal disruption of cell membranes, including the vacuolar membrane, leading to a partial or complete loss of the cell turgor pressure (De Vito et al., 2008; Lebovka et al., 2014). The turgor pressure in plant cells, upheld by the water-filled vacuole, maintains an internal hydrostatic pressure against the cell wall. The loss of turgor pressure causes cell debonding and reduction of the mechanical modulus of vegetable tissue, leading to tissue deformation. The extent of deformation depends on the strength of the electric field (voltage amplitude), on the time the sample is exposed to the field (treatment duration, i.e., pulse length times their number), and also on the composition (including the presence of starch or other components, cell packing density, tissue porosity), and on the viscoelastic properties of the tissue (Fincan and Dejmek, 2003; Mahnič-Kalamiza et al., 2015). Apple tissue comprises extracellular spaces that are occupied by air, accounting for between 25 % and 30 % of the tissue's entire volume, approximately. This tissue domain is characterised by high compressibility, resulting in a greater overall deformation of the sample compared to the densely packed tissue structure of potato (in Fig. 1 comparison between D and H). In our study, we found that under the PEF treatment conditions tested, apple tissue achieved a total volume reduction of about 15–16 % at the highest applied voltage amplitude (900 V, 1500 V/cm electric field calculated as the ratio between the applied voltage and the inter-electrode distance), compared to about 12 % reduction measured in potato tissue at the same applied voltage amplitude. However, it is still unclear how the proportion of disrupted cells relates to PEF-induced textural alterations. In tissues with large amount of interstitial air spaces, such as majority of apple varieties, an external applied load allows for the cells to re-arrange to fill the empty extracellular volume, while potatoes, with their low interstitial air content, exhibit very little overall volume reduction on the account of air loss. In potatoes, the volume loss is due almost entirely through loss of turgidity and intracellular liquid, meaning that for potato tissue to deform, cells must be sufficiently electroporated. These findings highlight the importance of considering both the technique of textural experimentation and the properties of plant cells when interpreting PEF data and suggest that comparison with other PEF assessment methods is necessary (e.g., impedance).

3.2. PEF-induced changes in animal tissues

To demonstrate that the deformation induced by PEF in plant tissue is indeed a consequence of 1) air loss and 2) intracellular liquid/turgor pressure loss, we conducted "control" experiments using chicken samples as reference animal tissue. Unlike plant tissues, animal tissues lack cell walls and do not possess the turgor pressure component observed in plant cells.

Fig. 2 presents the deformation analysis of a chicken sample compressed under a constant force of 30 N and exposed to various amplitudes of applied voltage (Fig. 2A). For the chicken sample, a higher

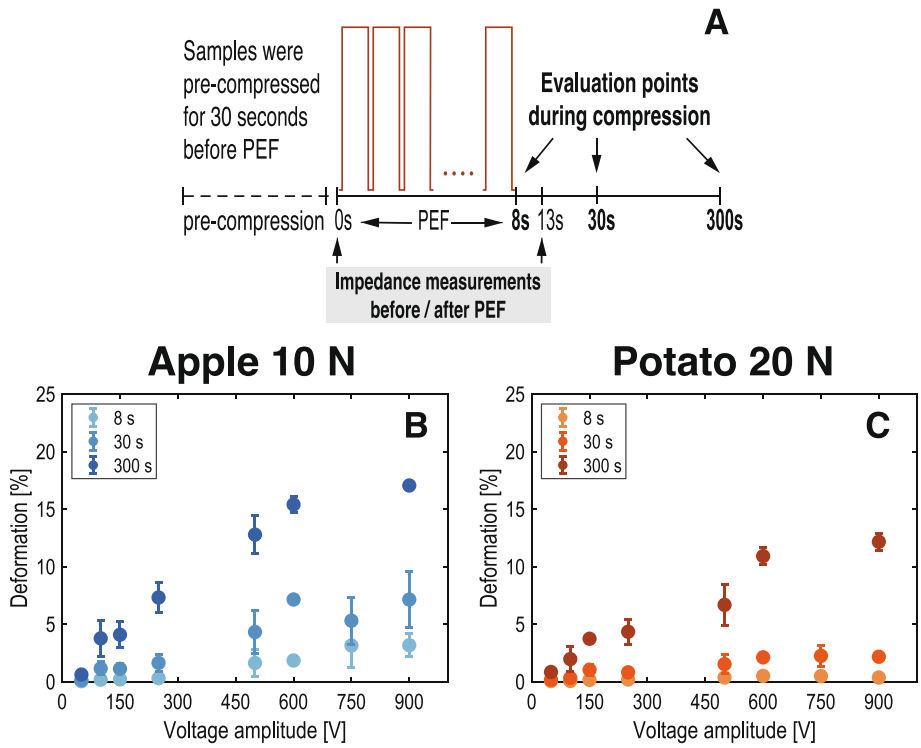

Fig. 3. Representation of the protocol and temporal evolution of measurements during the compression tests (**A**). Deformation (in % of original sample height) at different compression times: after 8 s (i.e. at the end of the delivery of the last pulse); after 30 s; and after 300 s of compression (i.e. at the end of the test) for apples compressed at a constant force of 10 N (**B**), and for potatoes compressed at a constant force of 20 N (**C**) as a function of the applied voltage amplitude. The results are given as means ± standard deviations (error bars) of n = 3.

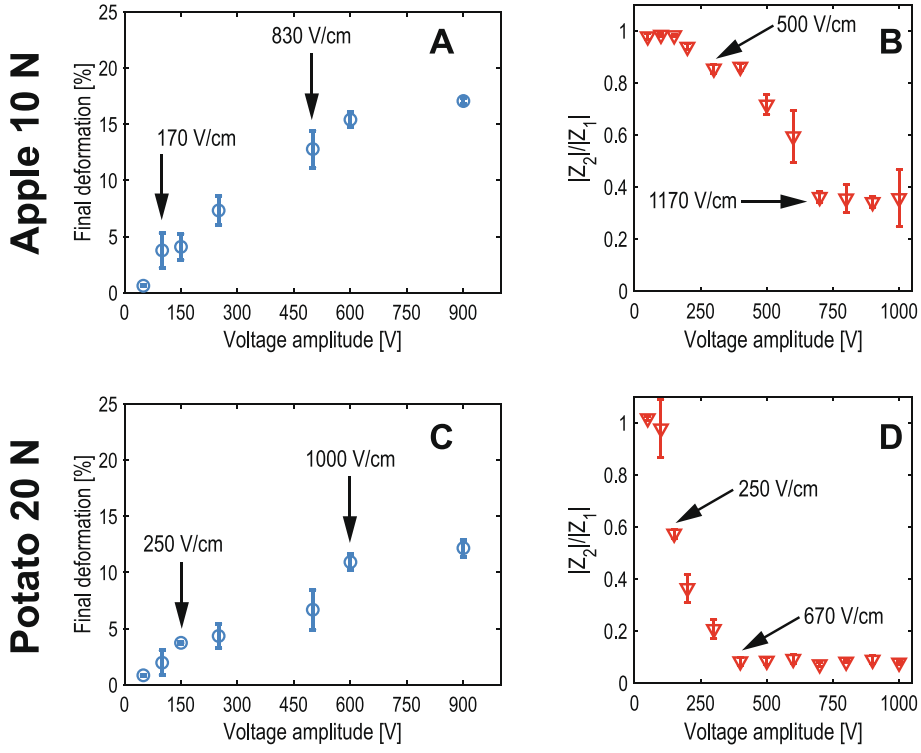

Fig. 4. Final deformation (in % of original sample height) at the end of the compression tests for apples compressed at a constant force of 10 N (**A**), and for potatoes compressed at a constant force of 20 N (**C**) as a function of the applied voltage amplitude. Normalized absolute impedance at a sampling frequency of 5 kHz for apples (**B**) and potatoes (**D**) subjected to various applied voltage amplitude. The results are given as mean values ± standard deviations (error bars) of n = 3.

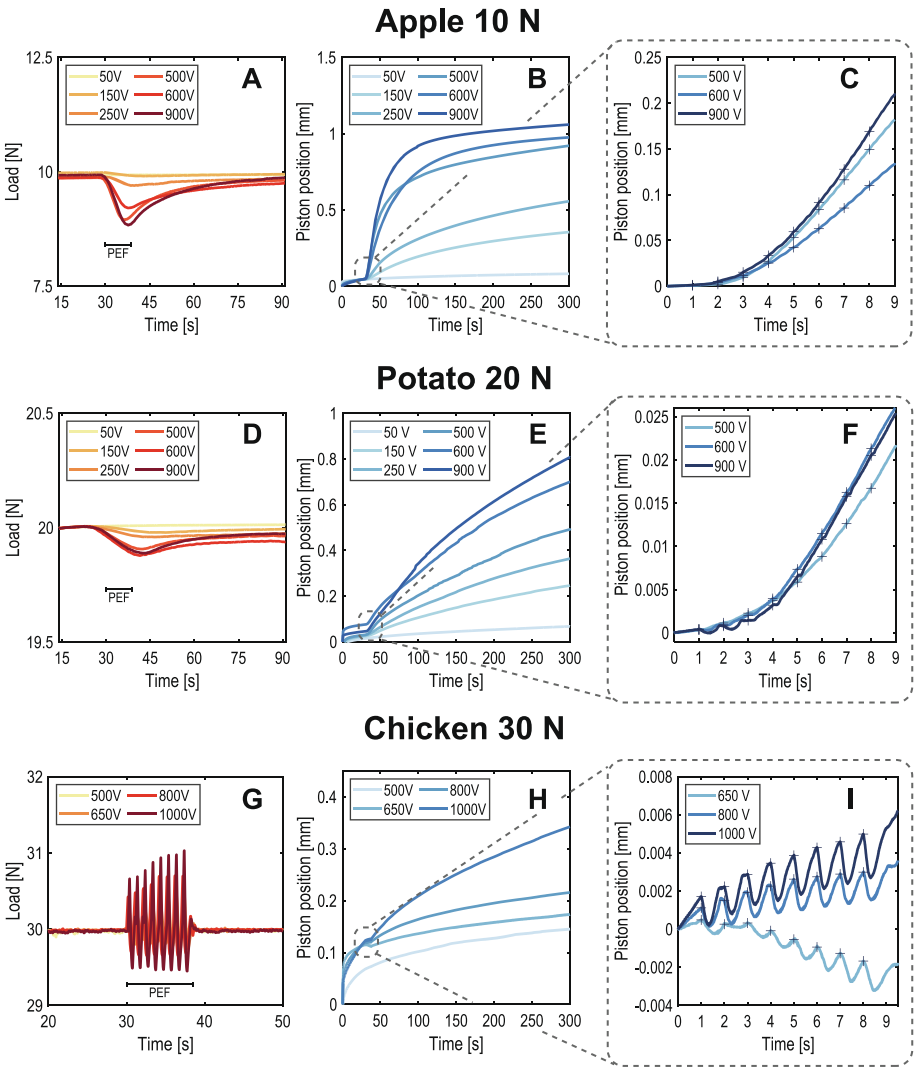

Fig. 5. Detailed view of the compression load for different voltage amplitudes applied to apples under a constant force of 10 N (**A**), potatoes under a constant force of 20 N (**D**), and chicken under a constant force of 30 N (**G**). Piston displacement during the application of various voltage amplitudes for apples (**B**), potatoes (**E**), and chicken (**H**). Zoomed-in view of piston displacement during the delivery of the pulses, that are marked with the + symbol. These pulses were delivered after the pre-compression stage and occurred at a repetition rate of 1 s^{-1}. Please note that not all the applied voltages are shown in the Fig. to ensure clarity of presentation, and time 0 corresponds to the pre-compression stage (**C**, **F**, **I**).

compressive force compared to plant samples was necessary to achieve uniform deformation of the tissue. The relationship between the deformation reached by the sample at the end of the compression test and the magnitude of the applied voltage is illustrated in Fig. 2B. When compared to plant tissues plotted on the same scale as in Fig. 1, the deformation of the animal tissue exhibits significantly smaller differences. However, when viewed on an expanded scale, the tissue deformation still appears to increase proportionally with the magnitude of the applied voltage (Fig. 2A - see insert). Nonetheless, the final deformation of the sample does not demonstrate a significant correlation with the amplitude of the applied voltages. This is indicated by the large error bars denoting the standard deviation (Fig. 2B – see insert).

The comparative analysis between plant and animal tissues has provided valuable insights into the deformation mechanisms induced by PEF treatment. It has been established that the observed deformation in plant tissue following PEF treatment is primarily attributed to a reduction in tissue turgidity, leading to increased compressibility. On the other hand, animal cells generally lack cell walls, making it impossible to observe the same deformation behaviour as seen in plant tissue. Consequently, the absolute compression value in animal tissue is lower compared to plant tissue.

3.3. Temporal evolution of deformation in plant tissues

Fig. 3A depicts the experimental protocol timeline and measurements of the apple and potato tissue deformation during the compression tests. The plots (Fig. 3B, C) illustrate the relation between the duration of the compression test and the deformation (%) of the studied plant tissues. If the compression test is stopped too soon after PEF treatment (i.e. at 8 or 30 s), the data on tissue deformation for both studied plant species appear to be inconsistent, especially at lower voltage amplitudes (< 250 V). We suppose that this behaviour can be attributed to two different mechanisms. Firstly, at lower electric fields, the slow deformation of tissue structure can occur due to a higher number of intact cells compared to electroporated cells. It has been reported that following the application of PEF treatment the structure of the tissue consists of a limited number of intact cells, as well as a partially damaged and deformed cell network (Faridnia et al., 2015; Fincan and Dejmek, 2002). Secondly, the release of the almost incompressible intracellular liquid from the inside to the outside of the tissue is time-dependent, which can also affect the deformation of the tissue during the compression tests. Furthermore, the compression of a PEF-treated cellular material triggers a complex series of simultaneous

phenomena, encompassing the expulsion of air, the disruption of cells, and the movement of fluid first from the intra-cellular to extra-cellular space, and through the extracellular space to ultimately vacating the tissue sample (Bouzrara and Vorobiev, 2003; Mahnič-Kalamiza and Vorobiev, 2014).

3.4. Electric field thresholds in plant tissues: Comparison between textural and electrical properties

In our study, we compared the deformation of apples and potatoes (under compressive forces of 10 N and 20 N, respectively, Fig. 4A, C) with the changes in electrical impedance of the tissues treated under the same PEF treatment conditions (Fig. 4B, D). The disruption of cell membrane caused by the electroporation phenomena permits a substantial outflow of the intracellular fluid, thus considerably reducing the cell turgor pressure (Lebovka and Vorobiev, 2017). Moreover, when electroporation occurs, the cell membrane permeability increases, which is reflected in the decrease of the electrical impedance (Castellví et al., 2017). The non-linear trend of both variables (deformation and impedance) made it possible to determine the critical electric field thresholds triggering cell membrane electroporation, together with the maximum membrane permeabilization achieved (deformation trends exhibit saturation behaviour within the tested range of pulse amplitudes). The critical electric field thresholds are marked in Fig. 4. The results show that apple tissue exhibits a significant increase ($p < 0.05$) in final deformation when exposed to an electric field of 170 V/cm (100 V of applied voltage) (Fig. 4A). A preliminary analysis of the data presented in Fig. 4 (A, B) indicates that texture analysis may offer greater sensitivity than electrical impedance analysis for detecting electroporation thresholds in apple tissue (170 V/cm and 500 V/cm, respectively). The results indicate that a significant reduction in normalized impedance, which represents the relative change in impedance of the tissue following PEF treatment, was only apparent in the apple tissue when an electric field of 500 V/cm (300 V of applied voltage) was used (Fig. 4B). Moreover, the maximum permeabilization of the apple cell membranes, as indicated by the point at which a plateau is reached, occurred at lower electric fields (830 V/cm) when considering the tissue deformation (Fig. 4A), rather than the maximum decrease in normalized impedance (1170 V/cm) (Fig. 4B) under the same treatment conditions of pulse number and duration. It might be argued that these observations suggest that measuring the changes in mechanical properties of the tissue induced by PEF treatment, such as deformation, may provide a more accurate and sensitive means of assessing electroporation in plant cells than relying solely on electrical impedance measurements. However, when evaluating plant tissue with varying degrees of structural complexity, such as potato tissue, changes of the mechanical and electrical properties were measured at similar critical electric fields. An electric field of 250 V/cm (150 V applied voltage) led to a significant ($p < 0.05$) increase in final deformation and a significant ($p < 0.05$) decrease in electrical impedance in potato tissue. Moreover, in contrast to what we observed with the apple tissue, the analysis of the electrical properties of the potato tissue shows that at 670 V/cm (Fig. 4D), a much lower electric field than that corresponding to the maximum deformation of the tissue (1000 V/cm, Fig. 4C), no further decrease in impedance occurs. As previously discussed in the work of Genovese et al. (2021), the interpretation of critical electroporation values derived from the electrical analyses is influenced by the specific properties and characteristics of the studied plant tissues. In the case of apple tissue, which is characterised by a highly disassociated domain with many air pockets, a higher electric field is required to induce a significant release and redistribution of intracellular fluid that can be detected by measurements of electrical impedance, whereas bulk texture modification can be detected even at lower electric fields. On the other hand, potato tissue shows a good correlation between the mechanical disintegration and the measured electrical properties, with the exception of the saturation trend in electrical impedance measurements at higher electric fields (670 V/cm, 400 V of applied voltage), presumably due to the abundance of extracellular liquid released. Yet, even at an electric field of 1000 V/cm (600 V of applied voltage), at which the point of maximum sample deformation is achieved, the changes in mechanical properties and turgor pressure reduction in potato tissue remain detectable. This means that, going by the electrical impedance measurements alone, we would have concluded that any treatment past the voltage-to-distance ratio of 670 V/cm in potato is meaningless, when, in fact, we are from that point on still seeing changes in response to treatment intensity, we are merely not able to detect them using electrical impedance alone.

It is worth to consider that the discrepancies between the two methods used can also be attributed to the temporal variation of the measured physical properties of tissue. In the case of impedance, measurements were taken between 3 and 5 s after the application of the last pulse, while the deformation measurements reflect the state of the sample after 300 s of compression (as schematically shown in Fig. 3A).

3.5. The (noticeable) effect of electroosmosis during pulse application

Fig. 5 provides a detailed analysis of the compression tests, focusing on the constant load applied (Fig. 5A, D, G), the displacement of the piston (Fig. 5B, C, E, F, H, I), and their relationship with the voltage amplitudes and the characteristics of the plant and animal samples.

In apples (Fig. 5A), potatoes (Fig. 5D), and chicken (Fig. 5G), the load curves exhibit a clear pattern during the simultaneous application of electroporation pulses (that is between 30 and 38 s), particularly at higher voltages ($V > 250$ for the plant tissues and $V > 500$ for the animal tissue). In plant tissues, distinct “dips” are noticeable along the axis of pressure application, aligned with the piston direction (Fig. 5A, D). These dips indicate a softening of the tissue, attributed to the disruption of cells induced by electroporation. However, in the chicken sample, the load curve displays multiple peaks corresponding to the number of pulses delivered during the PEF treatment (Fig. 5G). Each peak represents the impact of an individual pulse on the compression force.

The displacement curves (Fig. 5B, E, H) represent the movement of the piston compressing the plant and animal tissue samples, highlighting differences between vegetable tissues and skeletal muscle. In apples and potatoes, these curves demonstrate three distinct stages. Firstly, during the pre-compression phase (i.e. 30 s before PEF) the piston exerts a constant force on the plant tissue, counterbalanced by the cells’ turgor pressure. The intact cell walls ensure that the compression value remains constant during this phase. Secondly, the PEF treatment is delivered, leading to the disruption of cells and the outflow of the intracellular liquid. At this stage, cell turgor pressure is partially lost, and some cells experience complete loss of homeostasis. Finally, after the application of pulses, the compression increases proportionally with the applied voltage. This aligns with previous findings, where higher voltages lead to a higher probability of plant cell’s membrane disruption, resulting in a larger drop in turgor pressure, and ultimately, increased compression of the sample (Mahnič-Kalamiza and Vorobiev, 2014). In contrast, the compression variation in chicken sample (Fig. 5H), is smaller compared to plant tissues, as skeletal muscles lack cell walls, and thus turgidity, and possess a more flexible, gel-like structure.

A detailed view of the piston displacement during the application of the eight pulses (Fig. 5C, F, I), particularly at amplitudes above 500 V for plants and 650 V for chicken, reveals the stimulation of intracellular liquid movement through electroosmosis. Electroporation increases membrane permeability and conductivity in plant tissues, while the electric current induces electroosmosis, redistributing intracellular liquid within the treated sample, with water released from cells flowing in the direction of the cathode (Vorobiev and Lebovka, 2006). This electroosmosis behaviour manifested as an additional compressive pressure alongside the piston’s force (Fig. 5C, F, I). In potato tissue (Fig. 5F), this phenomenon is more pronounced than in apple tissue, with noticeable dips indicating a greater movement of fluid within the tissue structure channels. Conversely, the higher porosity and

dissociated domains in apple tissue caused by air pockets prevents the observation of the electroosmosis phenomenon (Fig. 5C), in line with previous work by Genovese et al. (2021). As animal cells are not subjected to cell turgor, the electroosmotic flow resulting from the PEF treatment is more pronounced and has a greater impact on the compression curve, as shown in Fig. 5I. This effect of electroosmosis is noticeable and pronounced even using relatively short, 100 μs pulses. In biomedical applications of electroporation, such as gene electrotransfer, pulses of millisecond or several milliseconds in duration are routinely used. Not much investigation has been dedicated to date to elucidating the importance of electroosmosis in the context of mass transport when using such long pulses (or even DC fields), but as our study inadvertently illustrates, the effect on mass transport could be significant, albeit only present during electroporation. Electroosmosis may as well prove to be a crucial transport mechanism both in plant and animal tissue electroporation, and thus we continue examining its importance in experimental as well as modelling studies (paper pending review).

4. Conclusions

To ensure accurate evaluation of PEF treatment effects, the use of texture analysis, which is often used in the literature to qualitatively characterise PEF-treated tissues, must be approached with caution, as the observed behaviours may in a small part be attributed to sample-to-sample variations and the dependence of the analysis method employed. Good repeatability needs to be achieved by careful control of the experimental conditions, and enough samples must be secured to be able to confidently draw conclusions on treatment effectiveness as a function of treatment intensity.

Nevertheless, even given a very limited number of samples and sample tissues, we have successfully demonstrated how two different methods of detecting PEF treatment effects in biological tissues can lead to vastly different values of treatment thresholds. For very turgid plant tissues with a highly conductive intracellular medium (relative to the extracellular compartment, e.g. potato), PEF treatment results in release of liquid that electrically homogenizes the tissue almost immediately (e.g., at low treatment intensity, at the beginning of pulse application, etc.), and we can observe further changes when increasing treatment intensity by using texture analysis that we have no way of detecting using impedance measurements alone. The ubiquitously used disintegration index (Z), in essence an index of normalised impedance change often calculated to determine when tissue has been "completely electroporated" (i.e., no further benefit would result from increasing treatment intensity), must be viewed from this perspective and not relied on absolutely when working with such tissues.

For animal tissues *ex vivo*, the texture analysis method turns out to be of just as limited use as is impedance analysis. This is in part due to the fact there is no osmotic imbalance in animal tissues that would lead to turgidity (and the loss of it during treatment), and in part due to the fact that electroporation of dead animal muscle several hours or days *post mortem* is probably not practical or even possible due to degradation of tissue that is, at that time, already too advanced.

In addition to providing supplementary information about PEF treatment effectiveness in plant tissues to electrical measurements, texture analysis reveals additional, highly relevant, and interesting phenomena associated with PEF. Not only does it highlight the effect of turgor loss, which is something by now quite well documented in literature, but also the effect of electroosmosis, about which not many studies have been conducted to date, but which might have a significant effect on mass transport during electroporation. In fact, it may as well prove to be a crucial transport mechanism both in plant and animal tissues during PEF treatment, and its importance should be examined in future studies.

Table A1
Voltage amplitudes (V), corresponding electric field strengths (V/cm), and total specific energy inputs (kJ/kg) used in this study. The electric fields were calculated as the ratio between the applied voltage and the electrodes distance (0.6 cm). The reported total energy inputs represent the minimum to maximum range calculated across various samples.

Voltage Amplitude [V]	Electric Field Strength [V/cm]	Total Specific Energy Input [kJ/kg]
50	83	0.000017–0.0005
100	167	0.0001–0.005
150	250	0.02–0.2
250	417	0.08–0.4
500	833	0.4–2.7
600	1000	0.5–3.0
900	1500	2.0–7.0

5. Authorship conformation

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

CRediT authorship contribution statement

Jessica Genovese: Investigation, Formal analysis, Validation, Data curation, Visualization, Methodology, Writing – original draft. **Pietro Rocculi:** Writing – review & editing, Resources, Funding acquisition. **Damijan Miklavčič:** Writing – review & editing, Funding acquisition. **Samo Mahnič-Kalamiza:** Conceptualization, Methodology, Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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Appendix

See Table A1.

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