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Pulsed electric field-assisted juice extraction of frozen/thawed blueberries

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Abstract

Pulsed electric field is an efficient method for cell membrane permeabilization of food tissues with most research being done on fresh plant cells. Freeze/thawing is also known to be capable of cell membrane permeabilization. In this work, frozen/thawed European blueberry (*Vaccinium myrtillus* L.) fruits were treated with pulsed electric field in order to further enhance the cell membrane permeabilization and, hence, the quality of blueberry juice during the subsequent pressing process. Blueberries tissues were exposed to 20 μs monopolar square wave pulses of different electric field strength (E = 1–3–5 kV cm^{-1}) and total specific energy input (W_T = 1–5–10 kJ kg^{-1}), with their permeabilization being characterized by electrical impedance measurements and cell disintegration index (Z_p). The juice, obtained after pressing (1.32 bar), was characterized for total polyphenols, anthocyanins content and antioxidant activity.

The cell disintegration index (Z_p) significantly ($p < 0.05$) increased from 0.2 up to 0.6 with increasing pulsed electric field treatment intensity (E and W_T). As a results, in comparison with control, pulsed electric field treatment induced a slightly higher release of polyphenols (up to +8.0%) and anthocyanins (up to +8.3%), thus improving the antioxidant activity of the juice (up to +16.7%). In conclusion, frozen/thawed blueberries could be pulsed electric field treated in order to further increase juice quality.

Key words: cell disintegration, juice, polyphenols, pulsed electric field, *Vaccinium myrtillus*.

Introduction

Cell membrane acts as a physical barrier for removing intracellular substances (water, juices and solutes) from the food tissues in common unit operations of food industry such as drying and extraction. Permeabilization of the cell membrane by means of food tissue pre-treatment may be an effective tool to promote mass transport processes, so that larger yields and shorter contact times can be achieved in the above mentioned unit operations. Cellular permeabilization can be performed by means of thermal treatment or by the addition of chemicals as well as of enzymes (Donsì et al., 2010 a; 2011). Unfortunately, these methods may induce the loss of sensory properties of manufactured products, as well as need of post-processing treatment stages (i.e. purification of the extract, removal of chemical residuals and inactivation of enzymes) with additional operational costs. On the other hand, freeze/thawing is also known to be an efficient but expensive physical method for cell membrane disintegration (Meyer, Richter, 2001). In many studies freeze/thawing of samples was assumed to be able to induce the maximum degree of cell membrane permeabilization (Pataro et al., 2012 a; b).

Pulsed electric fields (PEF) treatment is an innovative and promising method for non-thermal processing of foodstuff and a good alternative to conventional cell membrane permeabilization techniques (Knorr, 1999; Pataro et al., 2011). PEF allows obtaining a non-thermal permeabilization of cell membrane of vegetable tissue without post-processing operations and reducing energy consumption. Food tissue is placed between two electrodes and an electric field of moderate intensity (0.5–5 kV cm^{-1}) and relatively low total specific energy input (1–10 kJ kg^{-1}) is applied in the form of repetitive very short voltage pulses (typically from few μs up to 1 ms), which lead to only a negligible ohmic heating.

The mechanism of permeabilization involved is defined as electroporation: the application of the electric field to the cells forming the food tissue induces either the formation of pores or the local rupture of the cell membrane. Irreversible permabilization of both cell membrane and tonoplast causes the free flow of the intracellular water and valuable compounds including sugars, juices and bioactive compounds (Barsotti et al., 1999; Knorr, 1999; Pataro et al., 2011). The extent of cell membrane permeabilization mainly depends on the intensity of the external electric fields, pulse energy and number of pulses applied. In general, increasing the intensity of these parameters enhances the degree of membrane permeabilization (Angersbach et al., 2002; Lebovka et al., 2007). On the other hand, electroporation of plant tissue and the consequent mass transfer process are also complex functions of material properties which, in turn, are spatially dependent and highly inhomogeneous. There are several macroscopic methods for cell disintegration index (Z_p) evaluation: measurement of diffusion coefficient (Jemai, Vorobiev, 2002; Lebovka et al., 2007), complex impedance measurement (Angersbach et al., 2002; Donsì et al., 2010 a) and texture measurement of untreated and treated samples (Grimi et al., 2011).

Electric treatment can exert a selective permeabilization of the membranes (tonoplast and plasma membranes), while cell wall remains intact, improving the purity and the yield of the extracts. Thus, the use of PEF is advantageous in the extraction of cellular juice from fruits and vegetables (Bazhal et al., 2001; Jemai, Vorobiev, 2002; Pataro et al., 2012 a), in extraction of beet sugar (Bouzrara, Vorobiev, 2001), and in the recovery of valuable thermosensitive compounds, such as natural antioxidants which are present in agro-industrial wastes and by-products (Fincan et al., 2004; Corrales et al., 2008; Battipaglia et al., 2009). Polyphenols (anthocyanins, catechins, tannins and flavones) extraction is of particular importance, since they can be used as nutritive compounds, colorants and antioxidants or anti-inflammatory/anti-tumour agents, improving human health (Donsì et al., 2010 b). The most intense research on PEF-assisted polyphenol extraction has been done on grapes, since they contain large amount of different polyphenols, mostly located in the skin of grapes, and are widely used in wine industry (Donsì et al., 2010 a; b; Pataro et al., 2012 b). On the contrary, other fruits or berries received less investigation on PEF-assisted extraction of polyphenols. Nevertheless, it is known that different types of anthocyanins are found in fruit samples of black raspberries, red raspberries and highbush raspberries (Tian et al., 2005; Bobinaitė et al., 2012), strawberries (Kadivec et al., 2013) and black currants (Rubinskiene et al., 2005). In addition, blueberries contain large amount of polyphenols and all major anthocyanins such as delphinidin, cyaniding, petunidin, peonidin and malvidin (Burdulis et al., 2007; Koca, Karadeniz, 2009; Barba et al., 2012). However, only very few studies have been performed on polyphenol extraction from blueberries using PEF technology (Barba et al., 2012; Pataro et al., 2012 a), and in none of them the processing was carried out on frozen/thawed matrix.

The present work was designed to investigate the capability of PEF pretreatment to further enhance the degree of cell membrane disintegration of frozen/thawed blueberries with the aim of improving the quality of the expressed juice. For this purpose, the effect of different combinations of electric field strength and total specific energy input on the cell disintegration index and total polyphenol, anthocyanin content and antioxidant activity in juice was investigated.

Materials and methods

The experiments were performed in 2014 at University of Salerno, Italy and at Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry.

Chemicals and plant material. High performance liquid chromatography (HPLC) grade methanol, gallic acid, anhydrous sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl hydrate stable radical (DPPH$^{\bullet}$, 95%), concentrated hydrochloric acid, and Folin-Ciocalteu's phenol reagents were purchased from "Sigma-Aldrich" (Sigma-Aldrich Chemie, Germany). Methanol was purchased from "Fluka" (Sigma-Aldrich Chemie), sodium dihydrogen phosphate and potassium chloride were purchased from Carlo Erba reagents (Cornaredo, Italy).

European blueberry (*Vaccinium myrtillus* L.) fruits were purchased in the local market in Lithuania, frozen and stored at −20 ± 2°C until needed. Before the analysis berries were thawed in the refrigerator at 5 ± 2°C. Juices, that leaked during thawing process of blueberries, were poured out and not used in subsequent treatments and analysis.

Pulsed electric field (PEF) treatment. The PEF-assisted blueberries pressing was carried out loading 10 g of thawed blueberries in a specifically designed treatment chamber connected to an electric field generator (Fig. 1). Laboratory-made treatment cell consisted of a vertical spacer in polycarbonate, closed at both ends by two cylindrical stainless steel electrodes, with the upper one able to slide within the spacer under compression exerted by loading a weight on it. The lateral surface of the polycarbonate spacer was uniformly pierced (d = 0.5 mm) to permit the flow of the juice when compression (and eventually PEF treatment) was applied. The electrode area was 9.1 cm^2 while distance between the two electrodes could be adjusted (up to 5 cm) depending on the amount of the treated sample.

The two electrodes of the PEF chamber were electrically connected to a high voltage pulse generator "Modulator PG" (ScandiNova, Sweden) designed to provide monopolar square wave pulses with an independent setting of the applied voltage (0–25 kV cm^{-1}), pulse width (1–23 μs) and pulse repetition rate (1–450 Hz), limited only by the average power of 20 kW. The actual voltage and current signals at the treatment chamber were measured, respectively, by a high voltage probe (model P6015A, "Tektronix", USA) and a Rogowsky coil (Stangenes Inc., USA) connected to a 300 MHz digital oscilloscope (model TDS 3034B, "Tektronix"). The maximum electric field intensity (E, kV cm^{-1}) was evaluated as the peak voltage divided by the inter-electrode gap. The specific energy input per

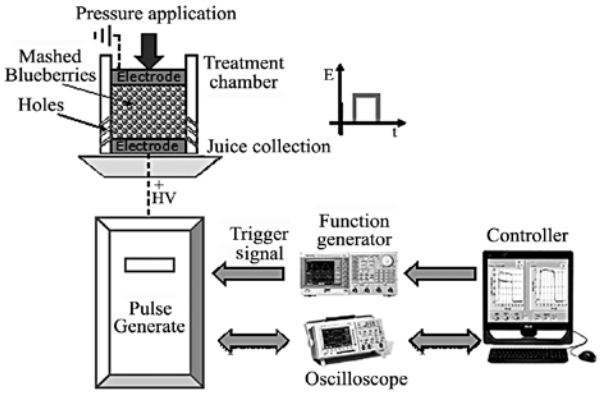


Figure 1. Schematic diagram of the pulsed electric field (PEF) system

pulse (W, kJ kg^{-1} pulse^{-1}) was calculated according to equation:

$$W = \frac{1}{m}\int_0^{\infty} U(t)\cdot I(t)dt \qquad (1),$$

where $U(t)$ and $I(t)$ represent, respectively, the voltage across the electrodes and the current intensity through the product at time t, m is the mass of the treated product. The total specific energy (W_T, kJ kg^{-1}) was calculated by multiplying W and the number of pulses applied. Blueberries were loaded and consolidated in the chamber under the weight of the upper electrode. After 2 min, PEF pretreatment of different field strengths (1, 3 and 5 kV cm^{-1}) and total specific energy inputs (1, 5 and 10 kJ kg^{-1}) at a constant frequency (20 Hz) and pulse width (20 s) were applied. Afterwards the sample was pressed by applying a constant pressure of 1.32 bar, and juice expression was carried out for additional 8 min. Control samples were collected after the application of the same protocol without PEF treatment. In all experiments the initial temperature of the samples was 20°C, and the final temperature never exceeded 25°C.

Impedance measurement. Measurement of electrical complex impedance of thawed blueberries in frequency sweep was used to characterize tissue permeabilization after PEF treatment according to the method described by (Donsì et al., 2010 a). The measurement was conducted by loading blueberries of untreated (thawed) or PEF-treated samples in a test vessel between the two parallel plate cylindrical electrodes (3 cm in diameter up to a 10 mm thickness). The electrodes were connected to an impedance analyser "Solartron 1260" (Solartron group, UK) consisting of a generator and an analyser. The generator produced a sinusoidal voltage of 1 V peak to peak for a frequency ranging between 1 kHz and 10 MHz. The analyzer provided a frequency response of the sample and calculated the electrical impedance as the ratio of the voltage drop across the sample and the current crossing though it during the test. Results were plotted as both the absolute value of the complex impedance $|Z(j\omega)|$ and phase angle θ as a function of the frequency for different treatment conditions. In order to quantify the cellular degree of permeabilization attained by each treatment, the cell disintegration index (Z_p) has been calculated on the basis of the measurement of the absolute value of complex impedance of untreated (Z_{untr}) and PEF-treated (Z_{tr}) tissue in the low (1 kHz) and high (10 MHz) frequency ranges (Donsì et al., 2010 a; Pataro et al., 2011):

$$Z_p = \frac{\left|Z_{untr(1\,kHz)}\right| - \left|Z_{tr(1\,kHz)}\right|}{\left|Z_{untr(1\,kHz)}\right| - \left|Z_{tr(1\,MHz)}\right|} \qquad (2).$$

The value of this index varies between 0 for untreated tissue and 1 for fully permeabilized tissue.

Determination of the total phenolic content (TPC) and total anthocyanins (TA). The TPC was determined using the Folin-Ciocalteu reagent (Slinkard, Singleton, 1977). The reagent was prepared by diluting a stock solution with distilled water (1/10, v/v). Then, 100 L of filtered blueberry juice was mixed with 5 mL of 80% aqueous methanol. The samples (1.0 mL, three replicates) were introduced into the test cuvettes and mixed with 5.0 mL of Folin-Ciocalteu's phenol reagent and 4.0 mL of Na_2CO_3 (7.5%). The absorbance was recorded at 765 nm in a spectrophotometer V-650 UV-Vis (JASCO Inc., USA) after incubation at ambient temperature for 1 hour. TPC was expressed in mg of gallic acid equivalents per L of juice.

The total anthocyanins content of centrifuged and filtered juice was determined using the pH differential method (Giusti, Wrolstad, 2001). Blueberry juice was filtered, added to buffer solutions (pH 1.0 and 4.5) and absorbance of the solutions was measured using spectrophotometer V-650 UV-Vis (JASCO Inc.) at 520 and 700 nm. The concentration of anthocyanins was expressed in mg of cyanidin-3-glucoside per L of juice.

Evaluation of radical scavenging capacity (RSC). For RSC assay 1 mL of centrifuged and filtered blueberry juice was diluted with 9 mL of aqueous methanol (80%), and for the blank sample measurements 1 mL of water was diluted with 9 mL of aqueous methanol. The RSC of juice against stable DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl hydrate stable radical) was determined by a slightly modified spectrophotometrical method (Brand-Williams et al., 1995). DPPH$^{\bullet}$ methanolic solution (3 mL, 6×10^{-5} M) was mixed with 30 µL of prepared sample at ambient temperature. The decrease of absorbance, due to the scavenging of DPPH$^{\bullet}$, was measured on a spectrophotometer V-650 UV-Vis (JASCO Inc.) at 515 nm after 30 min of incubation. The absorptions of blank samples (by applying the same analysis conditions) were tested each time before and after analysis. RSC was calculated by the following formula: RSC = [(AB – AS) / AB] × 100%, where AB is the absorption of the blank sample (t = 30 min), and AS is the absorption of the tested sample.

Statistical analysis. All the experiments were carried out in triplicate and each collected sample was analyzed in duplicate. The mean values and standard deviations of the experimental data were calculated. Differences among Z_p values as well as TPC, TA and RSC of samples before and after PEF treatment were determined by Student's t-test (SigmaPlot, USA). The differences were considered significant at * – $p \leq 0.05$ and ** – $p \leq 0.01$.

Results and discussion

Tissue permeabilization. The extent of tissue permeabilization due to the PEF treatment of different intensity was evaluated through impedance measurements

in frequency sweep on untreated (frozen/thawed) and treated blueberry tissue. Figures 2–4 report both the frequency-impedance spectra and frequency-phase angle spectra and the transition from untreated (frozen/thawed) to PEF-treated state in the frequency range investigated for the blueberry tissue.

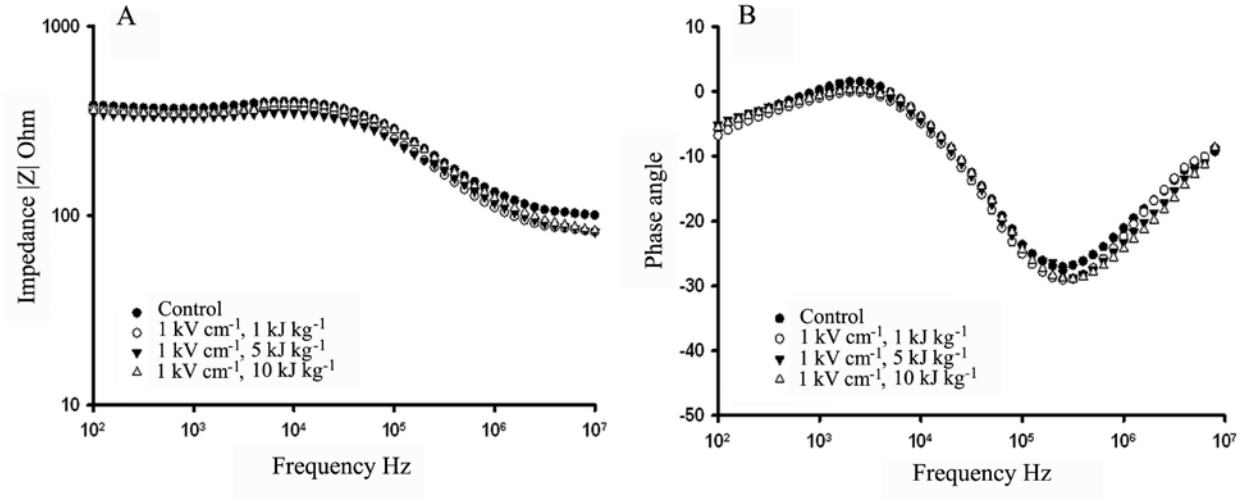


Figure 2. Complex impedance |Z| (A) and phase angle θ (B) for control (frozen/thawed) and PEF-treated blueberries at 1 kV cm^{-1} electric field strength and different energy input

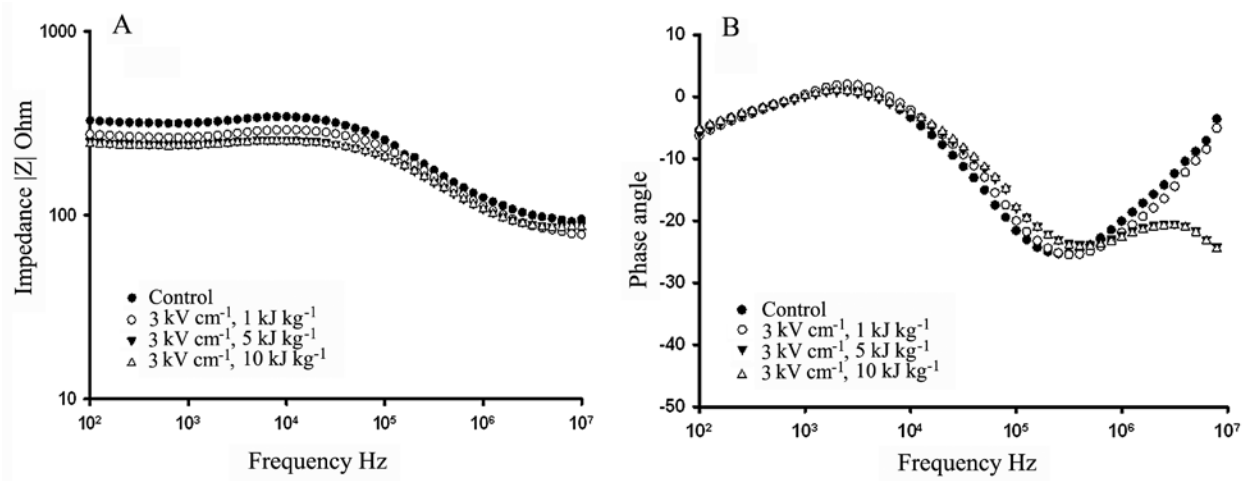


Figure 3. Complex impedance |Z| (A) and phase angle θ (B) for control (frozen/thawed) and PEF-treated blueberries at 3 kV cm^{-1} electric field strength and different energy input

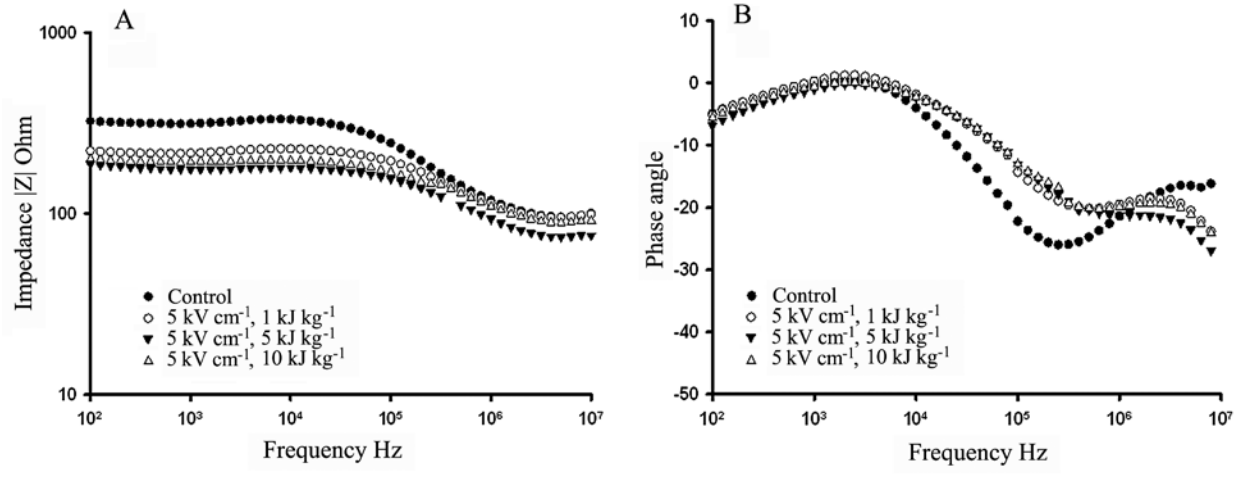


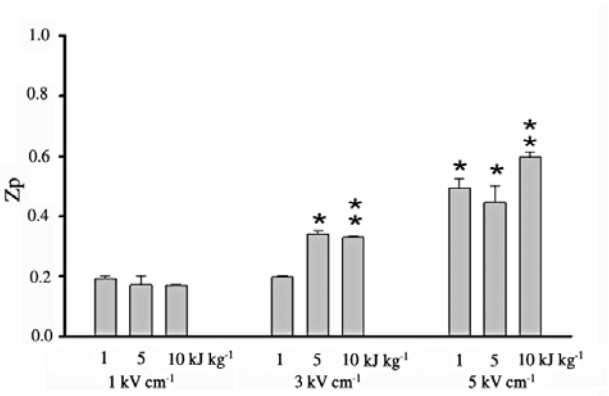
Figure 4. Complex impedance |Z| (A) and phase angle θ (B) for control (frozen/thawed) and PEF-treated blueberries at 5 kV cm^{-1} electric field strength and different energy input

The results of Figures 2A–4A show that the absolute impedance value of the untreated fruit tissue was frequency dependent, showing that the cell membranes of frozen and thawed blueberry tissues were not completely disintegrated due to freeze/thawing procedure. As a result, in the low frequency field, the cell membranes may still act as a capacitor preventing the flow of the electric current in the intracellular medium (ohmic-capacitive behaviour). Upon increasing frequency, the cell membranes became less and less resistant to the current flow. At very high frequency values (5 to 10 MHz), the membranes were totally shorted out and the absolute value of the complex impedance was representative of the contribution of both extra and intracellular medium (pure ohmic behaviour). Therefore, a further tissue permeabilization, induced by an external stress such as PEF treatment, may be detected in the low frequencies range by comparing treated and untreated samples (Donsì et al., 2010 b; Pataro et al., 2011).

Our results show that, in comparison to the untreated sample, PEF treatment may lead to a decrease of the absolute impedance values in the low frequency range but it depends on the treatment intensity. When low (1 kV cm^{-1}) electric field strength was applied, no appreciable differences between treated and untreated berries could be detected, independently of the energy input applied (Fig. 2A). It is likely that these PEF treatment conditions were too mild to induce further permeabilization of blueberry tissue, that was already permeabilized to some level by freeze/thawing procedure. However, as the electric field strength increased above 1 kV cm^{-1}, the absolute value of the impedance in the low frequency range started to decrease (Figs 3A and 4A). This is likely explained by the increase of the concentration of ionic species in the extracellular space, due to PEF-induced membrane permeabilization. Furthermore, the results also show that the higher PEF treatment intensity, i.e. the higher electric field strength and/or energy input, the higher degree of membrane permeabilization, even though it appears to depend mainly on the electric field strength applied rather than the energy input.

On the other hand, increasing the intensity of the PEF treatment also increased the value of the measured phase angle θ (Figs 2B and 4B). The phase angle of either intact or partially permeabilized tissue is in general negative, due to the capacitive behaviour of the cell membranes (Barsotti et al., 1999; Battipaglia et al., 2009). When the membrane disintegration progressed, the capacitance of the cell membrane became lower, which was reflected in the phase angle shift. The phase angle shift was mostly pronounced at medium frequencies and the phase angle shift occurred at a frequency of approximately 2 × 105 Hz (Figs 2B and 4B).

According to eq. 2, the extent of permeabilization can be quantified by evaluating the Z_p. Figure 5 reports Z_p values of blueberry tissue as a function of the field strength and energy input applied. Results show that in the range of the electric fields investigated (1–5 kV cm^{-1}) the permeabilization degree depended mainly on the applied electric field rather than on the energy input. Increasing the field strength from 1 to 5 kV cm^{-1}, the Z_p value significantly ($p < 0.05$) increased from 0.2 to 0.6, except for the treatment carried out at 3 kV cm^{-1} and 1 kJ kg^{-1}. On the other hand, results also show that increasing the energy input at a fixed field strength, Z_p value increased significantly ($p < 0.05$) only when the energy input was changed from 1 to 5 and 10 kJ kg^{-1} at 3 kV cm^{-1} and above 5 kJ kg^{-1} at 5 kV cm^{-1}. Z_p values are in agreement with other authors. For example, Z_p values of sugar beet after PEF treatment were between 0.2 and 0.9 (Maskooki, Eshtiaghi, 2012), of orange peel – between 0.1 and 0.3 (Luengo et al., 2013). Lebovka et al. (2014) have showed that both experimental and simulated Z_p values of potato tissue were between 0 and 0.8.

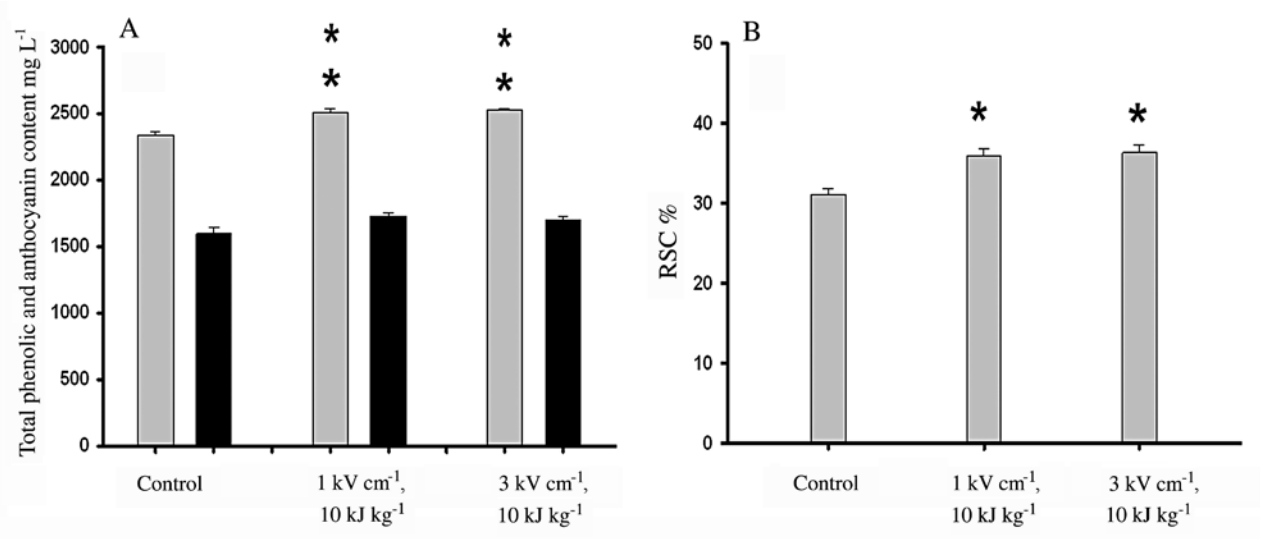


Note. * – significant at $p \leq 0.05$, ** – significant at $p \leq 0.01$; comparison is marked between different electric field strength.

Figure 5. Cell disintegration index (Z_p) dependence on different pulsed electric field (PEF) treatment conditions

In conclusion, our results seem to support the fact that cell membranes of frozen/thawed blueberries tissue are not completely destroyed, and that they can be further permeabilized if exposed to PEF treatment of proper electric field strength and energy input.

Chemical analyses. The results presented above show that cell membranes of blueberries were not completely disintegrated due to freeze/thawing process. Therefore, their exposure to PEF treatment of proper field strength and energy input may lead to a further increase of cell membrane permeabilization, which could be reflected in a greater yield of extraction of bioactive compounds from the inner part of the cell during the following pressing of the permeabilized tissue. For this purpose, the polyphenolic content, the anthocyanin content and the radical scavenging activity of the juice obtained after pressing (1.32 bar) of untreated (frozen/thawed) and PEF-treated blueberries were measured, with the results being reported in Figure 6. PEF treatments were carried out at 1 and 3 kV cm^{-1} and at a fixed energy input of 10 kJ kg^{-1}, despite the fact that the treatment at 5 kV cm^{-1} and 10 kJ kg^{-1} was able to provide the greatest value of Z_p. This is because of the observation that the application of electric field strength higher than 3 kV cm^{-1} often leads to arch formation between the electrodes of the treatment chamber, likely due to the presence of the holes on the lateral surface of the spacer insulator.



* – significant at $p < 0.05$, ** – significant at $p < 0.01$

Figure 6. Total phenolic (grey bars) and anthocyanin (black bars) content (A), and radical scavenging capacity (RSC) (B) of blueberry juice obtained after pressing (1.32 bar) of untreated (control) and PEF-treated blueberries

Results show that, in comparison with pressing of frozen/thawed sample, the application of PEF pretreatment may significantly ($p < 0.01$) promote polyphenols extraction (Fig. 6A), which increased from 7.4% after PEF treatment at 1 kV cm^{-1} and to up to 8.0% after PEF treatment at 3 kV cm^{-1}. Therefore, only pressing of freeze-thawed sample was evidently not sufficient to ensure total release of polyphenols in the juice, and the application of permeabilization techniques such as PEF can be of value since they may induce a further enhancement in the yield of extraction of bioactive compounds, probably being able to permeabilize also inner cell membranes. On the other hand, results of Figure 6A also show that anthocyanin content slightly increased after PEF treatment, with an increase of 8.3% after PEF treatment at 1 kV cm^{-1} and 6.7% after PEF treatment at 3 kV cm^{-1}. However, it was no significant difference with respect to the control ($p > 0.05$). Radical scavenging capacity in the juice obtained from PEF-treated samples was significantly higher ($p \leq 0.05$) than in the untreated juice, and exhibited a trend similar to that of the total polyphenols in comparison to control samples (Fig. 6A). As shown in Figure 6B, it increased from 31.1 ± 1% at control condition up to 35.9 ± 1% when 1 kV cm^{-1} and 10 kJ kg^{-1} was applied, and up to 36.3 ± 1% when 3 kV cm^{-1} and 10 kJ kg^{-1} was applied. The increase of anthocyanins and total polyphenols is in agreement with other authors. PEF pretreatment increased anthocyanin content by 17% of grape by-products (Corrales et al., 2008).

Conclusions

1. The present work showed that freeze/thawing process is not able to provide complete cell membranes disintegration of blueberry tissues.

2. Exposure of frozen/thawed fruit tissue to a pulsed electric field (PEF) treatment of sufficient intensity (up to 5 kV cm^{-1} and 10 kJ kg^{-1}) may promote a further increase of the cell membrane permeabilization, with an increase of the cell disintegration index (Z_p) up to 0.6.

3. In comparison to the untreated samples, the application of PEF treatment before pressing of thawed blueberries for juice production may add some quality advantage in terms of content of polyphenols (up to +8.0%), anthocyanins (up to +8.3%) and enhance the antioxidant activity of the obtained juice (up to +16.7%).

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Sulčių spaudimas iš sušaldytų/atšildytų mėlynių taikant impulsinio elektrinio lauko metodą

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Santrauka

Apdorojimas impulsiniu elektriniu lauku yra efektyvus metodas, taikomas siekiant padidinti ląstelių membranų pralaidumą. Dauguma tyrimų taikant impulsinį elektrinį lauką yra atlikta su šviežiais augaliniais audiniais. Sušaldymas/atšildymas taip pat padeda suardyti ląstelių membranas. Tyrimo metu, siekiant dar labiau padidinti ląstelių membranų pralaidumą ir kartu gautų sulčių kokybę, prieš sulčių spaudimą sušaldytos/atšildytos mėlynės buvo paveiktos impulsiniu elektriniu lauku. Mėlynės buvo veikiamos 20 µs trukmės monopoliniais stačiakampio pavidalo impulsais. Apdorojant mėlynes buvo taikytas skirtingo stiprumo elektrinis laukas (E = 1–3–5 kV cm^{-1}) ir suminė specifinė energija (W_T = 1–5–10 kJ kg^{-1}). Audinių pralaidumas apibūdintas išmatavus elektrinį impedansą ir apskaičiavus ląstelių dezintegracijos indeksą (Z_p). Po sulčių spaudimo (1,32 bar slėgiu) gautų sulčių kokybė apibūdinta įvertinus bendrą fenolinių junginių bei antocianinų kiekį ir antioksidacinį aktyvumą.
Didinant apdorojimo impulsiniu elektriniu lauku intensyvumą (E ir W_T), ląstelių dezintegracijos indeksas esmingai (p < 0,05) padidėjo nuo 0,2 iki 0,6. Apdorojimas impulsiniu elektriniu lauku, lyginant su kontroliniu bandiniu, padidino fenolinių junginių (iki +8,0 %) ir antocianinų (iki +8,3 %) išsiskyrimą į sultis ir jų antioksidacinį aktyvumą (iki +16,7 %). Todėl, siekiant pagerinti sulčių kokybę, apdorojant sušaldytas/atšildytas mėlynes gali būti taikomas impulsinio elektrinio lauko metodas.

Reikšminiai žodžiai: impulsinis elektrinis laukas, ląstelių dezintegracija, mėlynės, polifenoliai, sultys.