Impact of pulsed electric field treatment on juice yield and recovery of bioactive compounds from raspberries and their by-products

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Abstract
Pulsed electric field (PEF) is a non thermal treatment, which could be employed for plant tissue disintegration which leads to increased juice yield and enhanced extraction of bioactive compounds. Since 97% of red raspberries (Rubus idaeus L.) are sold processed into juices or other products, it is important to increase juice yield and bioactive compounds extraction.

This study investigated the effect of PEF pretreatment on red raspberries processing. After PEF pretreatment and mechanical pressing (1.32 bar for 6 min) the juice recovery from raspberries increased in the range of 9–25%. Compared to the untreated sample, press cake extracts contained significantly higher amounts of total phenolics (up to 22%), total anthocyanins (up to 26%) and higher ferric reducing antioxidant power (FRAP) (up to 24%). Mild PEF pretreatment (1 kV cm−1 electric field strength and 6 kJ kg−1 total specific energy) was sufficient to achieve higher raspberry juice recovery and to enhance extraction of bioactive compounds from raspberry press cake left after the juice pressing. PEF pretreatment of red raspberries is a promising technique to improve the efficiency of industrial processing of raspberries.

Key words: anthocyanins, cell disintegration, juice recovery, pulsed electric field, Rubus idaeus.

Introduction
Red raspberries (Rubus idaeus L.) are one of the richest sources of antioxidant phytonutrients among the common fruits and vegetables (Beekwilder et al., 2005). The total anthocyanin content of raspberries is 79–92 mg 100 g−1 of fruit weight, 98% of which are cyanidin glycosides (Koponen et al., 2007). The content of anthocyanins and their profile may vary depending on raspberry genotype (Bobinaitė et al., 2012). However, ellagitannins, not anthocyanins, were identified as the most active principles in red raspberries (Zhang et al., 2010; Bobinaitė et al., 2013). Raspberries contain 297.3 mg 100 g−1 fruit weight of ellagitannins (Koponen et al., 2007). The most common ellagitannins present in red raspberries are lambertianin C (trimer) and sanguin H-6 (dimer) (Kähkönen et al., 2012). Free ellagic acid levels are generally low in red raspberries, only 1.4% of the total ellagic acid content (Chemistry and biology..., 2009).

Ellagitannins were found to contribute up to 60% of the total antioxidant capacity of R. idaeus fruits (Beekwilder et al., 2005), whereas the contribution of anthocyanins was reported to be only 17% (Borges et al., 2010) or 25% (Beekwilder et al., 2005).

Red raspberries also contain small amounts of other polyphenolic compounds, such as quercetin-3-glucuronide, kaempferol-3-glucuronide and (−)-catechin (Maatta-Riihinen et al., 2004). Furthermore, raspberries contain high amounts of vitamin C (~26.2 mg 100 g−1 of fresh weight), as well as many other vitamins and minerals (Rao, Snyder, 2010).

It is important to note that only 3% of red raspberries are sold fresh and the rest are sold as juices and other processed products such as jams, jellies and yogurts (Vladisavjevic et al., 2013). High (90°C) temperature and high (up to 600 MPa) pressure are often
used for increased juice yield and also for pasteurization. However, it was shown that both high temperature and high pressure treatments may cause anthocyanin degradation in raspberry products (Verbeyst et al., 2011). Mechanical pressing and extraction with solvents are widely used in the food industry for juice or other compounds (colorants, antioxidants, essential oils, etc.) extraction from fruits or vegetables. Unfortunately, the quality of extracted products (turbidity, colour, flavour, nutrients, etc.) may be degraded after conventional raw material pretreatments, such as grinding, heating or addition of chemicals/enzymes (Vorobiiev, Lebovka, 2010). Therefore, an alternative, preferably non-thermal treatment is required for maximal preservation of valuable compounds in fruit juice.

Pulsed electric field (PEF) treatment is an innovative and promising method for non-thermal processing of foodstuffs. It was shown that the application of PEF on plant material increased mass transfer from processing of foodstuffs. It was shown that the application of innovative and promising method for non-thermal compounds is required for maximal preservation of valuable 2010). Therefore, an alternative, preferably non-thermal treatment is required for maximal preservation of valuable compounds in fruit juice.

Pulsed electric field (PEF) treatment is an innovative and promising method for non-thermal processing of foodstuffs. It was shown that the application of PEF on plant material increased mass transfer from processed plant tissues (Knorr, 1999). Typically, high electric field strength (1–10 kV cm⁻¹), but short (μs–ms) repeated pulses of electric field, which permeabilize the cell membranes, are applied. Pretreatment with PEF before juice pressing may increase juice yield from grapes (Donisi et al., 2010), apples (Schilling et al., 2007), oranges (Demirdöven, Baysal, 2015). In addition to increased juice recovery, due to PEF non-thermal action selective permeabilization of the membranes (tonoplast and plasma membrane) could be affected, while the cell wall remains intact, thus improving the purity and the quality of the extracts (Praporscic et al., 2007). While the most studies were focused on PEF treatment for the most common fruits, such as apple, grapes or oranges, only few studies were performed on other fruits or berries. It was shown by our group that PEF can increase juice recovery from blueberries (Bobinaitė et al., 2015). To the best of our knowledge, no research was performed to investigate PEF treatment impact on raspberries processing.

This study was designed to investigate the effect of PEF pretreatment on red raspberries with the aim of enhancing the recovery and the quality of the pressed juice as well as the subsequent extraction of bioactive compounds from the press cake left after PEF assisted pressing.

Materials and methods

Chemicals and raw material. The experiments were performed in 2014−2015 at University of Salerno, Italy, and Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Methanol, gallic acid, anhydrous sodium carbonate, concentrated hydrochloric acid, iron (III) chloride hexahydrate (FeCl₃·6H₂O), 2,4,6-tripyridyl-s-triazine (TPTZ) and Folin and Ciocalteu’s phenol reagent were purchased from Sigma-Aldrich ("Sigma-Aldrich Chemie", Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from "Acros Organics" (Belgium). Ethanol (70%) and sodium acetate were supplied from "Fluka" (Switzerland). Potassium chloride was purchased from "Carlo Erba Reagents" (Italy). Cultivated red raspberries (Rubus idaeus L.) were purchased on 30th June at a local market in Lithuania and stored for maximum 30 days at 4°C until processing. In addition, part of the berries were frozen and stored at −20 ± 2°C until needed.

Pulsed electric field (PEF) treatment. The PEF assisted pressing of raspberries was carried out loading 14 g of raspberries in a specifically designed treatment chamber connected to an electric field generator. The two electrodes, each of them having the area of 9.1 cm², were electrically connected to a high voltage pulse generator Modulator PG (“ScandiNova”, Sweden). Monopolar square wave pulses were used. 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”, USA). 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 pulse (W, kJ kg⁻¹ pulse⁻¹) was calculated according to the equation (1):

\[ W = \frac{1}{m} \int U(t) \cdot I(t) \, dt \]  

(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 \( W_r \, \text{kJ kg}^{-1} \) was calculated by multiplying \( W \) and the number of pulses applied.

Raspberries were loaded and consolidated in the chamber under the weight of the upper electrode. After 2 min of pressing, PEF pretreatment of different field strengths (1 and 3 kV cm⁻¹) and total specific energy inputs (1, 6 and 12 kJ kg⁻¹) at a constant frequency (20 Hz) and pulse width (20 μs) was applied. Afterwards the sample was pressed by applying a constant pressure of 1.32 bar, and juice pressing 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 did not exceed 25°C.

Impedance measurement. Measurement of electrical complex impedance of thawed raspberries in frequency sweep was used to characterize tissue permeabilization after PEF treatment (Donisi et al., 2010). The measurement was conducted by loading raspberries 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 analyzer, model 1260 ("Solartron", UK) consisting of a generator and an analyzer. 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 \( \theta \) 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 ($Z_p$) was calculated on the basis of the measurement of the absolute value of complex impedance of untreated ($Z_{unt}$) and PEF treated ($Z_p$) tissue in the low (1 kHz) and high (10 MHz) frequency ranges (Donsi et al., 2010):

$$Z_p = \frac{Z_{unt}(j\omega) - Z_{fr}(j\omega)}{Z_{unt}(j\omega) - Z_{fr}(j\omega)} \quad (2).$$

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

**Collection of juice and estimation of juice recovery.** The juice collected during the pressing phase was centrifuged at 5000 rpm for 10 min at 5°C centrifuge ALC PK 130R (DJB Labcare Ltd., UK) to separate the pulp from the juice. Then the juice was weighed in order to evaluate the yield of extraction, which was expressed as the grams of juice per 100 g of fresh weight of raspberries. The extracted juice was stored at 4 ± 1°C until analyzed.

**Extraction of phenolic compounds from raspberry press cake.** The press cake obtained after PEF assisted pressing of raspberries was weighed and immediately immersed in the extraction solvent (50% ethanol, 0.5% HCl, v/v). The solvent to press cake ratio was 6:1 (mL g<sup>-1</sup> ethanol, 0.5% HCl, v/v). The extraction process was carried out for 24 hours at ambient temperature with constant shaking at 150 rpm. The extracts obtained were filtered (Whatman No. 1 filter paper) and stored at 4°C until analyzed.

**Determination of the total phenolic content (TPC).** The TPC was determined using the Folin and Ciocalteu’s reagent, as previously described (Bobinaitė et al., 2012). The reagent was prepared by diluting a stock solution with distilled water (1/10, v/v). Then, 100 µL of filtered raspberry juice was mixed with 5 mL of aqueous methanol (80%). 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<sub>2</sub>CO<sub>3</sub> (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 (GAE) per L of juice or per 100 g of press cake.

**Analysis of total anthocyanins (TA).** The total anthocyanins content of centrifuged and filtered juice was determined using the pH differential method (Lee et al., 2005). Raspberry juice or press cake extracts were filtered and added to buffer solutions (pH 1.0 and 4.5) and absorbance of the solutions was measured using a spectrophotometer V-650 UV-Vis (Jasco Inc., USA) at 520 and 700 nm. The concentration of anthocyanins was expressed in mg of cyanidin-3-glucoside per L of juice or per 100 g of press cake.

**Evaluation of ferric reducing antioxidant power (FRAP).** FRAP assay of control and PEF treated samples of both raspberry juice and raspberry press cake extract was carried out according to the method described by Benzie and Strain (1996) with some modifications. For FRAP measurement of juice, 1 mL of raspberry juice was diluted with 5 mL of aqueous methanol (50%), whereas for the blank sample measurements, 1 mL of water was diluted with 5 mL of aqueous methanol (50%). For FRAP measurement of raspberry press cake extract, 1 mL of extract was diluted with 3 mL of aqueous methanol (50%), whereas for the blank sample measurements, 1 mL of extraction solvent was diluted with 3 mL of aqueous methanol (50%). For the analysis, 2 mL of freshly prepared FRAP working solution and 20 µL of diluted juice or extract were mixed and incubated for 30 minutes at ambient temperature. The change observed in the absorbance was due to the reduction of ferric-tripyridyltriazine (Fe III-TPTZ) complex by the antioxidants present in the samples, which was monitored at 593 nm using a Genesys-10 UV/Vis (“Thermo Spectronic”, USA) spectrophotometer. The adsorptions of blank samples (applying the same analysis conditions) were tested each time before the analysis. Trolox (“Acros Organics”, Belgium) was used as the standard for the calibration curve, and the FRAP values were expressed as µmol of Trolox equivalents (µmol TE) per mL of juice or per g of fruit weight berry press cake.

**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 using software SPSS 20 (SPSS Inc., USA). Mean values were further compared using Turkey’s test, and differences were considered to be statistically significant at $p \leq 0.05$.

**Results and discussion**

**Tissue permeabilization and juice quality.** Permeabilization of the cell membrane improves the mass transfer through the membrane, which leads to improved juice recovery or extraction of various biochemical compounds. The extent of tissue permeabilization due to the PEF treatment was quantified by using an impedance measurement method. During all experiments, the pulse width was set to 20 μs and the pulse repetition frequency was 20 Hz. Only electric field strength and total specific energy values were changed. 1, 3 or 5 kV cm<sup>-1</sup> electric field strength and from 1 to 12 kJ kg<sup>-1</sup> total specific energy was used to evaluate tissue permeabilization. For 5 kV cm<sup>-1</sup> electric field strength, the maximal specific energy was only 3 kJ kg<sup>-1</sup>, since at higher values the arching occurred.

Figure 1 shows the dependence of Zp values on the electric field strength and the total specific energy input. The highest cell disintegration index was achieved when 3 kV cm<sup>-1</sup> electric field strength and from 3 up to 10 kJ kg<sup>-1</sup> total specific energy values were applied. In this case, Zp of 0.51–0.53 was reached, which shows partial tissue permeabilization and juice quality.

In addition, some authors reported that with increased electric field applied, the Zp was increasing until a threshold saturation value was reached (Luengo et al., 2013). Our results supported this observation. When electric field strength of 1 kV cm<sup>-1</sup> was used, the maximal Zp value was reached at the total specific energy 6 kJ kg<sup>-1</sup>. Further increase of the total specific energy did not increase cell permeabilization (Fig. 1).
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The same phenomenon was observed when the electric field strength of 3 kV cm$^{-1}$ was used: $Z_p$ reached maximum at 3 kJ kg$^{-1}$ total specific energy and no significant increase in $Z_p$ was observed at further specific energy increase to 6 or 12 kJ kg$^{-1}$. In our study we also applied PEF to frozen/defrosted raspberries; however, it seems that freezing and then thawing of raspberries disrupted their cell membranes to the extent that subsequent PEF treatment did not further increase their cell disintegration index (data not shown). Interestingly, our previous study has shown that exposure of frozen/thawed blueberries to PEF treatment of sufficient intensity may promote further increase of the cell membrane permeabilization, with an increase of the cell disintegration index ($Z_p$) up to 0.6 (Lamanauskas et al., 2015b). These findings are not surprising, since permeabilization of plant tissues and the consequent mass transfer process is a complex function of the interaction between the PEF parameters and the material properties. Further investigations of PEF pre-treatment of fresh raspberries were carried at 1 and 3 kV cm$^{-1}$ with energy input of 1, 6 and 12 kJ kg$^{-1}$.

Figure 2 shows the juice yield from fresh raspberries pretreated by PEF at different electric field intensities (1 and 3 kV cm$^{-1}$) and different energy input (1, 6 and 12 kJ kg$^{-1}$). At control conditions, without application of PEF, the juice recovery from berries was 37.6 ± 4.49%. The highest increase in juice content was reached when 3 kV cm$^{-1}$ electric field strength and 12 kJ kg$^{-1}$ total specific energy were applied; the juice recovery increased by 25%, up to 47.0 ± 2.14%. PEF pretreatments of lower intensity (1 or 3 kV cm$^{-1}$ and 1 or 6 kJ kg$^{-1}$) slightly increased the juice content but the differences were not statistically significant. The improvement of the juice recovery after PEF pretreatment was reported previously for other plant tissues, but the reported juice recovery increase varies in a wide range, most probably due to different intrinsic characteristics of the plant tissues and/or PEF treatment parameters (Jaeger et al., 2012). After PEF pretreatment the juice yield of apple mash increased just 4.1% or 6% (Schilling et al., 2007; Turk et al., 2012). On the other hand, the juice content increase after PEF pretreatment is as high as 24% for grapes variety ‘Muscadelle’, ‘Sauvignon’ and ‘Semillon’ (Praporscic et al., 2007) or by more than 30% for blueberries (Bobinaité et al., 2015). However, in the present study freezing and thawing of red raspberries resulted in even higher increase in the juice content, which was 54.3 ± 0.24%. It indicates that cell disintegration of fresh raspberries due to PEF pretreatment was not complete.

PEF had no impact on qualitative characteristics of the raspberry juice, i.e. there was no significant difference in chemical composition or ferric reducing antioxidant power between control juice and juice obtained from PEF pretreated raspberries. The total phenolics content of the control juice obtained from fresh raspberries was 1033.9 ± 32.35 mg GAE L$^{-1}$ of juice. The total phenolics content of the juice was similar after PEF pretreatments of raspberries at all treatment intensities (Fig. 3). On the other hand, the juice obtained from frozen-thawed raspberries had significantly higher content of total phenolics; in this case it was 1219.3 ± 44.37 mg GAE L$^{-1}$ of juice. Anthocyanin content also remained unchanged after PEF pretreatment. The juice obtained from fresh raspberries contained 311.4 ± 19.20 mg L$^{-1}$ of anthocyanins, and similar content was in the juice obtained from fresh PEF pretreated raspberries at all treatment intensities (Fig. 3). Similarly to what was observed in the case of total phenolics, the juice obtained from frozen-thawed raspberries had statistically
significantly higher content of anthocyanins, $342.2 \pm 29.20 \text{ mg L}^{-1}$ of juice. This shows that PEF application did not result in complete disintegration of raspberry cell tissues, thus PEF pretreatment had different impact on juice yield and total phenolics/anthocyanins content. However, the extraction of valuable compounds from the plant tissues by pressing is never fully accomplished (Viskelis et al., 2009; Laroze et al., 2010; Bobinaith et al., 2013). More recently it has been shown that red raspberry press cake extracts exhibit significant antioxidant, proapoptotic and antibacterial activity suggesting that raspberry press cake may be regarded as a potential resource of nutraceuticals (Cetojevic-Simin et al., 2015). Based on these considerations, efficient extraction methods should form part of the industrial practices to recover valuable substances from raspberry press cake.

Figure 3. Total phenolics content (a) and total anthocyanins content (b) of raspberry juice obtained after pressing untreated (control) and PEF treated berries

Note. Different letters above the bars indicate significant differences between the mean values ($p \leq 0.05$).

Raspberry press cake results. During juice pressing, part of the anthocyanins and other valuable compounds found in berries are recovered in the juice.
amount of these compounds in the extract by 25.7%, or to 50.7 ± 3.38 mg 100 g⁻¹. Similarly as in the case of total phenolics, the increase in anthocyanins content in the extracts did not depend on PEF treatment intensity and reached saturation even at low PEF intensities. When PEF treatment of 3 kV cm⁻¹ and varying energy input (1–12 kJ kg⁻¹) were applied, the content of anthocyanins in the press cake extracts fluctuated from 43.4 to 49.6 mg 100 g⁻¹ of press cake. Interestingly, the content of anthocyanins in the press cake extract obtained from frozen-thawed raspberries was quite low – 43.7 ± 2.15 mg 100 g⁻¹.

Improved extraction of phenolic compounds and anthocyanins from PEF pretreated press cake is in agreement with other studies. Previously it was found that PEF pretreatment increases extraction yields of total phenolics by 50% and anthocyanins by 17% from grape by-products (Corrales et al., 2008). Similar observation was also documented for blueberries: after PEF pretreatment total phenolics content increased by 63% and anthocyanins content increased by 78%, when extracted from blueberries press cake (Bobinaitė et al., 2015).

The extracts from raspberry press cakes obtained applying PEF assisted juice pressing resulted in higher FRAP (Fig. 6). In comparison to the untreated fresh sample, PEF pretreatment of raspberries increased FRAP of their press cake extracts by 12–24% (for 3 kV cm⁻¹ 6 kJ kg⁻¹ and 1 kV cm⁻¹ 6 kJ kg⁻¹ treatments, respectively). Interestingly, in the case of press cake extracts for the improvement of bioactive compounds extraction, the lowest applied PEF treatment parameters 1 kV cm⁻¹ 6 kJ kg⁻¹ and 3 kV cm⁻¹ 1 kJ kg⁻¹ were almost as effective as freezing-thawing.

Note. Different letters above the bars indicate significant differences between the mean values (p ≤ 0.05).

Figure 6. Ferric reducing antioxidant power (FRAP) of extracts from press cake obtained by pressing untreated (control) and PEF treated raspberries

Conclusions

1. After pulsed electric field (PEF) pretreatment of red raspberries (Rubus idaeus L.) juice recovery increased by 9–25%.

2. Total phenolics and anthocyanin content in red raspberries juice did not change after PEF pretreatment; however, they increased in red raspberries press cake extracts. In the press cake extracts, the total phenolics content increased by 20%, while anthocyanin content increased by 26%.

3. Mild PEF treatment (0.5 or 1 kV cm⁻¹ electric field strength and 6 kJ kg⁻¹ total specific energy) was sufficient for increasing juice recovery and extraction of bioactive compounds. Thus PEF pretreatment of red raspberries is a promising technology when applied with standard technologies, such as mechanical pressing and extraction with solvents.

4. After freezing-thawing of red raspberries the juice recovery increased by 46%, total phenolics content by 18% and anthocyanin content by 10%.

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Impulsinio elektrinio lauko įtaka sulčių ir bioaktyvių medžiagų išgavimui iš aviečių ir jų produktų

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Santrauka

Apdorojimas impulsiniu elektriniu lauku yra neterminis apdorojimo būdas, naudojamas augalų ląstelėms suardyti, dėl to padidėja sulčių išeiga arba bioaktyvių medžiagų išgavimą. Kadangi 97 % aviečių parduodamos perdirbtos, yra svarbu padidinti sulčių arba bioaktyvių medžiagų išgavimą. Tyrimo metu nustatyta, jog avietes apdorojus impulsiniu elektriniu lauku ir mechaniškai spaudžiant (1,32 bar, 6 min), sulčių išeiga padidėjo 9–25 %. Lyginant su šviežų uogų išspaudomis, impulsiniu elektriniu lauku apdorojus aviečių išspaudų ekstraktai turėjo iki 22 % daugiau fenolinų junginių bei iki 26 % daugiau antocianinių ir pasižymėjo iki 24 % didesniu antioksidacinio aktyvumo. Šiltna impulsinis elektrinis laukas (1 kV cm⁻¹ elektrinio lauko stiprumas ir 6 kJ kg⁻¹ bendroji specifinė energija) buvo pakankamas, siekiant padidinti sulčių išeigą ir bioaktyvių medžiagų išgavimą iš aviečių išspaudų.

Reikšminiai žodžiai: antocianai, impulsinis elektrinis laukas, ląstelių dezintegracija, Rubus idaeus, sulčių išeiga.

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