

Improving the Extraction Yield of Juice and Bioactive Compounds from Sweet Cherries and their by-products by Pulsed Electric Fields

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The influence of pulsed electric fields (PEF) pre-treatment of sweet cherry (*Prunus Avium*) fruits of “Duroni Nero” variety both on the extraction yield and antioxidant properties of juice obtained by pressing, and on the subsequent extraction with solvent of bioactive compounds from cherry by-products (press-cake) was investigated. PEF pre-treatments were performed at constant specific energy input ($W_T = 10$ kJ/kg) and different electric field strength ($E = 0.5\text{--}3$ kV/cm) before applying a pressure of 1.64 bar for 5 min. PEF-assisted pressing ($E = 1$ kV/cm) led to a significant increase of juice yield (+40%), anthocyanins (+80%), and antioxidant power (+27%) with respect to untreated samples. However, PEF treatment intensity higher than 1 kV/cm did not significantly improved the quantitative and qualitative characteristics of juice. Compared to the untreated samples, the extracts from electroporated press-cakes achieved after PEF-assisted pressing of cherry fruits at 0.5 kV/cm showed the highest increases in anthocyanins (+38%) and antioxidant activity (+21%). No evidence of selective release or degradation of individual anthocyanins in juice and press cake extracts due to PEF application was observed. Overall, the results of this investigation demonstrated the potential of PEF as a mild technology to improve the efficiency of industrial processing of cherry fruits.

1. Introduction

Sweet cherries (*Prunus avium* L.) are an excellent source of many nutrients and antioxidant phenolics such as phenolic acids (hydroxycinnamate), flavonols, and especially anthocyanin pigments, which are mostly concentrated in cherry skins and may account for more than 70% of total phenolics (Chaovanalikit and Wrolstad, 2004; Kim et al., 2005). Cyanidin 3-rutinoside and cyanidin 3-glucoside have been identified as the major anthocyanins in cherry fruits, with pelargonidin 3-rutinoside, peonidin-3-O-rutinoside and peonidin 3-glucoside being present in much lower amounts (Chaovanalikit and Wrolstad, 2004). Several studies have reported the health beneficial effects associated to the consumption of cherry fruits, which potentially reduces the risk of cancer and alleviates arthritis and gout-related pain (Wang et al., 1999).

Due to their seasonal nature and reduced shelf-life, an increasing amount of cherries and other red fruits are commonly processed into various food products. In particular, juices obtained by pressing of red fruits are increasingly commercialized in recent years due to their reported nutritional properties and health-beneficial effects. As a result, red fruits processing unavoidably generates significant amount of by-products or wastes (press cake residues or pomace), which mainly consist of skins and seeds (Lee and Wrolstad, 2004). They often represent an environmental burden, either in terms of the impact of their use in low-added value products (i.e. animal feed, compost), or of their disposal. However, red fruits processing residues may also represent an opportunity to contribute to the economic and social benefit, since they typically retain high amount of anthocyanins and polyphenols, which could be efficiently valorised as natural colorants, nutraceuticals, and antioxidants if adequately recovered (Bobinaite et al., 2014). In this frame, demand is

increasing for developing novel processing methods able to improve the efficiency of industrial processing of red fruits, while valorising plant processed wastes with a positive effect on the environmental impact.

Pulsed electric fields (PEF) is a novel physical method, which has been shown promise as a valuable alternative to the traditional cell disintegration techniques (grinding, heating, or enzymatic maceration), in order to improve mass transfer rates of juices and intracellular compounds contained in plant cell materials during the subsequent pressing or extraction operations (Donsi et al., 2010a). During PEF treatment, the plant tissues is exposed to short duration electric field pulses of moderate intensity (0.5-10 kV/cm) and relatively low energy (1-10 kJ/kg), which develops pores in the cell membranes (electroporation), resulting in increased membrane permeability and facilitated loss of the cell content (Donsi et al., 2010a). Several studies have shown the positive effects of the application of PEF pre-treatment on the yield and antioxidant properties of juices expressed by mechanical pressing of apples, carrots and blueberries (Grimi et al. 2011; Jaeger et al. 2012; Bobinaite et al., 2014). Furthermore, PEF treatment applied before extraction with solvents has been found to be advantageous to enhance the extraction yield of antioxidants from processing wastes of blueberries (Bobinaite et al., 2014), orange peel (Luengo et al., 2013) and mango peel (Parniakov et al., 2016), among others. However, to our knowledge, no previous study focused on PEF-assisted processing of sweet cherries and their by-products (press cake). Moreover, only in the study of Bobinaite et al. (2014), it was demonstrated that pores formed in cell membranes of PEF treated blueberry fruit tissues enhanced not only the extraction yield and antioxidant properties of the expressed juice, but also facilitated the subsequent recovery by extraction with solvent of bioactive compounds (phenolics and anthocyanins) from berry by-products (press cake left after PEF-assisted pressing). The validation of this approach to other plant materials could foster the integration of PEF technology in food industry, as it will allow the use of the entire fruit, which could have economic benefits to producers and a beneficial impact on the environment, leading to a greater diversity of products.

Therefore, the objective of this work was to demonstrate that PEF applied to fresh cherry fruits prior to pressing induces permeabilization of cell membranes, which enables not only enhanced yields and quality of the expressed juice, but also increased recovery of anthocyanins from cherry press cake extracts.

2. Materials and Methods

2.1 Raw materials

Sweet cherries of "Duroi Nero" variety (Campania region, Italy) were purchased from a local supermarket and stored at 4°C until needed. Before being processed, cherries of uniform colour were selected and after the removal of the kernels, were chopped into strips with a length of 7 mm and a thickness of 2 mm.

2.2 Chemicals

Cyanidin 3-O-rutinoside was obtained from TransMIT (Giessen, Germany). HPLC grade methanol, ethanol, acetonitrile and 2,4,6-tripyridyl-S-triazine (TPTZ) were supplied from Sigma-Aldrich (Steinheim, Germany). Analytical grade formic acid was purchased from Riedel-deHaën (Seelze, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Acros Organics (Geel, Belgium).

2.3 PEF equipment

PEF treatments were performed by using the PEF unit previously described by Bobinaite et al. (2014). Briefly, the system consists of a high voltage pulsed power (25 kV-500 A) generator (Modulator PG, ScandiNova, Uppsala, Sweden) able to generate monopolar square wave pulses (3-25 μ s, 1-450 Hz). The generator was connected by a high voltage cable to a batch cylindrical treatment chamber (3.4 cm in diameter, electrode gap up to 5 cm) specifically designed to be used also as a mechanical press (Bobinaite et al., 2014). The actual voltage and current signals at the treatment chamber were measured by a high voltage probe (Tektronix, P6015A, Wilsonville, OR, USA) and a Rogowsky coil (2 - 0.1 Stangenes, Inc., USA) connected to a 300 MHz digital oscilloscope (Tektronix, TDS 3034B, Wilsonville, OR, USA).

2.4 PEF assisted pressing and extraction

For each experiment, 12 g of chopped cherries were loaded into the treatment chamber and subjected to PEF pre-treatment at variable field strength ($E = 0.5$ -3 kV/cm) and at constant total specific energy input ($W_T = 10$ kJ/kg) applied by changing the voltage across the electrodes and the number of pulses at constant frequency (5 Hz) and pulse width (20 μ s). These processing conditions were identified through preliminary tests based on the impedance analyses (data not shown). After the PEF treatment, according to the protocol used by Bobinaite et al. (2014), the samples were pressed in the same PEF chamber at a constant pressure of 1.64 bar for 5 min. The liquid expressed during each experiment was collected in a plastic tube and then centrifuged at 5000 rpm for 10 min at 4°C (PK121R model, ALC International, Cologno Monzese, Milan, Italy)

in order to obtain a clear juice. The latter was weighed to evaluate the extraction yield, expressed as g of juice per 100 g of fresh weight (fw) cherries, and then stored at 4°C until analysed. Untreated (control) samples were collected after the application of the same protocol without PEF treatment. The remaining processing waste (press cake left after pressing of either untreated or PEF treated cherries) were subjected to solid-liquid extraction with acidified aqueous ethanol (50% ethanol; 0.5% HCl, v/v) at a solvent to cake ratio of 5:1 (mL/g). After 24 h extraction at 25 °C with constant shaking at 160 rpm, samples were filtered as previously described (Bobinaite et al., 2014) and stored at 4 ± 1°C until analyzed.

2.5 HPLC-DAD analysis of anthocyanins

Sweet cherry juice and press cake extracts were diluted 1:5 (v/v) with a solution consisting of 90% solvent A (10 % aqueous formic acid) and 10% solvent B (acetonitrile–methanol, 85:15, v/v). Prior to HPLC analysis diluted juices and extracts were filtered through regenerated cellulose, syringe-tip filters (0.45 µm, 13 mm). An HPLC system Waters 2695 provided with a photo diode array detector (DAD) Waters 2998 (Waters Corporation, USA) was used for analysis of anthocyanins. Analytical separation of anthocyanins was carried out with a ACE Excel 5 Super C18 column (5µm; 250 × 4.6 mm; Aberdeen, Scotland) by following the procedure of Wang et al. (2014). The temperature of the column oven was set at 25°C. An elution gradient consisting of Solvent A and Solvent B was applied at a flow rate of 1.0 mL/min as follow: 5 to 12% of solvent B in 30 min, from 12 to 25% solvent B in 20 min. The injection volume of each sample was 10 µL, and the individual anthocyanins were detected at 520 nm. Data were recorded from 200 to 600 nm. Anthocyanins were quantified with a semi-quantitative method using a linear regression of a commercial standard. Cyanidin 3-rutinoside ranging from 3.125 to 100 µg/mL was dissolved in solvent B (10%) and solvent A (90%) to generate six-point external standard calibration curve ($R^2=0.999$).

2.6 Determination of antioxidant activity

The antioxidant activity of juice and press cake extracts was determined by ferric reducing antioxidant power (FRAP) assay according to the method reported by Benzie and Strain (1996) with some modifications, as thoroughly described elsewhere (Bobinaite et al. 2014). Trolox was used as the standard for calibration curve and the FRAP values were expressed as µmol of trolox equivalents (µmol TE) per mL of juice or g of cherry press cake.

2.7 Statistical analysis

PEF treatments were carried out in triplicate and each collected sample was analysed in duplicate. The mean values and standard deviations (SD) of the experimental data were calculated. Differences among mean values were analyzed by means one-way ANOVA using SPSS 20 software (SPSS Inc., Chicago, USA). When significant differences were detected, the Tukey test was performed to determine which particular means were significantly different ($p \leq 0.05$).

3. Results and discussion

3.1 Sweet cherry juice: effect of PEF on extraction yield

The effect of PEF pre-treatments ($E = 0.5$ -3 kV/cm; $W_T = 10$ kJ/kg) on the extraction yield of sweet cherry juice is reported in Table 1.

Table 1: Juice yield obtained after pressing of untreated (0 kV/cm) and PEF-treated ($W_T = 10$ kJ/kg) cherries. Different letters indicate significant differences between the mean values ($p \leq 0.05$).

E (kV/cm)	0	0.5	1	3
Juice Yield (g/100 g fw)	20.2±1.9a	24.5±0.4b	28.3±1.4c	23.2±1.4ab

Results show that the application of PEF treatments at 0.5 and 1 kV/cm significantly ($p \leq 0.05$) increased the juice yield as compared with the untreated sample, which rose by 20 and 40 %, respectively. However, for further increasing of the field strength applied up to 3 kV/cm, no significant difference ($p > 0.05$) could be observed with respect to the untreated sample. In agreement with previous findings (Jaeger et al., 2012; Bobinaite et al., 2014), it is likely that high intensity PEF treatment caused softening of cherry tissues leading to unfavourable de-juicing conditions, such as compaction and closing of the capillaries in press cake. From these results, it can be concluded that the permeabilization effect induced by PEF treatment at 1 kV/cm and 10 kJ/kg as well as the corresponding tissue softening due to the electroporation, resulted in the most favorable de-juicing properties of cherry fruits.

3.2 Sweet cherry juice: effect of PEF on anthocyanins content and antioxidant power

Individual and total anthocyanins content (TAC) of juice obtained after pressing of untreated (0 kJ/kg) and PEF-treated cherries are shown in Table 2.

Table 2: Concentrations (in mg/100mL of juice) of individual and total anthocyanins content (TAC) of cherry juice obtained after pressing of untreated (0 kJ/kg) and PEF-treated ($W_T = 10$ kJ/kg) sweet cherries. Different letters in the same line indicates significant differences between the samples ($p \leq 0.05$)

Peak	R _t (min)	Anthocyanin	0 kV/cm	0.5 kV/cm	1 kV/cm	3 kV/cm
1	21.6	Cyanidin-3-Glucoside	2.2±0.7a	2.6±0.7a	3.7±0.7ab	2.8±0.8ab
2	24.6	Cyanidin-3-Rutinoside	10.4±0.9a	14.8±1.9ab	21.0±1.1b	17.4±0.9ab
3	31.5	Pelargonidin-3-Rutinoside	0.7±0.3a	0.8±0.4a	0.9±0.3a	0.8±0.5a
4	35.8	Peonidin-3-Rutinoside	2.9±0.2a	3.4±0.5ab	3.6±0.3b	3.6±0.4ab
Total			16.2±1.4a	21.6±1.1ab	29.2±1.1b	24.6±2.1ab

TAC in the untreated sample (0 kV/cm) was 16.2 mg/100mL. Moreover, HPLC chromatogram profiles (data not shown) revealed the presence of four main anthocyanin compounds in the untreated sample, with cyanidin-3-rutinoside being the predominant compound, while the content of the other three compounds, namely peonidin-3-rutinoside, cyanidin-3-glucoside, and pelargonidin-3-rutinoside, was 3.6, 4.7, and 14.9 times lower than the cyanidin-3-rutinoside content.

The application of a PEF pre-treatment clearly contributed to a further increase in the extraction of all the anthocyanin compounds detected in the untreated sample, whose total content rose approximately to 33, 80, and 52 % when the cherry fruits were PEF-treated at 0.5, 1, and 3 kV/cm, respectively. However, a significant increase in TAC as compared with the untreated sample was observed only for the PEF-treated sample at 1 kV/cm. These results are consistent with those previously observed for the juice yield (Table 1), indicating that more intense treatment conditions (>1 kV/cm) for fresh cherries might not be necessary to intensify the release of intracellular compounds into juice. Similar results were previously observed by Bobinaite et al. (2014), who found that the TAC of blueberry juice significantly increased by 60, 78, and 44 % when the berry fruits were PEF-treated at 1, 3, and 5 kV/cm, respectively. Interestingly, HPLC analyses (data not shown) also revealed no evidence of selective release or degradation of individual anthocyanins due to PEF application to cherry fruits.

The antioxidant activity of the cherry juice is reported in Figure 1. Results show that, compared to the juice obtained from the untreated cherries, PEF pre-treatments at 0.5, 1, and 3 kV/cm increased the FRAP values of the juices by 10.0, 27.4, and 15.2 %, respectively. Similarly, it has previously been reported that PEF treatment also improves the antioxidant activity of juices obtained after pressing of grapes, apples and blueberry fruits (Donsi et al. 2010b; Grimi et al., 2011; Bobinaite et al., 2014). However, it should be noted that, as compared with the untreated sample, significant ($p \leq 0.05$) differences in the antioxidant activity of the cherry juices were detected only for the samples obtained after PEF treatment at 1 kV/cm.

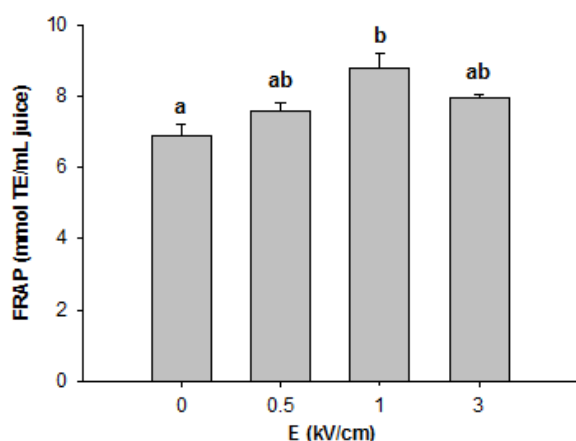


Figure 1: Ferric Reducing Antioxidant Power (FRAP) of juices obtained after pressing of untreated (0 kV/cm) and PEF-treated ($E = 0.5-3$ kV/cm; $W_T = 10$ kJ/kg) sweet cherries. Different letters above the bars indicate significant differences between the mean values ($p \leq 0.05$).

These results were well correlated ($R^2 = 0.989$) with those of anthocyanin content (Table 2). Partially in contrast with these findings, Chaovanalikit and Wrolstad (2004) observed that total phenolics were a much better predictor of antioxidant properties than TAC.

3.3 Sweet cherry extracts: effect of PEF on anthocyanin content and antioxidant activity

In fresh cherries, anthocyanins and other phenolic compounds are mostly accumulated in the skins with lesser amounts being present in the fruits flesh (Chaovanalikit and Wrolstad, 2004). As cherry press cake is mainly composed of skins, it is likely that it retains high amounts of anthocyanins and other phenolics.

Table 3 shows the individual and TAC of the extracts obtained from the press cakes of untreated and PEF treated cherries.

Table 3: Concentrations (in mg/100g press cake) of individual and total anthocyanins content (TAC) of press cake extracts obtained after pressing of untreated (0 kJ/kg) and PEF-treated ($W_T = 10$ kJ/kg) sweet cherries. Different letters in the same line indicate significant differences between the samples ($p \leq 0.05$)

Peak	R _t (min)	Anthocyanin (mg/100 g)	0 kV/cm	0.5 kV/cm	1 kV/cm	3 kV/cm
1	21.6	Cyanidin-3-Glucoside	25.9±1.2a	36.9±1.8b	28.1±2.2ab	25.6±1.7a
2	24.6	Cyanidin-3-Rutinoside	124.7±3.2a	171.5±4.3b	174.5±8.9b	171.7±6.5b
3	31.5	Pelargonidin-3-Rutinoside	1.8±0.4a	2.3±0.7a	2.2±0.2ab	2.3±0.4a
4	35.8	Peonidin-3-Rutinoside	5.1±0.5a	7.3±0.2ab	6.8±0.3ab	6.7±0.6ab
Total			157.5±11.2a	218.0±14.8b	211.6±3.1b	206.3±8.5b

TAC of untreated extracts was 157.5 mg/100g press cake, which confirmed that a substantial amount of anthocyanin compounds was still retained inside the plant cells. Similarly to the results previously observed for cherry juice (Table 2), 4 individual anthocyanins were also characterized in press cake extracts, with cyanidin-3-rutinoside being again identified as the major anthocyanin followed by cyanidin-3-glucoside and pelargonidin-3-rutinoside, while peonidin-3-rutinoside was present in much lower amount. HPLC chromatogram profiles also revealed that the number and type of these anthocyanins were not affected by the PEF pre-treatment (data not shown). However, as compared with the untreated sample, the total amount of these compounds increased by 38.4, 34.3, and 31.0% when they were extracted from press cakes obtained after PEF-assisted pressing of fresh fruits at 0.5, 1, and 3 kV/cm, respectively. In agreement with previous findings (Bobinaite et al., 2014), it is likely that pores formed during the PEF pre-treatment of fresh fruit were still open after pressing. Some previous literature data have shown that PEF treatment remarkably enhanced the extraction of anthocyanins from grape by-products (Corrales et al. 2008) and red cabbage mash (Gachovska et al., 2010), even though in these studies PEF treatment was applied directly to the food wastes rather than the fresh fruits. Extracts obtained from the press cakes of PEF-treated cherries possessed a significantly ($p \leq 0.05$) stronger antioxidant activity than the untreated samples (Figure 2). Similarly, the positive effect of PEF pre-treatment on extractability of antioxidant compounds from blueberry by-products (Bobinaite et al., 2014) was previously reported.

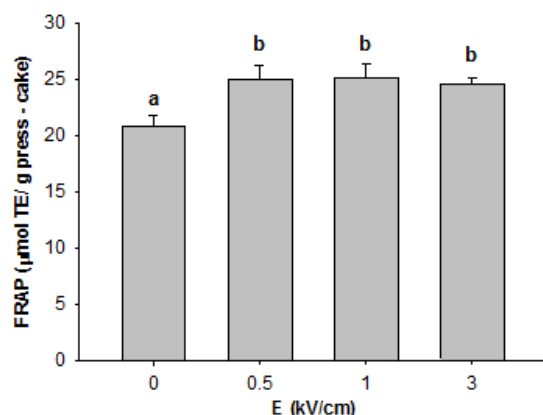


Figure 2: Ferric Reducing Antioxidant Power (FRAP) of extracts from cherry press – cakes obtained after pressing of untreated (0 kV/cm) and PEF-treated ($E = 0.5-3$ kV/cm; $W_T = 10$ kJ/kg) sweet cherries. Different letters above the bars indicate significant differences between the mean values ($p \leq 0.05$).

In comparison to the control extract, PEF treatments at 0.5, 1 and 3 kV/cm significantly ($p \leq 0.05$) increased the FRAP values of the press-cake extracts by 20.6, 21.0 and 18.2 %, respectively. Moreover, the antioxidant activity of the blueberry press cake extracts correlates ($R^2 = 0.998$) with the content of anthocyanins in the extracts, as previously observed for cherry juice. However, it is worth noting that no significant difference was detected among the PEF treated samples. This is consistent with the fact that, in this study, significant changes of anthocyanins content in the press cake extracts of PEF pre-treated samples at different field strength were not observed.

4. Conclusions

The results of this study have demonstrated that the electroporation effect induced by PEF pre-treatment at relatively low field strength ($E = 0.5\text{--}1$ kV/cm) and energy input ($W_T = 10$ kJ/kg) appeared to be sufficient for the improvement of juice yield as well as for the intensification of the anthocyanins extraction from both cherry fruits and their by-products (press cakes). Moreover, HPLC analyses revealed that there was no evidence of degradation of individual anthocyanins due to PEF application.

Finally, these promising results confirm the potential of PEF technology to improve the efficiency of the red fruits transformation process, adding value to food product and allowing, at the same time, the valorization of the food processing waste leading to a greater diversity of products.

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