



Optimization of Pulsed Electric Field Treatment for the Extraction of Bioactive Compounds from Blackcurrant

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Received: 29 January 2019 / Accepted: 12 April 2019
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Abstract

The objective of this work was to enhance the bioactive compounds extraction of blackcurrant using pulsed electric fields (PEF) technology. An experimental design was performed to find the best PEF conditions using the desirability approach and response surface methodology. The effect of the electric field strength and the treatment time over the total polyphenolic content (TPC) and the antioxidant activity (AA) was analyzed. The optimum treating conditions were found to be 1318 V/cm and 315 pulses, and resulted in increments of 19%, 45%, and 6%, for TPC, AA, and total monomeric anthocyanins, respectively. Two initial temperatures were studied (10 and 22 °C) during electroporation. A significant effect of temperature over PEF treatment was observed. The PEF treatment was appropriate for increasing the extraction of bioactive compounds, leading to improved blackcurrant juices that could be used as ingredients for functional foods.

Keywords PEF · Polyphenols · Anthocyanins · Antioxidant activity · Blackcurrant

Introduction

The increase of fruit intake in everyday diet has been one of the health concerns around the world for the past decade. The health benefits related to their consumption are associated to the ingestion of dietary fiber, vitamins, and minerals, as well as other bioactive components (Chang et al. 2016). Blackcurrant (*Ribes nigrum* L.) is a berry

fruit which has an exceptional content of bioactive compounds, mainly polyphenols and among them, mostly anthocyanins (Bakowska-Barczak and Kolodziejczyk 2011; Corrales et al. 2008). Several health benefits and biological activities of blackcurrant have been reported: anti-inflammatory and immuno-modulatory responses, antioxidant properties, antimicrobial, and anticarcinogenic activities (Gopalan et al. 2012).

Many processing technologies have been tested to enhance juice recovery and promote the release of the bioactive compounds from vegetable tissue. With this purpose, thermal and enzymatic treatments have been used for many years in the industry. However, these technologies usually consume large amounts of energy and time and may result in nutritional and sensory deterioration (Ricci et al. 2018). The search for faster, environmentally friendly, and more efficient extraction methods has driven to the development of new techniques such as ultrasound-assisted extraction (Chemat et al. 2017), microwaves (Bagherian et al. 2011; Drosou et al. 2015), and pulsed electric field (PEF) (Barba et al. 2015a; Boussetta et al. 2012, 2014; Grimi et al. 2011; Loginova et al. 2011). PEF treatment is a non-thermal technology that consists in the application of an electric field, exposing the sample to repetitive short voltage pulses of relatively low energy and moderate intensity (Bobinaité et al. 2015). In the case of plant tissues, the effect of PEF application is the permeabilization of the cell

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membranes that in turn promotes the release of liquid and important compounds from the cells (Barba et al. 2015b; Bobinaitė et al. 2015). PEF-assisted extraction increases mass transfer without exposing the material to severe temperature conditions thus reducing heat-sensitive compounds degradation (Barba et al. 2015b). Moreover, the short processing times required by this technique as well as the lower energy consumption compared to other conventional extraction methods makes it a very interesting technology for the food industry (Vorobiev and Lebovka 2016; Frey et al. 2017).

PEF treatments were effectively used to improve the yield and quality of juices obtained from several fruits and vegetables: apples (Carbonell-Capella et al. 2016; Grimi et al. 2011), carrots (Jaeger et al. 2012), grapes (Donsi et al. 2010), and blackberries (Barba et al. 2015a). Pataro et al. (2017a) applied PEF treatments on blueberries and their by-products and reached promising results regarding extraction efficiency of juice and antioxidants. However, to the best of our knowledge, no studies of PEF treatments applied over blackcurrant have been conducted yet.

In order to effectively electroporate the cell membrane, the applied electroporating pulses have to reach a minimum value of field strength, called the “threshold of electroporation” (Asavasanti et al. 2010). This critical field strength induces the critical transmembrane voltage needed for the onset of enhanced permeability. To achieve the threshold of electroporation, different treatment conditions may be required depending on the food matrix and the electroporation system. Response surface methodology (RSM) is a very powerful technique for optimization purposes that enables the study of the effect of different factors and their interactions (Baş and Boyacı 2007; Suárez et al. 2014). On the other hand, the desirability function allows the simultaneous optimization of multiple responses, giving the optimum value of each independent variable that results in the desired response for all and each dependent variables (Olaiz et al. 2014; Silva et al. 2013).

The use of natural components as ingredients in different foodstuffs is a very popular current trend in food science and industry. In this context, blackcurrant extracts represent a very attractive and potentially healthy natural ingredient to be considered for a broad range of food products. The aim of this work was to optimize the PEF conditions treatment to improve the extraction of bioactive compounds from blackcurrant to produce an extract with high antioxidant capacity, suitable to be used as a healthy food ingredient.

Materials and Methods

Materials

Individually quick-frozen (IQF) blackcurrants (*R. nigrum* L., Titania cultivar) were purchased at a local market and stored at

– 18 °C until use. The rest of the reagents were analytical-grade.

Methodology

The frozen fruit was thawed under controlled conditions (water bath at 40–45 °C for 25 min), reaching a temperature of 10 °C before applying the pulsed electric field (PEF) treatment. Following the PEF procedure, juice was obtained by cold pressing using a commercial slow juicer (Peabody by Hurom PE-HSJO2, China). The cold pressing process parameters were power = 150 W, velocity = 70 RPM, and pressing time = 1.5 min. Control samples were prepared following the same procedure but without the application of PEF.

PEF Setup

PEF treatment of the thawed blackcurrant was carried out in batches of 60 g of fruit in a customized laboratory-scale chamber that consisted of an acrylic cuvette of 21 cm × 5 cm × 1 cm. The thawed fruit was placed inside the empty chamber with little volume of the natural fruit juice moistening the sample and providing a better contact between the individual fruits. The electrode consisted in two confronted rows of 22 needles distanced by 80 mm. The treatment chamber was immersed in ice in order to minimize the temperature effect for the corresponding experiments. Electroporation treatment was applied with a square wave electroporator (BTX ECM 830, Harvard Apparatus Inc., MA, USA). The pulse voltage and current were measured by a high-voltage probe (100×) and a shunt resistor (1 ohm) in series with the chamber, using an oscilloscope DSOX2012A, 100 MHz, and two channels (Agilent Technologies, CA, USA). These data were recorded all along the treatment. The pulse width was 100 ms for all the experiments. The temperature was measured just before applying the PEF treatment and at the end of it with an infrared thermometer Benetech GM 320 (USA).

Experimental Design

A factorial design was applied for two independent variables: electric field (V/cm) and number of pulses (constant pulses width). The effect of different levels of each variable on the total phenolic content (TPC) and the antioxidant activity (AA) was studied with the purpose of maximizing both responses. A total of 24 experiments were conducted based on two-factor and 4 × 3 level experimental design, with one replicate of the whole design. Table 1 shows the levels of the experimental design. Level 0 corresponds to the control samples, without PEF treatment. The maximum value of electric field was limited by the occurrence of sparks, due to the high conductivity of the sample, while the selected number of pulses was based

Table 1 Experimental design used to optimize the PEF treatment conditions and results for the two response variables selected

Number	Factor values		Response values	
	Electric field (V/cm)	Number of pulses	TPC (mg GA/g extract)	AA (mg GA/g extract)
1	0	50	3.2 ± 0.3	1.6 ± 0.1
2	0	275	3.1 ± 0.2	1.3 ± 0.1
3	0	500	3.27 ± 0.07	1.29 ± 0.01
4	650	50	3.4 ± 0.2	1.40 ± 0.04
5	650	275	3.85 ± 0.08	1.6 ± 0.1
6	650	500	3.6 ± 0.3	1.59 ± 0.03
7	1300	50	4.1 ± 0.4	1.7 ± 0.1
8	1300	275	4.1 ± 0.3	1.9 ± 0.2
9	1300	500	4.0 ± 0.3	1.8 ± 0.2
10	1950	50	3.7 ± 0.2	1.63 ± 0.06
11	1950	275	3.8 ± 0.2	1.7 ± 0.2
12	1950	500	3.6 ± 0.3	1.6 ± 0.2
13	0	50	3.12 ± 0.08	1.66 ± 0.06
14	0	275	3.2 ± 0.1	1.6 ± 0.1
15	0	500	3.3 ± 0.3	1.69 ± 0.05
16	650	50	3.40 ± 0.08	1.65 ± 0.07
17	650	275	3.36 ± 0.07	1.8 ± 0.1
18	650	500	3.35 ± 0.04	1.72 ± 0.09
19	1300	50	3.71 ± 0.09	1.81 ± 0.05
20	1300	275	3.7 ± 0.1	1.9 ± 0.1
21	1300	500	3.7 ± 0.2	1.83 ± 0.03
22	1950	50	2.98 ± 0.01	1.65 ± 0.08
23	1950	275	3.51 ± 0.09	1.78 ± 0.08
24	1950	500	3.1 ± 0.9	1.8 ± 0.1

on previous experiments. The experiments were led in randomized order to minimize the effect of the variability due to extraneous factors (Nath and Chattopadhyay 2007).

Optimization

Response surface methodology (RSM) was used to obtain the optimum PEF treatment conditions using the desirability approach (Derringer and Suich 1980). Several models were tested, and the cubic model (Eq. 1) showed the best fitting to the experimental data.

$$y = a + bX_1 + cX_2 + dX_1X_2 + eX_1^2 + fX_2^2 + gX_1^2X_2 + hX_1X_2^2 + iX_1^3 + jX_2^3 \quad (1)$$

where X_1 = electric field and X_2 = number of pulses.

Effect of Temperature

The effect of temperature was assessed at the optimal conditions found with the experimental design. A control sample without PEF application was also studied. Two initial

temperatures were tested, 10 and 22 °C, by immersing the treatment chamber in an ice bath or by keeping it at room temperature, respectively. The temperature was monitored every 25 pulses during all the treatment time with an infrared thermometer Benetech GM 320 (USA), and in both cases, it increased less than 5 °C during the PEF application.

Physicochemical Properties

The physicochemical properties were measured on the juices obtained after cold pressing, with and without the previous application of PEF.

Soluble Solids, Total Acidity, and pH The fruit was characterized according to AOAC methods: moisture (920.62), soluble solids (932.12), acidity (945.26), and pH (945.27) (AOAC 2000).

Bioactive Compounds Content and Antioxidant Activity

These properties were measured on the juices obtained after cold pressing, with and without the previous application of

PEF. A spectrophotometer ultraviolet-visible Jasco V-630 (Tokyo, Japan) was used in all cases.

Blackcurrant Extracts Methanolic extracts were used for total phenolic content (TPC) and antioxidant capacity (AA) determinations. For extract preparation, 0.5 g of juice was homogenized in 5 mL of absolute methanol, shaken for 5 min using a magnetic stirrer and filtered under vacuum using a Büchner funnel. The pellet was extracted again with 5 mL of absolute methanol. The extracts were combined, and methanol was added to constitute a total volume of 10 mL.

Ethanol extracts were used for monomeric anthocyanin content (TMA) determination. For extract preparation, 0.5 g of juice was homogenized in 10 mL of ethanol acidified with hydrochloric acid, shaken for 15 min using a magnetic stirrer and filtered under vacuum using a Büchner funnel. The pellet was extracted two more times with 5 mL of ethanol acidified with hydrochloric acid. The extracts were combined, and ethanol was added to constitute a total volume of 25 mL.

Total Phenolic Content Total phenolic content was determined using the Folin–Ciocalteu reagent following Archaina et al. (2018) procedure. One hundred microliters of methanolic extract were mixed with 900 μL of water, 100 μL of Folin–Ciocalteu reagent, and 600 μL of 20% sodium carbonate in 1.5 N NaOH. After 25-min incubation at 40 °C and centrifugation (10,000 rpm, 5 min), the supernatant absorbance was measured at 765 nm. A calibration curve was constructed with gallic acid ($R^2 = 0.9989$) and the results were expressed as gallic acid equivalents in milligrams per 1 g of sample.

Antioxidant Activity Antioxidant activity was measured using the bleaching method of 2,20-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] radical cation (ABTS⁺), according to Coria Cayupán et al. (2011). ABTS⁺ was dissolved in distilled water to a final concentration of 7 mM. Radical cation solution was prepared by incubating the ABTS⁺ solution with a 2.45 mM potassium persulfate solution for 16 h in the dark at room temperature. Subsequently, the solution was diluted with phosphate buffer pH 7.40 to an absorbance of 1.00 ± 0.01 at 734 nm. Then, 1.9 mL of the final ABTS⁺ solution was added to aliquots of 0.1 mL of sample extract, incubated at 25 °C for 30 min and the decrease in absorbance at 734 nm was measured. A calibration curve was done with gallic acid as standard ($R^2 = 0.9956$). The results were expressed as gallic acid equivalents in milligrams per 1 g of sample.

Monomeric Anthocyanin Content TMA was determined using the pH differential method (Giusti and Wrolstad 2001). A 1:3 (methanolic extract/buffer) dilution was performed using

buffer solution of pH 1.0 or 4.5. After 15-min incubation in the dark, the absorbances at 510 and 700 nm were measured. TMA was expressed as cyanidin-3-glucoside (molecular weight (MW) = 445.2 and a molar extinction coefficient $\epsilon = 26,900 \text{ L cm}^{-1} \text{ mol}^{-1}$) per 1 g of sample using Eqs. 2 and 3.

$$A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5} \quad (2)$$

$$TMA = (A \times MW \times DF \times 1000) / (\epsilon \times l) \quad (3)$$

where MW is the molecular weight of cyanidin-3-glucoside, DF is the dilution factor, and l is the optical path length.

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 6 (CA, USA, 2014). An ANOVA analysis and the Tukey's test were carried out to detect differences ($p < 0.05$) between samples. All measurements were made in triplicate and the average values were informed. Design Expert 10 software (Minneapolis, USA) was used for response optimization (RSM) of the extraction conditions.

Results and Discussion

Table 1 shows the 24 experiments conducted according to the experimental design used to find the optimum PEF treatment conditions, and the results obtained for the selected response variables (TPC and AA) in each experiment. The statistical analysis showed that both independent variables presented positive effects over the response variables being the electric field the parameter of highest influence ($p < 0.05$). A cubic model was fitted to the experimental data and the following equations were obtained for TPC (Eq. 4, $R^2 = 0.818$) and AA (Eq. 5, $R^2 = 0.724$):

$$y = 3.81 + 0.74X_1 + 0.008181X_2 - 0.035X_1X_2 - 0.42X_1^2 - 0.11X_2^2 + 0.024X_1^2X_2 - 0.16X_1X_2^2 - 0.50X_1^3 + 0.0000X_2^3 \quad (4)$$

$$y = 1.75 + 0.35X_1 + 0.053X_2 + 0.042X_1X_2 - 0.12X_1^2 - 0.025X_2^2 - 0.067X_1^2X_2 - 0.064X_1X_2^2 - 0.23X_1^3 + 0.0000X_2^3 \quad (5)$$

where X_1 = electric field and X_2 = number of pulses.

The statistical analysis performed to evaluate the goodness of fit showed the models were adequate ($p < 0.05$). The adjusted R^2 values were 0.714 and 0.649 for TPC and AA, respectively. Also the lack-of-fit of the regression models was not significant for both responses.

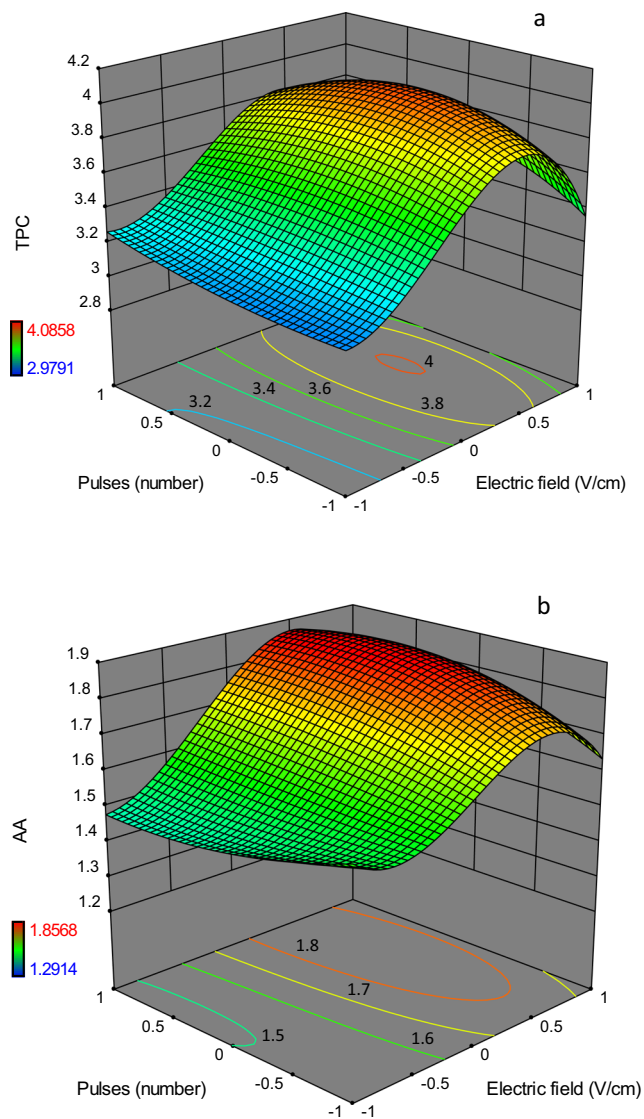


Fig. 1 Surface and contour plots for TPC (a) and AA (b) as a function of the electric field and number of pulses. Electric field intensity ranging from 0 (encoded level - 1) to 1950 V/cm (encoded level 1) and pulses from 50 (encoded level - 1) to 500 (encoded level 1)

Figure 1 shows the surface plot of TPC (Fig. 1a) and AA (Fig. 1b) as a function of electric field and number of pulses. An increase in both variables can be observed when the electric field increases up to 1300 V/cm. This behavior could be related to an increment in the release of bioactive compounds that provide antioxidant activity, as Delsart et al. (2014) observed in their study on Cabernet Sauvignon grape berries. They analyzed the phenolic compounds release after PEF treatment and showed that PEF caused the depolymerization of tannins in the skin, resulting in smaller molecules that could diffuse more easily. Conversely, when the electric field is raised above 1300 V/cm, AA and TPC suffered a decrease, probably due to bioactive compounds deterioration. It has been already observed by other authors that high levels

of electrical energy delivered during PEF treatment may cause deterioration and degradation of valuable compounds (Mahnič-Kalamiza et al. 2014). Bobinaitė et al. (2015) found a slight decrease in both TPC and AA on blueberries juice with the application of a PEF pre-treatment, when increasing the electric field up to 5 kV/cm. Fig. 2 shows the surface response for the desirability function versus electric field values and number of pulses. The desirability function reached a value of 0.869, which indicates that a good maximization was achieved. The optimum treatment conditions suggested by the design were an electric field of 1318 V/cm and a number of pulses of 315, which would result in a TPC value of 3.8 and AA value of 1.8 mg GA/g as predicted by the model. With PEF treatment under the best operative conditions, TPC, AA, and TMA increased 19%, 45%, and 6%, respectively. The actual current that circulated through the fruit during the PEF treatment was measured and the total energy received by the sample was calculated. For the optimum conditions, the total received energy was 30 ± 2 kJ/kg.

With the purpose of verifying the accuracy of the optimization process for predicting the optimum response values, three new samples were subjected to the optimum treatment conditions predicted by the model. The experimental values were consistent with the predicted values, reaching an average TPC value of 3.8 ± 0.2 mg GA/g and AA value of 1.88 ± 0.06 mg GA/g.

PEF treatment has shown a positive effect on the extraction of bioactive compounds from diverse vegetal sources. Nevertheless, due to the differences in the treatment conditions, sample preparation, and electrode design, and the lack of information about experimental procedures, a comparison between PEF treatments applied among different authors is a

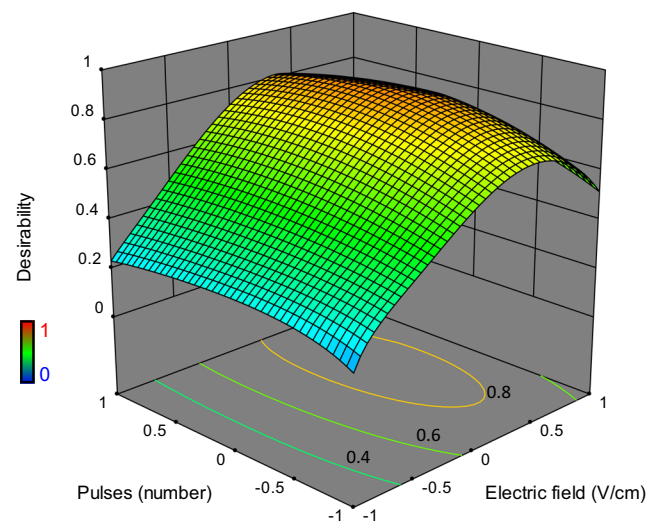


Fig. 2 Desirability surface and contour plot as a function of electric field and number of pulses, obtained by optimization process of multiple response variables. Electric field intensity ranging from 0 (encoded level - 1) to 1950 V/cm (encoded level 1) and pulses from 50 (encoded level - 1) to 500 (encoded level 1)

Table 2 Characterization of the extract obtained under the optimum extraction conditions (1318 V/cm, 315 pulses) and a control sample

	Control	PEF
Total soluble solids ($^{\circ}$ Brix at 25 $^{\circ}$ C)	13.1 \pm 0.1 ^a	14.3 \pm 0.7 ^a
pH	3.14 \pm 0.04 ^b	2.88 \pm 0.05 ^a
Total acidity (mg citric acid/100 g extract)	2.41 \pm 0.02 ^a	2.60 \pm 0.01 ^b
TPC (mg GA/g extract)	3.18 \pm 0.08 ^a	3.8 \pm 0.2 ^b
AA (mg GA/g extract)	1.296 \pm 0.007 ^a	1.88 \pm 0.06 ^b
TMA (mg cyd-3-glu/g extract)	1.301 \pm 0.008 ^a	1.38 \pm 0.02 ^b

Means within rows with a different lower case superscript letter are significantly different ($p < 0.05$)

complex analysis. In addition, there are not many studies about optimization of extraction of bioactive compounds by PEF treatment in vegetal material. Donsi et al. (2011) showed that a PEF treatment of 20 kJ/kg applied to grape skins after maceration of red wines allowed an increase of AA of 25% (ABTS⁺ method). Bobinaitė et al. (2015) applied electric fields for the production of juice and extraction of bioactive compounds from blueberry fruits, and observed an AA increase of 36% (FRAP method) when the applied energy was 10 kJ/kg. Pataro et al. (2017b) applied a 10 kJ/kg treatment to sweet cherries and reached a 27% increase on AA (FRAP method). In the present study, we reached 45% AA increase while working at the optimum conditions (30 \pm 2 kJ/kg).

Table 2 shows the characterization of the optimum extract and a control sample. The PEF-treated sample presented a significantly higher total acidity and lower pH value. Also, among bioactive compounds, there was a slight raise in the extraction of anthocyanins, and as previously mentioned, an increase in AA and TPC was observed. This is of great interest as anthocyanins and other polyphenols are one of the most valuable compounds which confer many of the beneficial assets to berry fruits.

Figure 3 shows the effect of temperature over the PEF-assisted extraction of bioactive compounds, at initial temperatures of 10 and 22 $^{\circ}$ C. Comparing both control samples, it was found that an increase in temperature caused an increment of 15% in TPC (Fig. 3a) and 21% in AA (Fig. 3b). Comparing each treatment with the corresponding control at the same temperature, the effect of both the electric field and the temperature can be noticed.

Figure 4 shows the relationship between the electric current and the temperature during the PEF process. The initial temperatures were 10 and 22 $^{\circ}$ C, and the corresponding electrical currents were 54 \pm 3.6 and 71 \pm 4.4 A, respectively. As expected, the electrical current was higher for the higher initial temperature. Additionally, starting from both temperatures, during the PEF treatment, a raise of up to 5 $^{\circ}$ C was observed, showing a linear relationship between the measured temperature and the electric current which could be attributed to the

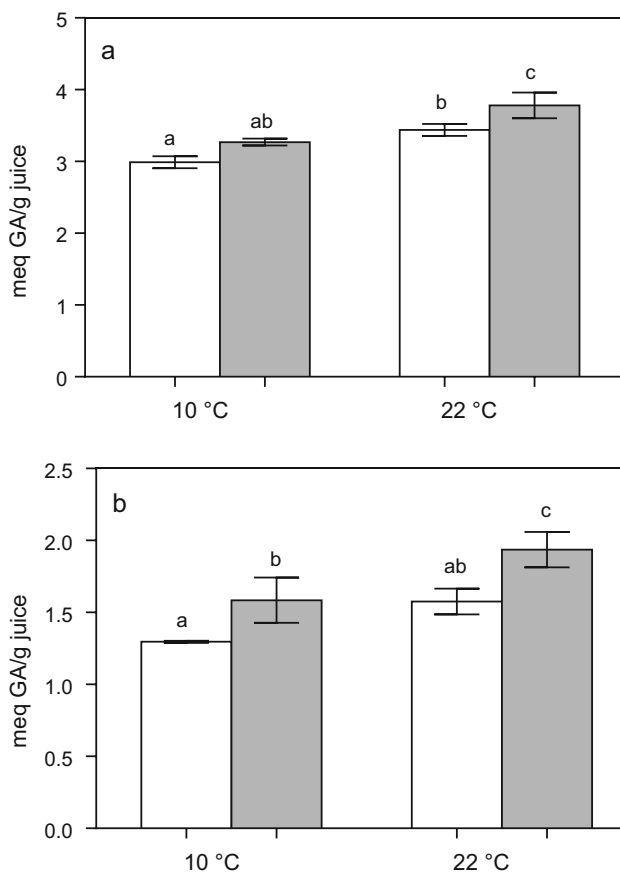


Fig. 3 TPC (a) and AA (b) of the extracts obtained by the optimum PEF treatment conditions (grey) and the corresponding controls (white) at 10 and 22 $^{\circ}$ C

Joule effect (Frey et al. 2017). Experiments with sugar beet showed that at treatment temperatures below 7 $^{\circ}$ C, a significant decrease of the electroporation efficiency occurred (at $E = 6$ kV/cm) (Frey et al. 2017). The authors state that a raise in the treatment temperature leads to an increase in the conductivity

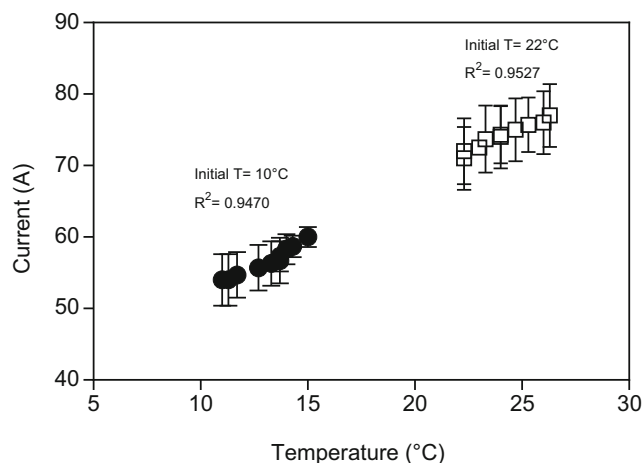


Fig. 4 Electric current vs. measured temperature for the experiments under controlled temperature conditions. Black circles = initial temperature = 10 $^{\circ}$ C; open squares = initial temperature = 22 $^{\circ}$ C

of the sample, causing a higher electric current during pulse application. Therefore, it is expected that a higher extraction would be observed when temperature is raised. These findings emphasize the relevance of monitoring the temperature when applying PEF treatments as it has a relevant effect on the final outcome. According to the type of product, the selection of a low treating temperature should be considered to avoid microbial growth, (since in some cases, like our blackcurrant extract, no subsequent pasteurization step will be applied), and minimize quality deterioration due to loss of nutrients, changes in color, and development of off-odors or off-tastes (Ling et al. 2015).

Conclusions

This work studied the effect of PEF treatment on the extraction of bioactive compounds of blackcurrant. The used experimental design and the response surface methodology were useful for the selection of the best extraction conditions, finding an optimum electroporation treatment with the following process conditions: 1318 V/cm and 315 pulses, which resulted in a total energy of 30 ± 2 kJ/kg received by the sample.

On the other hand, the studies conducted at different temperatures showed that temperature has a significant influence over the effect of PEF treatment. These findings make evident the importance of controlling the temperature conditions when designing a PEF protocol.

This work shows that the application of PEF technology followed by juice extraction by cold pressing can be a very powerful tool to improve the extraction of bioactive compounds and obtain high-quality juices from blackcurrant, having a 45% increase in antioxidant capacity when compared to the control sample.

Therefore, these results are encouraging as they demonstrate that the PEF treatment is suitable for incrementing the extraction of the bioactive components, thus enabling obtaining juices that could be consumed as fresh products, incorporated as ingredients for functional foods, and as food colorants due to their high anthocyanins concentration.

Funding The authors acknowledge the financial support of UBACYT 20020150100188BA, UNCOMA 04/L007, UBACYT 20620130100027, PIP 11220170100863CO, and PICT 2014/2250.

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