# Optimization of Pulsed Electric Fields-Assisted Extraction of Polyphenols from Potato Peels Using Response Surface Methodology

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**Abstract**

In this work, optimal Pulsed Electric Fields-assisted extraction conditions were selected in order to intensify the extractability of polyphenol compounds with high antioxidant activity from potato peels. Effectiveness of PEF as cell disintegration technique was confirmed using both impedance measurements and Scanning Electron Microscopy (SEM). Solid-liquid extraction (SLE) for both untreated and PEF pre-treated potato peels was optimized to determine the most effective solvent concentration (0-100% ethanol in water) as well as extraction temperature (20-50°C) and time (30-240 min) using response surface methodology. Total phenolic compounds (TPC) and antioxidant activity (DPPH) of the extracts were determined. Results showed that the application of PEF prior to SLE has the potential to reduce duration, temperature and consumption of solvent to achieve the same recovery yield of phenolic compounds. Under optimized conditions (54% ethanol, 233 min and 50°C for SLE; 52% ethanol, 230 min and 50°C for PEF), the extracts obtained from PEF pre-treated samples showed higher total phenolics yield (10%) and antioxidant activity (9%) as compared to the control extraction. Finally, HPLC-DAD analysis revealed the major classes of the detected polyphenolic compounds as chlorogenic, caffeic, syringic, protocatechuic and *p*-coumaric acids, and no significant degradation of individual polyphenols due to PEF application was observed.

**Keywords**: Pulsed Electric Fields (PEF), Solid Liquid Extraction (SLE), Potato peels by-product, Polyphenols, HPLC, Response Surface Methodology.

## 1. Introduction

Potato (*Solanum tuberosum*) is the fourth largest crop harvested worldwide after maize, wheat, and rice and represents one of the major staple foods of the human diet. The amount of potato produced worldwide in 2017 was 388 million of tons and Europe alone accounted for approximately 31% share with a total annual production of 122 million of tons (FAOSTAT 2017). Most of the potatoes produced are consumed in a processed form such as chips or French fries. In addition, potatoes are also canned or used in the preparation of ready to eat meals or as dehydrated potato flakes.

During their industrial processing, potatoes are usually peeled thus generating huge amount of waste, accounting on average for 100 thousand tons per year (Chang 2011), which are currently disposed in landfills thus incurring environmental and disposal costs to processors. However, the potential of potato peels as a cheap source of high-value compounds such as polyphenols has long been recognized (Akyol et al. 2016).

Specifically, polyphenols are mostly concentrated in potato peels, at levels 10 times greater than those detected in the flesh, thus accounting for approximately 50% of all polyphenols in a potato tuber (Friedman 1997). Phenolic compounds are an extremely heterogeneous class of secondary plant metabolites that protect plants against biotic stress caused by herbivores, insects, or pathogens as well as abiotic stress caused by excessive UV light or free radicals (Schieber and Saldaña 2009). Moreover, polyphenols have properties which are potentially beneficial for humans as they can act as antioxidants with potential benefits including the prevention of LDL-lipoprotein oxidation, platelet aggregation or red blood cell damage. In addition, they have the potential to act as antimutagens, anticarcinogens, metal chelators, antimicrobial or clarifying agents. In food, polyphenols influence color, sensory profile, astringency and bitterness and their antioxidant activity retards oxidative degradation of lipids, resulting in an overall improvement of the quality and nutritional value of the food (El Gharras 2009).

Conventional solid-liquid extraction (SLE) process is widely used to recover intracellular compounds from plant-based matrices. The successful application of this extraction technique, which typically

involves the intimate contact between the food matrix and organic solvent or solvent mixture with high affinity for the target compounds, is a function of the mass transfer processes based on diffusion and permeation of solvent and solute (Huang et al. 2013). The use of conventional SLE process for the extraction of polyphenols from potato peels has been widely investigated (Amado et al. 2014; Anastácio and Carvalho 2013). Moreover, due to their high antioxidant activity, potato peels extracts have been successfully added to vegetable oil as a natural alternative to synthetic antioxidants like BHT and BHA (Samarin et al. 2012), or used to retard lipid peroxidation in irradiated lamb meat (Kanatt et al. 2005).

However, in order to enhance the extraction yield, these methods often require energy intensive pre-treatments of comminution and/or drying of the raw material, as well as relatively high extraction temperatures and large volumes of organic solvents, which are very often potentially toxic and harmful to the environment. In addition, they may induce either the loss of valuable compounds or the co-extraction of undesirable components, thus increasing the downstream processing costs (Luengo et al. 2014; Pataro et al. 2018).

Due to the various drawbacks associated to conventional extraction methods, several novel extraction methodologies have been emerging in the recent years (Galanakis 2012). Some of these technologies have been proven to be successful in enhancing the extraction of phenolic compounds from potato peels such as microwave assisted extraction (Singh and Saldaña 2011), pressurized liquid extraction (Wijngaard et al. 2012) and ultrasound assisted extraction (Hossain et al. 2014; Paleologou et al. 2016).

In this line, pulsed electric fields (PEF) technology has been proposed as an intensification pre-treatment in the extraction of valuable intracellular compounds from food residues. The technique involves the exposure of plant tissue to an electric field of moderate intensity (0.5-10 kV/cm) and relatively low energy (1-20 kJ/kg), applied in the form of repetitive very short voltage pulses (typically from few μs up to 1 ms), which induces the selective permeabilization of the cell membranes (tonoplast and plasma membrane), thus facilitating the penetration of solvent into the cell

and the subsequent diffusion of solubilized valuable compounds from the inner parts of the cells (Pataro et al. 2018; Roselló-Soto et al. 2016).

For example, PEF pre-treatments were successfully applied to increase the extraction rate of natural pigments and bioactive compounds (polyphenols) from various food processing by-products such as blueberry and grape pomace, cocoa bean shell, papaya peels, as well as to shorten the extraction time and reduce solvent consumption and/or lower extraction temperatures (Barba et al. 2015; Barbosa-Pereira et al. 2018; Bobinaitė et al. 2015; Boussetta et al. 2012; Parniakov et al. 2014; Pataro et al. 2017; Puértolas et al. 2013).

However, to our knowledge, no studies have yet been published on the PEF effect and optimization of the PEF-assisted extraction procedure of phenolic compounds from potato peels.

Thus, the main aim of this study was to investigate the potential of PEF pre-treatment in combination with SLE to intensify the extractability of polyphenolic compounds from potato peels. Specifically, the effect of different combinations of field strength (E) and total specific energy input ($W_T$) on the cell disintegration index of potato peel tissues was evaluated with the aim to define optimal PEF pre-treatment conditions to be applied before the subsequent SLE phase. In addition, response surface methodology was used to optimize the extraction process of polyphenolic compounds from potato peels with regards to ethanol concentration, extraction temperature and extraction time during both conventional SLE and PEF-assisted extraction steps. Then, the effect of optimized SLE and PEF-assisted extraction process on total content and composition of polyphenols in the extracts was evaluated using HPLC-DAD analyses and the antioxidant activity was assessed by DPPH method.

## 2. Materials and methods

### *2.1. Chemicals and raw material*

Fresh potatoes of the "*Desiree*" variety were purchased from a local market and stored in dark at 4°C for up to 5 days before being processed. Potatoes of uniform shape and colour were selected and

manually peeled with a sharp knife. The peels were then cut using a hollow metal cylinder to obtain discs 1cm in diameter, which were subsequently subjected to impedance analyses as well as to SLE and PEF-assisted extraction tests. All reagents used in this work were purchased from Sigma-Aldrich (Steinheim, Germany), unless otherwise stated.

*2.2. PEF system*

PEF pre-treatment of potato peels before either impedance analysis or solvent extraction were carried out using a laboratory scale batch system previously described in detail by Bobinaitė et al. (2015). Briefly, the system consisted of a high voltage pulsed power (25 kV-500 A) generator (Modulator PG, ScandiNova, Uppsala, Sweden) capable of generating near rectangular shape monopolar pulses with different pulse widths ($\tau$=3-25 μs) and pulse repetition frequencies (f=1-450 Hz). The generator was connected by a high voltage cable to a batch cylindrical treatment chamber, which consisted of two parallel plate cylindrical electrodes separated by a polycarbonate tube. The area of the electrodes was 7.1 $cm^2$ while the distance between the electrodes could vary up to 5 cm, depending on the mass of sample to be treated (Bobinaitė et al. 2015). Voltage and current signals in the treatment chamber were measured independently using a high voltage probe (Tektronix, P6015A, Wilsonwille, OR, USA) and a Rogowsky coil (2 - 0.1 Stangenes, Inc., USA) connected to a 300 MHz digital oscilloscope (Tektronix, TDS 3034B, Wilsonwille, OR, USA). The maximum electric field intensity (E, in kV/cm) and total specific energy input ($W_T$, in kJ/kg) were calculated as detailed by Bobinaitė et al. (2015).

*2.3. Determination of tissue permeabilization via impedance analyses*

Cell disintegration index ($Z_p$) was used to quantify the degree of cell membrane permeabilization of potato peel tissues induced by PEF treatment before extraction. The determination of $Z_p$ via impedance analyses was carried out according to the method described by Bobinaitė et al. (2015)

with some modifications. For the impedance measurements, approximately 5 g of untreated and PEF treated discs of potato peels were loaded in the treatment chamber along with distilled water, necessary to ensure electrical continuity between the electrodes. The ratio of peels to distilled water was 1:1 (g/mL). The electrodes were connected to an impedance analyzer (Solartron 1260, UK), which was working in the frequency range of $10^2$ - $10^6$ Hz. For each treatment condition investigated (E = 0.25 – 3 kV/cm; $W_T$ = 1 – 20 kJ/kg, treatment time ($t_{PEF}$)=0.07÷400 ms, τ=20 μs, f=10 Hz), the $Z_p$ value, ranged between 0 (for intact tissue) and 1 (for fully permeabilized tissue), was calculated on the basis of the measurement of the absolute value of the complex impedance of untreated ($Z_{untr}$) and treated tissue ($Z_{tr}$) in the low (0.1 kHz) and high (1 MHz) frequency ranges (Donsì et al. 2010; Pataro et al. 2011).

$$Z_p = \frac{\left|Z_{untr\ (0{,}1\ kHz)}\right| - \left|Z_{tr\ (0{,}1\ kHz)}\right|}{\left|Z_{untr\ (0{,}1\ kHz)}\right| - \left|Z_{tr\ (1\ MHz)}\right|} \quad (1)$$

All the measurements were carried out in triplicate.

*2.4. PEF-assisted extraction experiments*

For PEF-assisted extraction experiments, approximately 5 g of potato peels were loaded into the treatment chamber and PEF pre-treated under the optimal conditions identified through the $Z_p$ determinations. In all experiments, the initial temperature of the samples was 20±1 °C and the final temperature never exceeded 23±1°C.

After the electro-permeabilization treatment, potato peels were immediately placed into a 200 mL Pyrex flask, where a water-ethanol mixture was added at a constant solid to liquid ratio (1:20 g/mL). The flasks were then introduced in an orbital incubator S150 (PBI international, Milan, Italy) where the extraction process was carried out under constant shaking at 160 rpm for different times (0-240 min), temperatures (20-50°C) and ethanol concentration (0 - 100%), as derived from the experimental design described in the following. An identical experimental design was used for untreated (control)

potato peels, which were subjected to conventional SLE process using the same extraction protocol but without the application of the PEF pre-treatment. At the end of the diffusion step, the extracts from untreated and PEF treated potato peels were centrifuged at 5700 × g (PK121R model, ALC International, Cologno Monzese, IT) for 10 min at 4 °C to separate the supernatant, which was then filtered through 0.45 μm syringe filters. The final extracts were then stored at -20 °C until further analysis.

*2.5 Experimental design and statistical analysis*

Response surface methodology was used to identify the relationship between response variables and process variables, as well as to determine the optimal conditions that maximise the extraction yield of phenolic compounds and antioxidant activity of potato peels extracts from untreated (control) and PEF treated samples. PEF pre-treatments were carried out at optimal conditions, in terms of field strength and energy input, which were identified from the impedance analyses as the lowest treatment intensity required to achieve the highest permeabilization degree of cell membrane of potato peel tissues. A three-factors face centred central composite design (FC-CCD) was used to investigate the effects of ethanol percentage ($X_1$, 0 - 100%, v/v) in a water-ethanol solvent mixture, extraction time ($X_2$, 30 - 240 min) and extraction temperature ($X_3$, 20 - 50 °C). Total phenolic content ($Y_1$) and antioxidant activity ($Y_2$) of untreated and PEF treated samples were used as response variables. Each experimental design consisted of 33 sets of treatment conditions including five replicates of central points and two replicates of factorial and star points (Table 1). The effects of unexplained variability in the observed response due to extraneous factors were minimized by randomizing the order of experiments.

A second order polynomial model reported in the Equation (2) was applied to predict the response variables as a function of the investigated independent factors:

$$Y_k = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (2)$$

where *Y* is the predicted response variable (total phenolic content and antioxidant activity), $X_i$ and $X_j$ are independent variables, $\beta_0$, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ are the intercept, regression coefficients of the linear quadratic and interaction terms of the model, respectively.

*2.6 Scanning electron microscopy (SEM)*

The morphological characteristics of untreated and PEF treated potato peels were observed prior extraction by using Scanning Electron Microscopy (SEM) analysis, with the samples prepared following the method described by Carullo et al. (2018) with some modifications. Potato peel discs were initially immersed in a 2 % (v/v) glutaraldehyde phosphate buffer solution for 30 min in order to fix cellular structures. Afterwards, the buffer was removed and the discs were dehydrated by means of ethanol solutions of increasing concentration (25%, 50%, 75%, and 100%, v/v). Finally, the so obtained samples were critically point dried with supercritical $CO_2$ in a Quorum K850 critical point dryer (Quorum Technologies Ltd, London, UK) and then coated with a golden layer in an Agar Auto Sputter Coater 103A (Agar Scientific Ltd, Stansted, UK), before being analyzed in a high-resolution ZEISS HD15 Scanning Electron Microscope (Zeiss, Oberkochen, Germany).

*2.7 Determination of total phenolic content (TPC) and antioxidant activity of the extracts*

The total phenolic content (TPC) of potato peel extracts was determined using the Folin-Ciocalteau method as described by Bobinaitė et al. (2015), with some modifications. Briefly, 1 mL of extract, which was diluted up to four times (0.25 mL extract + 0.75 mL water) when required, was mixed with 5 mL of 10% (v/v) Folin-Ciocalteau reagent and allowed to stand for 5 min at room temperature. Afterwards, 4 mL of sodium carbonate (7.5%, w/v) was added to the mixture. After shaking, the mixture was incubated for 60 min at room temperature in a dark place. The absorbance of the reacting mixture was then measured at 765 nm using a V-650 Spectrophotometer (Jasco Inc. Easton, MD, USA). Gallic acid dissolved in ethanol/water mixtures (0 – 50 – 100 % v/v) was used to generate

five-point external standard calibration curves (shown in Figs. S1-S3 of Supplementary Material) in a concentration range comprised between 1 and 100 mg/L, which was defined by preliminary experiments (data not shown). The results of TPC were expressed as milligrams of gallic acid equivalents (GAE) per kg of fresh weight potato peel (FW PP).

The antioxidant power of extracts from untreated and PEF treated potato peels was evaluated by DPPH spectrophotometric assay using the procedure reported by Rapisarda et al. (1999). Briefly, 3.9 mL of a 25 ppm DPPH solution in methanol was mixed with 0.1 mL of undiluted potato peels extract and immediately incubated in the dark for 5 min. The absorbance of the reacting mixture was measured at fixed wavelength (515 nm). Ascorbic acid was used as the standard for the calibration curve and the results were expressed as milligrams of ascorbic acid equivalent (AAE) per kg of FW PP.

*2.8 HPLC-DAD analyses of extracts*

Prior to HPLC analysis, potato peels extracts from untreated and PEF pre-treated samples were evaporated to dryness using a Genevac MiVac sample concentrator (IPSWICH, UK) set at 60°C overnight; residues were then dissolved in 0.5 mL of 50% ethanol. HPLC analyses were performed using an Agilent 1200 Series (Palo Alto, USA) HPLC system equipped with a diode assay detector (DAD), via the methodology described by Singh and Saldaña (2011) with some modifications. In particular, analytical separation of polyphenols was carried out in an ACE C18-PFP (150 mm x 4.6 mm ID, 5µm particle size) column (Phoenomenex, Macclesfield, UK). The mobile phase consisted of (A) formic acid (0.5%; v/v) in water and (B) formic acid (0.5%, v/v) in methanol. For compound separation, the following gradient was used; over the first 15 min the proportion of B was changed from 16 to 19 %, over a further 10 min to 27% and another 1 min to 41 % before being increased to 65% over 10 min and 100% B over 8 min; the column was re-equilibrated at 16% B for 5 min, resulting in a total run time of 50 min. The volume injected was 50 µL and the flow rate was 1

mL/min. The polyphenols quantified were chlorogenic acid (CGA), caffeic acid (CFA), syringic acid (SGA), protocatechuic acid (PCA), and *p*-coumaric acid (CMA). The signal for each polyphenol compound quantified was recorded at its wavelength of maximum absorbance. In particular, 325 nm was the wavelength selected to quantify caffeic and chlorogenic acid, 309 nm for *p*-coumaric acid, 278 nm for syringic and 260 nm for protocatechuic acid. Polyphenol compounds in the extracts were identified according to HPLC retention times (RT) as well as UV spectra in comparison with commercial standards. All commercial standard phenolic compounds were dissolved into 50% ethanol solution to generate seven-point external standard calibration curves (concentration range was from 1 to 100 mg/L), displaying excellent linearity ($R^2 > 0.99$).

*2.9 Statistical analysis*

Experiments and analysis of collected samples were performed in triplicates unless otherwise specified, and the mean values and standard deviations (SD) of experimental data were calculated. Statistically significant difference ($p \leq 0.05$) between the means were evaluated using two-tailed t-test or one-way analysis of variance (ANOVA) and the Tukey's test. Statistical analyses were carried out using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA).

The adequacy of the model was predicted through the regression analysis ($R^2$) and ANOVA analysis ($p \leq 0.05$). The FC-CCD and the corresponding analysis of the data including the generated 3-D response surfaces and the determination of the optimal set of process parameters that maximise the recovery of phenolic compounds and antioxidant activity, was carried out using the software package Design Expert Version 9.0.3 software (Minneapolis, MN). Five replicates of the optimal conditions were performed to validate the models.

## 3. Results and discussion

*3.1. PEF-induced cell damages and optimization of PEF treatment conditions*

The assessment of the cell disintegration index ($Z_p$) via electrical impedance measurements has been successfully used as a reliable indicator of the degree of cell membrane permeabilization induced upon the application of PEF treatment in diverse fruit and vegetable tissues such as blueberry (Bobinaitė et al. 2015), grape pomace (Barba et al. 2015), orange peels (Luengo et al. 2013), papaya peels (Parniakov et al. 2014) and mango peels (Parniakov et al. 2016). In the present work, $Z_p$ was used to select the optimal PEF treatment conditions, with regard to minimal field strength and energy input (or treatment time) required to induce the highest cell membrane permeabilization degree, thus intensifying the recovery yield of target intracellular compounds during the subsequent solvent extraction step.

Figure 1 depicts the influence of the total specific energy input ($W_T$) and field strength (E) on the $Z_P$ of potato peel tissues. Results showed that, regardless of the electric field applied, the extent of cell membrane permeabilization significantly increased with increasing the energy input up to a saturation point at about 5 kJ/kg, above which no further cellular damages could be detected. For the lowest investigated electric field values (E = 0.25 – 0.5 kV/cm) the $Z_p$ reached a maximum value of 0.89, while for higher treatment intensities (E ≥ 1 kV/cm) an almost complete disintegration of cell membranes was observed, with a maximum $Z_P$ value of 0.96. However, results of ANOVA showed that, at any energy input, significant differences ($p \leq 0.05$) were detected only when the field strength was increased from 0.5 to 1 kV/cm. According to these results, 1 kV/cm and 5 kJ/kg ($t_{PEF}$ =6 ms) were assumed as the optimal PEF treatment conditions to be used as a pre-treatment of potato peels before the subsequent extraction process.

The general trend of the influence of field strength and energy input on $Z_P$ values observed in our research is in agreement with previously reported data for other plant tissues such as onions (Asavasanti et al. 2010), blueberries (Bobinaitė et al. 2015), orange peels (Luengo et al. 2013), potatoes as well as apples and carrots (Lebovka et al. 2002). Moreover, these results are in agreement with those obtained in the work of Lebovka et al. (2002), in which it was shown that the highest value

of the cell disintegration index obtained in potatoes was attained when applying electric fields equal to or greater than 0.6 kV/cm.

*3.2. Effect of PEF pre-treatment on kinetic extraction of phenolic compounds*

Extraction kinetics of phenolic compounds from untreated and PEF treated potato peels under the optimal electrical conditions (1 kV/cm, 5 kJ/kg) were performed by using 50% water-ethanol mixture as extracting solvent at a temperature of 35°C for up to 24 h.

The aim of this preliminary analysis was to assess the potential of PEF in increasing the recovery of phenolic compounds from potato peel tissues when compared with SLE, and to define a suitable interval for the extraction time to be further investigated in the FC-CCD. As shown in Figure 2, regardless of the application of PEF pre-treatment, the TPC initially increased sharply and then tended to level off to a relatively constant value as the extraction time increased. The majority of the phenolic compounds were recovered approximately during the first 240 min of extraction, while longer diffusion times did not produce any substantial increment of the amount of total phenolics.

However, it is worth noting that the pre-treatment of potato peel tissues with PEF before solvent extraction significantly ($p \leq 0.05$) increased the extraction rate of phenolics as compared with untreated samples. For instance, the application of the PEF pre-treatment markedly reduced the extraction time for obtaining the same amount of total phenolics (1062 mg GAE/kg FW) in the untreated and PEF-treated samples from 240 to 144 min, respectively.

The positive impact of PEF pre-treatment on extraction of polyphenols from food by-products was also previously observed for orange peels (Luengo et al. 2013), grape seeds (Boussetta et al. 2012), grape pomace (Barba et al 2015), cocoa bean shell (Barbosa-Pereira et al. 2018), papaya peels (Parniakov et al. 2014), mango peels (Parniakov et al. 2016) and blueberry pomace (Bobinaitė et al. 2015; Pataro et al. 2017).

The increase in phenolic extraction rate can be explained by the structural cell damages caused by PEF (Fig. 1), as also illustrated by the SEM images of untreated and PEF treated potato peel tissue

presented in Figure 3. In particular, it can be noted that PEF treatment induced damages on the surface of plant cells leading to the formation of cavities and increasing surface roughness which suggest disorganization of the cell envelop (cell wall/membrane) (Pillet et al. 2016). This likely facilitated the penetration of the solvent into the cytoplasm and the subsequent mass transfer of the solubilized intracellular compounds, thus intensifying the extractability of phenolic compounds.

According to the results of Figure 2, in additional studies aimed at investigating the influence of the extraction time on the recovery yield of phenolic compounds based on response surface methodology (FC-CCD), the extraction time was set in the range between 30 and 240 min.

*3.3. Model Fitting*

The FC-CCD was constructed to investigate the effect of three factors, namely extraction temperature, extraction time and ethanol percentage on TPC and antioxidant activity (DPPH) of potato peels extracts, achieved from either PEF-assisted extraction or conventional SLE process (Table 1).

Results show that the application of PEF pre-treatment to potato peels markedly enhanced the TPC (10%) and antioxidant activity (9%) of extracts, as compared with the control extraction, with significant differences observed especially when pure water was used as a solvent.

The data obtained from the FC-CCD were fitted to a second-order polynomial equation (Eq. 2). The values and significance of the regression coefficients of the predicted polynomial models and corresponding *p* values for each variables are shown in Table 2.

For the extracts of both untreated and PEF pre-treated samples all the investigated factors resulted in a significant linear and quadratic effect on TPC, with most of the interactions between single factors being significant. In contrast to the untreated samples, a dependency of the ethanol concentration on the extraction temperature was detected in the case of PEF pre-treated samples. However, the interaction between extraction time and temperature was not significant for the PEF pre-treated samples, but was significant in the case of the untreated samples. This suggests that, in the investigated variables domain, PEF pre-treatment reduced the influence of extraction temperature on

diffusion time while amplifying the interaction between extraction temperature and ethanol percentage. Regarding the antioxidant activity of the extracts obtained upon SLE process, results show that the ethanol percentage-time and time-temperature interactions were significant and the concentration of ethanol also had a significant quadratic effect. Similar effects were detected in the case of the PEF-treated samples except for the linear effect of ethanol concentration which was not significant.

The relationship between either TPC or antioxidant activity and extraction parameters had determination coefficient ($R^2$) values ranged between 0.92 and 0.99, which indicate a good correlation between the experimental data and those predicted by the model. Moreover, analysis of variance indicated that the selected model was significant ($p<0.0001$) for all responses and the lack of fit test was not significant ($p>0.05$), thus confirming the validity of the model to describe the experimental data.

*3.4. RSM analysis and optimization of extraction process*

Response surfaces reported in Figure 4 for both untreated and PEF pre-treated samples, show the complex interactions among ethanol concentration, extraction time and extraction temperature on the TPC level of the extracts. In the whole investigated domain, PEF pre-treatment increased the amount of extracted phenols as compared with the untreated samples. Even though all the investigated variables had a statistically significant effect on TPC ($p \leq 0.05$), ethanol concentration, with its parabolic trend, was the factor that mostly influenced the observed response. This can be attributed to the fact that ethanol concentration affects the polarity of the solvent mixture, thus allowing the recovery of polyphenols with a broad spectrum of polarities. The highest TPC value was achieved at 50°C, 54% ethanol, and 233 min for control samples (1180 mg GAE/kg FW PP) and at 50°C, 52% ethanol, and 230 min for PEF pre-treated samples (1295 mg GAE/kg FW PP, + 10% over SLE). Five replicates using the optimised conditions were performed to successfully validate the models (data not shown).

In agreement with previous findings (Amado et al. 2014; Wijngaard et al. 2012), results of Figure 4 clearly show that ethanol concentration is one of the main factors affecting the recovery of phenolic compounds from potato peels. The optimal concentration of ethanol determined in our study (52-54%) was slightly lower than that detected in previous works (59-60%) upon SLE of polyphenols from potato peels (Paleologou et al. 2016; Wu et al. 2012). The application of PEF prior to SLE can be successfully used not only to increase the amount of TPC in the extract, but also to reduce both duration and temperature of the extraction process as well as the ethanol concentration to achieve the desired extraction yield. For instance, as compared to SLE process, the application of a PEF permeabilization step prior to SLE reduced the extraction time, the diffusion temperature and the ethanol concentration by 134 min, 11°C and 26%, respectively, to achieve the same amount of TPC (1180 mg GAE/kg FW PP).

It is worth noting that, in comparison with the control extraction, the highest positive impact of PEF-pre-treatment on the extractability of phenolic compounds was detected when pure water was used as solvent, showing a dramatic increase of TPC from 397.48 mg GAE/kg FWPP up to 794.05 mg GAE/kg FWPP. This may be ascribed to the capability of ethanol to affect the barrier properties of the cell membrane of the plant tissues (Patra et al. 2006), thus reducing the permeabilization effect induced by PEF on the subsequent extraction phase.

Response surfaces of antioxidant capacity of extracts from untreated and PEF treated potato peels are shown in Figure 5. From these results it clearly appear that the extracts obtained from the PEF-treated potato peels possessed a higher antioxidant activity than that observed in the control extracts, even though significant differences ($p \leq 0.05$) were detected only when pure water was used as solvent. Moreover, in agreement with previous findings (Barbosa Pereira et al. 2018; Pataro et al. 2017), a strong positive correlation was found between the TPC and antioxidant activity with a Pearson correlation coefficient equal to 0.902, suggesting that phenolic compounds mostly contribute to the global antioxidant activity of the potato peel extracts. The values of the factors that maximise antioxidant activity were 62% ethanol, 240 min and 50°C for control extracts, and 57% ethanol 240

min and 50°C for PEF extracts, resulting in an antioxidant activity of 803.08 mg AAE/kg FWPP and 877.17/kg FWPP, respectively.

*3.5. Quantification of the main phenolic compounds of extracts via HPLC-DAD analysis*

HPLC-DAD measurements were performed to evaluate the effect of PEF pre-treatment on the content and composition of polyphenols of potato peels extracts from untreated and PEF treated samples obtained under the same extraction conditions (50% ethanol, 240 min).

After preliminary analysis showing a poor retention of gallic acid (capacity k' = 0.385) and peak broadening observed for late-eluting compounds (peak width = 1.02 min), the $C_{18}$ column was substituted with a PFP column due to its ability to form $\pi$-$\pi$ interaction with phenols. This changing increased k' value from 0.385 to 0.992 for gallic acid, while also significantly decreased the peak width of *p*-coumaric acid from 1.02 min to 0.81 min (a reduction of 20%). Although a k' value less than 2 is recommended for robust methods, the increment of k' by 150% represents a significant improvement in the retention of gallic acid. Thus, the use of PFP-column instead of $C_{18}$-column significantly improved chromatography and enhanced the ability to quantify lower levels of late-eluting polyphenols in potato peels extracts.

HPLC chromatograms profiles of the extracts from untreated and PEF treated potato peels after extraction at 50°C are compared in Figure 6. Similar HPLC profiles were obtained at temperatures of 20 and 35°C (data not shown). The quantification of individual phenolic compounds is, instead, reported in Table 3 for all the investigated extraction temperatures. Results show that, regardless of the application of a PEF pre-treatment, the two major phenolic compounds detected in the extracts were chlorogenic (peak 2) and caffeic (peak 3) acids, whereas protocatechuic (peak 1), syringic acid (peak 4), and *p*-coumaric acid (peak 5) were present in lower amount, which is in agreement with findings previously reported by other scientists (Onyeneho and Hettiarachchy 1993; Singh & Saldaña 2011). It is worth noting that the abundance of chlorogenic acid in the potato peels extracts might be

of particular importance, as it has been demonstrated to show several beneficial properties for human health due to its potential antioxidant, anticarcinogenic, anti-inflammatory, analgesic, antimicrobial, neuroprotective, and cardioprotective effects; moreover, it has also been reported to potentially increase insulin sensitivity, to decrease the gut glucose absorption and to prevent gluconeogenesis (Akyol et al. 2016).

Additionally, in agreement with the observations reported by other authors (Bobinaitė et al. 2015; López et al. 2009; Luengo et al. 2013; Pataro et al. 2017), the extracts obtained from untreated and PEF pre-treated potato peels presented similar phenolic profiles (Fig. 6), confirming that no degradation/modification of individual phenolic compounds occurred due to the application of PEF pre-treatments.

Results reported in Table 3 show that the increase in the extraction temperature significantly ($p<0.05$) increased the content of phenolic compounds in the extracts of both control and PEF treated samples, even though the increment in the recovery yield of phenolic compounds was more evident when temperature was increased from 20 to 35°C. It is likely that the increase in the solubility of the material being extracted and its diffusivity with temperature could explain the improvement in the extractability of the compounds of interest. Independently on the extraction temperature, PEF pre-treatment significantly ($p\leq0.05$) increased the extraction yield of phenolic acids, as compared to the control extraction. These results further reflect a positive effect of PEF application for the intensification of the extractability of phenolic compounds.

## 4. Conclusions

The results of this study have demonstrated that a PEF pre-treatment at 1 kV/cm and at 5 kJ/kg is sufficient to achieve high level of potato peel tissues permeabilization, thus intensifying the recovery yield of phenolic compounds during the conventional extraction process. The effects of diffusion temperature, process duration and ethanol concentration on TPC and antioxidant activity were determined using a FC-CCD and response surface methodology. The variables were significant and

the quadratic model accurately predicted the TPC and antioxidant activity for potato peels extracts. Optimum processing parameters of PEF-assisted extraction can either enhance the extractability of high-added value molecules (polyphenols) with strong antioxidant power, or to reduce the extraction time and the consumption of solvent as compared to conventional SLE. Interestingly, the effect of PEF was noticeably higher when water was used as a solvent, making the use of PEF particularly suitable to design a green extraction process, thus reducing downstream purification costs. The HPLC analyses revealed that chlorogenic acid and caffeic acid were the most abundant phenolic compounds in the potato peels extracts. Moreover, PEF pre-treatment kept the composition of the control extracts while showing higher content of polyphenolic compounds.

These promising results confirm the potential of PEF technology to promote the valorisation of the food processing wastes allowing to either intensify the extractability of high-added values compounds or reduce the consumption of energy, amount of solvent and processing time.

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**Figure captions**

**Figure 1** Influence of total specific energy input ($W_T$) and electric field strength (E) on the cell disintegration index ($Z_p$) of potato peels tissues.

**Figure. 2** Extraction kinetics of total phenolic compounds (mg GAE/kg FW) of 50% EtOH extracts of untreated (control) and PEF treated (E = 1 kV/cm; $W_T$ = 5 kJ/kg) potato peel samples. Extraction temperature was set at 35°C.

**Figure 3** Scanning electron microscopy (SEM) before (Control) and after PEF treatment (E = 1 kV/cm, $W_T$ =5 kJ/kg) of cells of potato peel tissues. Red arrows indicate cell surface damages.

**Figure 4** TPC response surfaces of extracts obtained from untreated (control) and PEF pre-treated (E = 1 kV/cm; $W_T$ = 5 kJ/kg) potato peels. The extraction temperature was set at 50°C in the response surfaces on the left, while the extraction time was set at 135 min in the response surfaces on the right.

**Figure 5.** TPC and DPPH response surfaces of extracts from untreated (control) and PEF pre-treated (E = 1 kV/cm; $W_T$ = 5 kJ/kg) potato peels. The extraction temperature was set at 50°C in the response surfaces on the left, while the extraction time was set at 240 min in the response surfaces on the right.

**Figure 6** HPLC chromatograms (λ = 325 nm) of 50% ethanol extracts obtained after 240 min extraction at 50°C from untreated (control) (a) and PEF pre-treated (b) potato peels samples. Peak identification: protocatechuic acid (1), chlorogenic acid (2), caffeic acid (3), syringic acid (4), *p*-coumaric acid (5).

**Figure 1**

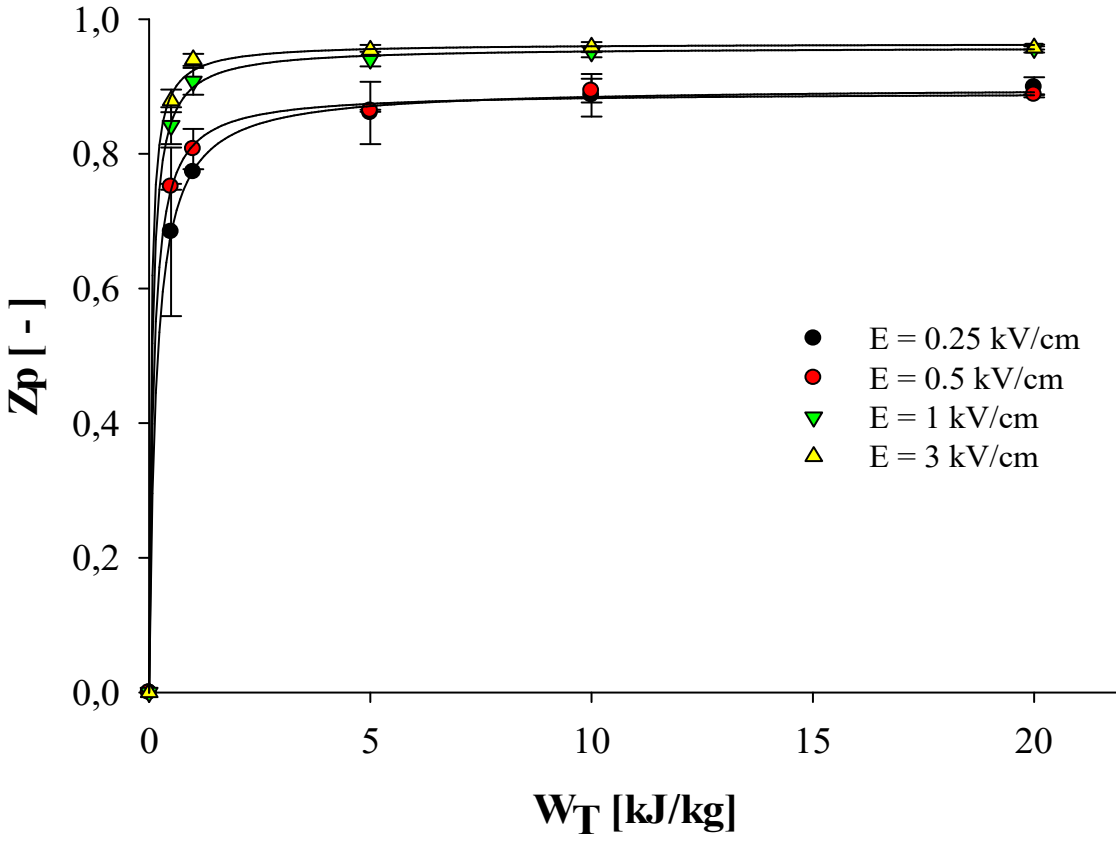

1,0
0,8
0,6
0,4
0,2
0,0
Zp [ - ]
0
5
10
15
20
W_T [kJ/kg]
E = 0.25 kV/cm
E = 0.5 kV/cm
E = 1 kV/cm
E = 3 kV/cm

**Figure 2**

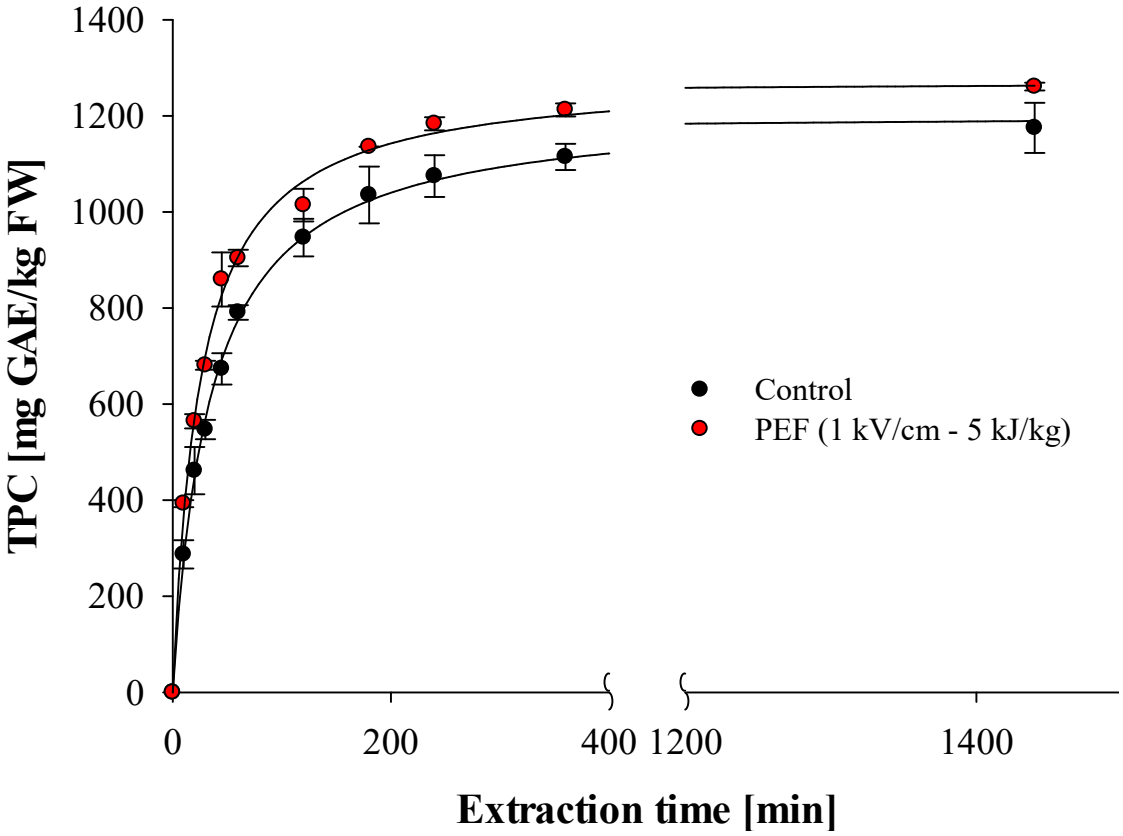

1400
1200
1000
800
600
400
200
0
TPC [mg GAE/kg FW]
0
200
400
1200
1400
Extraction time [min]
Control
PEF (1 kV/cm - 5 kJ/kg)

**Figure 3**

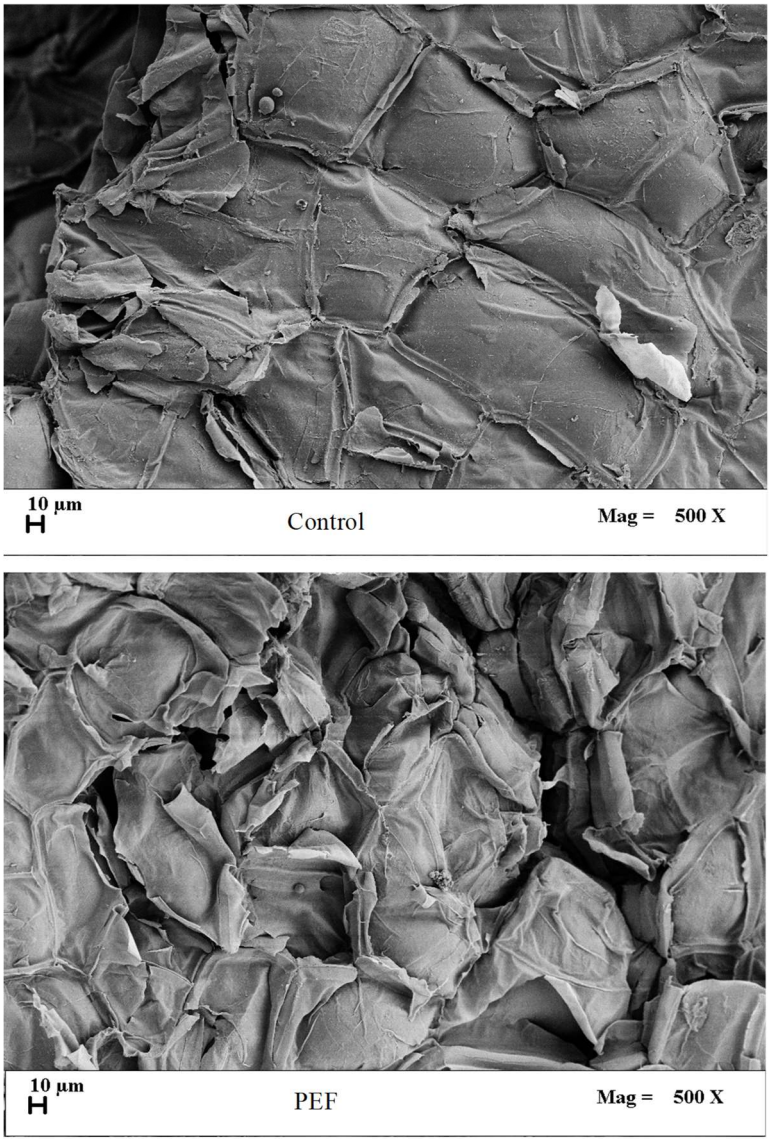


**Figure 4**

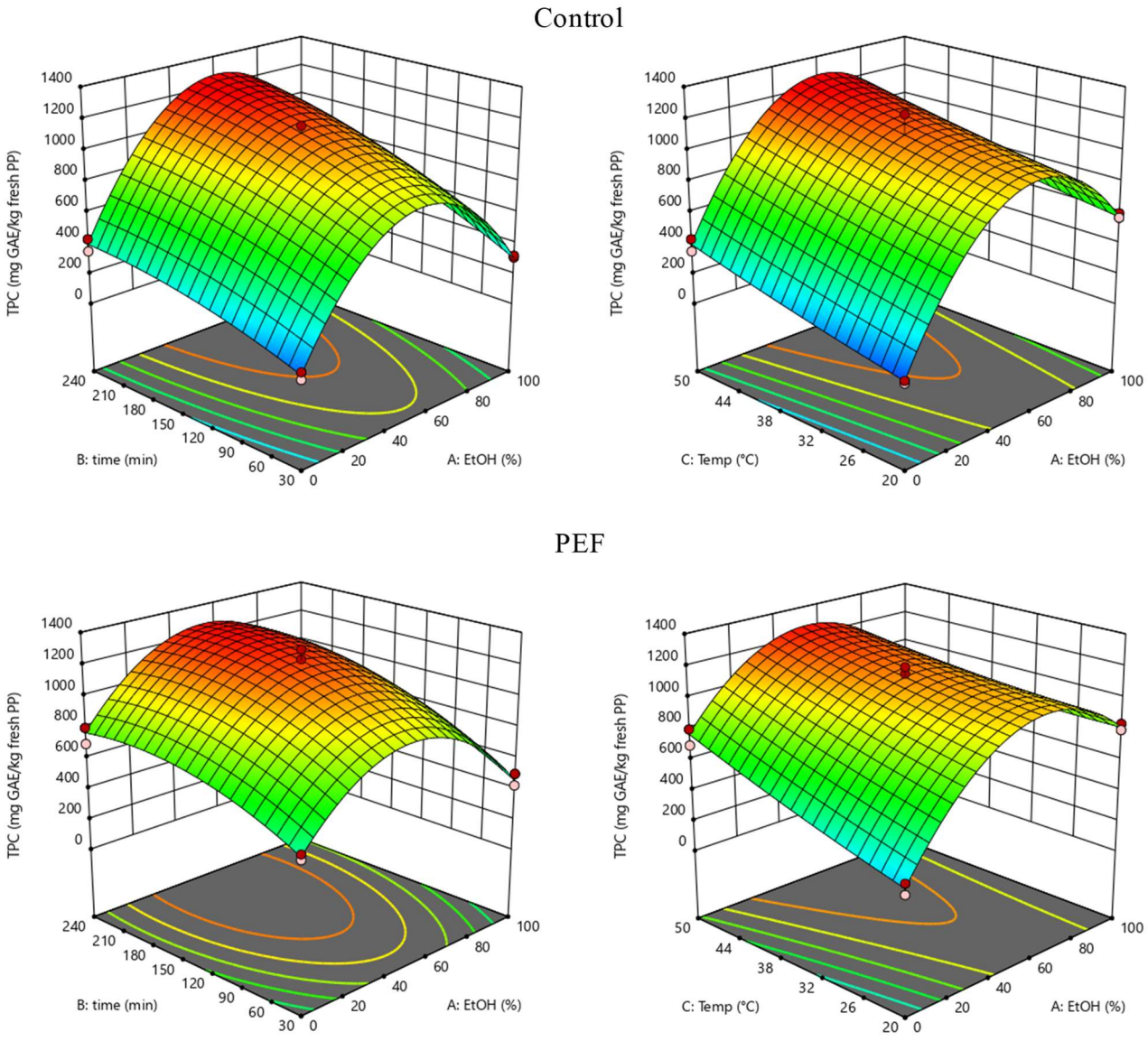
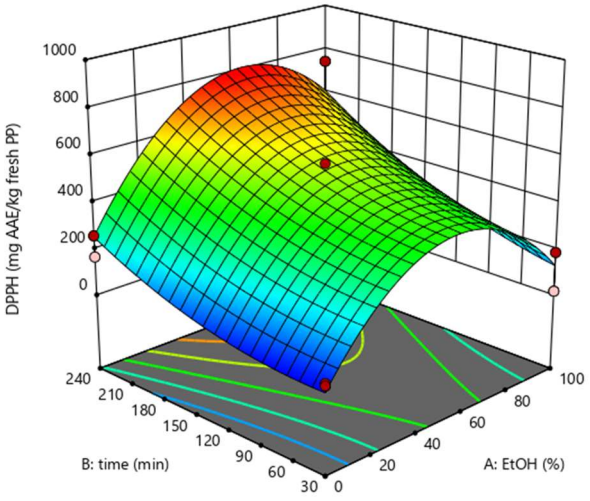
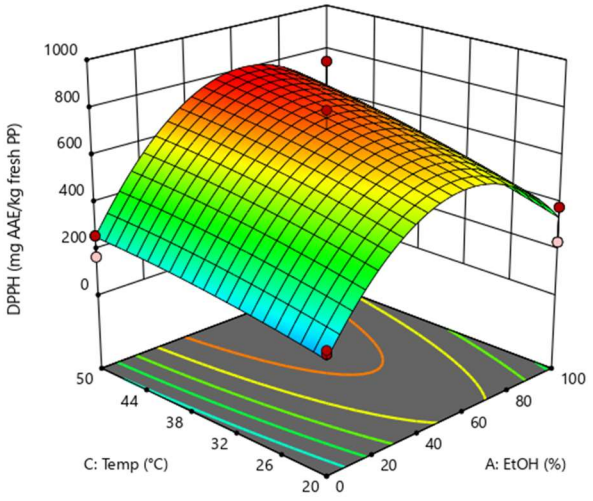

Control
TPC (mg GAE/kg fresh PP)
1400
1200
1000
800
600
400
200
0
B: time (min)
240
210
180
150
120
90
60
30
A: EtOH (%)
0
20
40
60
80
100
C: Temp (°C)
50
44
38
32
26
20
PEF

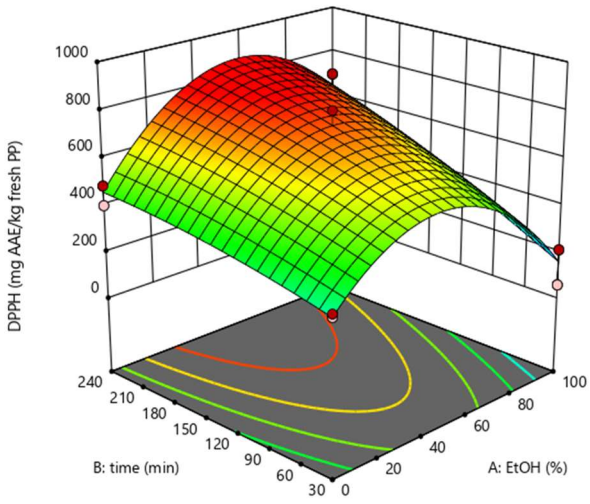
**Figure 5**

Control




PEF



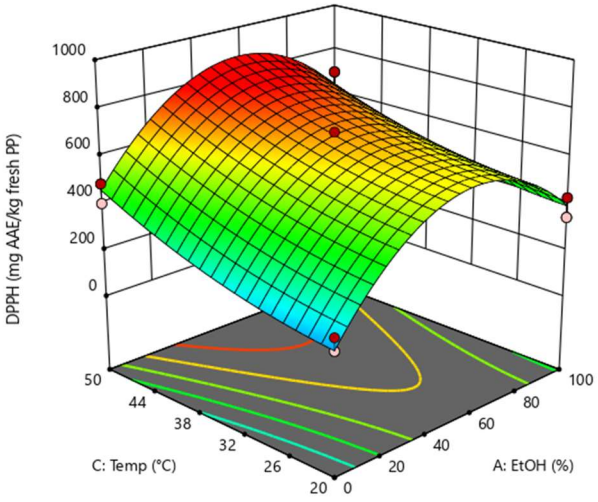



**Figure 6**

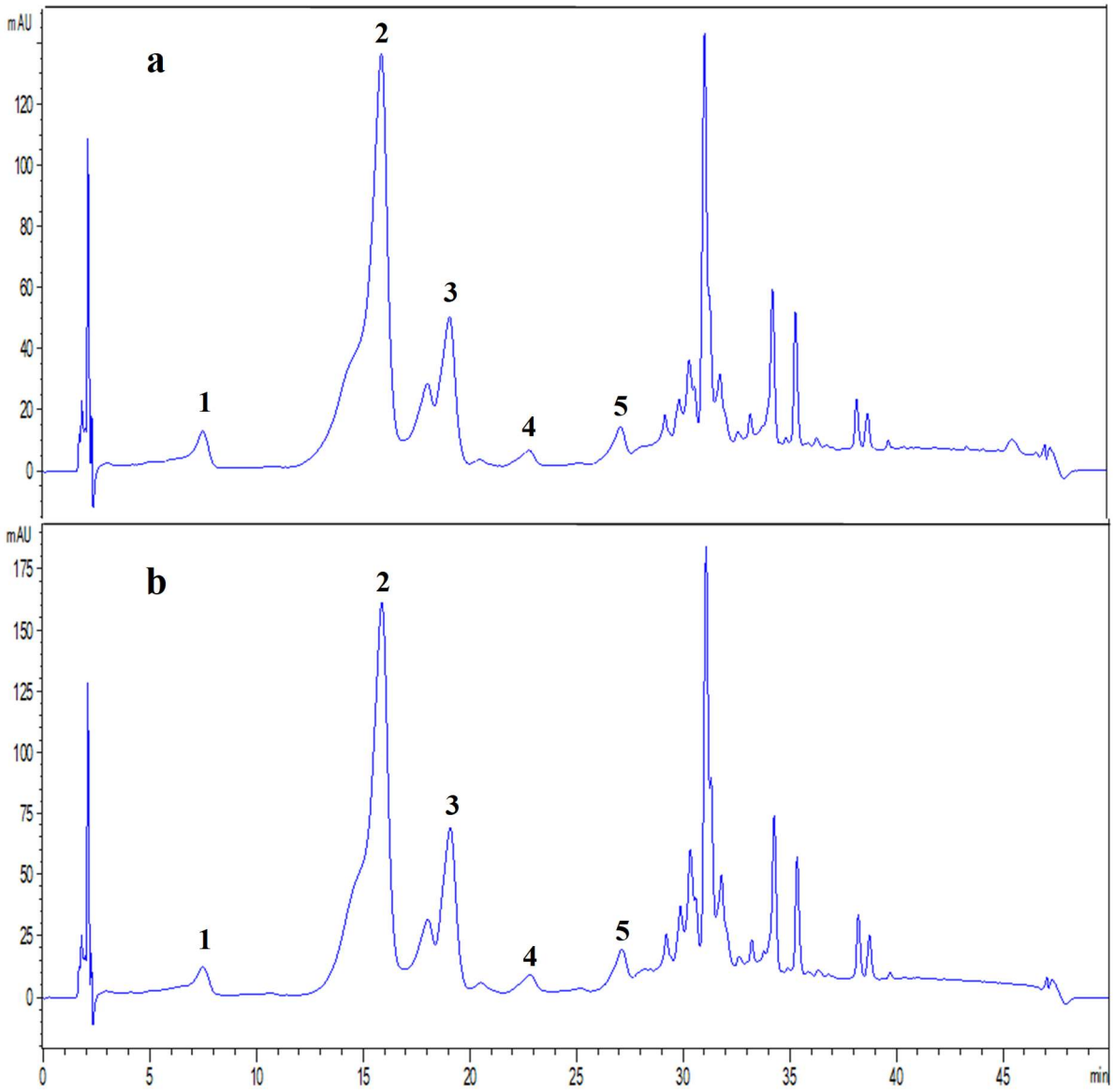


**Table 1** Actual and Coded values (in bracket) of the three variables investigated and TPC and DPPH values in potato peel extracts from either conventional SLE or PEF-assisted extraction process. TPC is expressed in mgGAE/kg FW PP and DPPH in mgAAE/kg FW PP.

| Trial no. | Variables | | | SLE | | PEF-assisted extraction | |
|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | TPC$^{(*)}$ | DPPH$^{(**)}$ | TPC$^{(*)}$ | DPPH$^{(**)}$ |
| 1 | 0 (-1) | 30 (-1) | 20 (-1) | $4.1 \pm 2^{b}$ | $31.1 \pm 13^{B}$ | $99.9 \pm 19^{a}$ | $226.7 \pm 50^{A}$ |
| 2 | 100 (+1) | 30 (-1) | 20 (-1) | $217.6 \pm 24^{b}$ | $89.0 \pm 69^{A}$ | $453.6 \pm 44^{a}$ | $114.2 \pm 64^{A}$ |
| 3 | 0 (-1) | 240 (+1) | 20 (-1) | $79.1 \pm 11^{b}$ | $166.2 \pm 11^{A}$ | $317.6 \pm 48^{a}$ | $200.7 \pm 38^{A}$ |
| 4 | 100 (+1) | 240 (+1) | 20 (-1) | $584.4 \pm 20^{b}$ | $308.5 \pm 107^{A}$ | $815.1 \pm 28^{a}$ | $384.5 \pm 60^{A}$ |
| 5 | 0 (-1) | 30 (-1) | 50 (+1) | $116.0 \pm 33^{b}$ | $38.4 \pm 8^{B}$ | $497.3 \pm 24^{a}$ | $320.8 \pm 9^{A}$ |
| 6 | 100 (+1) | 30 (-1) | 50 (+1) | $314.5 \pm 8^{a}$ | $101.5 \pm 121^{A}$ | $462.7 \pm 54^{a}$ | $133.9 \pm 109^{A}$ |
| 7 | 0 (-1) | 240 (+1) | 50 (+1) | $388.8 \pm 57^{b}$ | $213.6 \pm 66^{A}$ | $746.8 \pm 73^{a}$ | $443.5 \pm 59^{A}$ |
| 8 | 100 (+1) | 240 (+1) | 50 (+1) | $831.4 \pm 9^{a}$ | $651.5 \pm 131^{A}$ | $844.4 \pm 45.8^{a}$ | $649.55 \pm 65^{A}$ |
| 9 | 0 (-1) | 135 (0) | 35 (0) | $191.0 \pm 20^{b}$ | $90.9 \pm 30.2^{B}$ | $527.3 \pm 29^{a}$ | $277.2 \pm 41^{A}$ |
| 10 | 100 (+1) | 135 (0) | 35 (0) | $516.9 \pm 35^{b}$ | $206.9 \pm 96^{A}$ | $737.2 \pm 26^{a}$ | $311.7 \pm 61^{A}$ |
| 11 | 50 (0) | 30 (-1) | 35 (0) | $748.4 \pm 16^{a}$ | $450.0 \pm 60^{A}$ | $785.4 \pm 9^{a}$ | $509.22 \pm 82^{A}$ |
| 12 | 50 (0) | 240 (+1) | 35 (0) | $1024.55 \pm 15^{b}$ | $675.8 \pm 56^{A}$ | $1173.3 \pm 30^{a}$ | $674.9 \pm 41^{A}$ |
| 13 | 50 (0) | 135 (0) | 20 (-1) | $880.2 \pm 65^{b}$ | $442.3 \pm 29^{A}$ | $1054.1 \pm 54^{a}$ | $570.2 \pm 54^{A}$ |
| 14 | 50 (0) | 135 (0) | 50 (+1) | $1130.9 \pm 37^{a}$ | $545.5 \pm 65^{A}$ | $1263.5 \pm 43^{a}$ | $758.8 \pm 60^{A}$ |
| 15 | 50 (0) | 135 (0) | 35 (0) | $1003.4 \pm 40^{b}$ | $525.4 \pm 93^{A}$ | $1089.6 \pm 8^{a}$ | $607.6 \pm 72^{A}$ |

The results are expressed as mean ± standard deviation (n=2 for factorial and axial points, n=5 for central point).
PEF treatment conditions:1 kV/cm; 5 kJ/kg.
$X_1$: ethanol concentration (%), $X_2$: extraction time (min), $X_3$: extraction temperature (°C).
$^{(*)}$ Values with different superscript lowercase letter within the same row are significantly different ($p \leq 0.05$).
$^{(**)}$ Values with different superscript uppercase letter within the same row are significantly different ($p \leq 0.05$).

**Table 2.** Analysis of variance (ANOVA) of the second polynomial models for the TPC and antioxidant activity (DPPH) in potato peel extracts from either conventional SLE or PEF-assisted extraction process.

| Coefficients | SLE | | | | PEF-assisted extraction | | | |
|---|---|---|---|---|---|---|---|---|
| | TPC (mgGAE/kg FW PP) | | DPPH (mgAAE/kg FW PP) | | TPC (mgGAE/kg FW PP) | | DPPH (mgAAE/kg FW PP) | |
| $\beta$0 (Intercept) | 1.4574 | *** | 106.3567 | *** | -93.4552 | *** | 422.1469 | *** |
| $\beta$1 (EtOH %) | 26.82752 | *** | 12.7373 | *** | 25.2435 | *** | 11.5641 | ns |
| $\beta$2 (Time) | 1.92627 | *** | -1.6964 | *** | 4.3566 | *** | -0.3868 | *** |
| $\beta$3 (Temperature) | -6.733 | *** | 1.0986 | ** | -0.6379 | *** | -13.4944 | *** |
| $\beta$12 (EtOH % × Time) | 0.01276 | *** | 0.0109 | ** | 0.0065 | ** | 0.0157 | *** |
| $\beta$13 (EtOH % × Temperature) | -0.01298 | ns | 0.0501 | ns | -0.1313 | *** | -0.0137 | ns |
| $\beta$23 (Time x Temperature) | 0.02762 | *** | 0.0294 | * | 0.0041 | ns | 0.0336 | ** |
| $\beta$11 (EtOH % x EtOH %) | -0.24724 | *** | -0.1433 | *** | -0.1928 | *** | -0.1289 | *** |
| $\beta$22 (Time x Time) | -0.00759 | *** | 0.005 | ns | -0.0122 | *** | -0.0022 | ns |
| $\beta$33 (Temperature × Temperature) | 0.14896 | * | -0.0593 | ns | 0.1972 | * | 0.212 | ns |
| p-value of the Model | <0.0001 | *** | <0.0001 | *** | <0.0001 | *** | <0.0001 | *** |
| $R^2$ | 0.9940 | | 0.9195 | | 0.9907 | | 0.9407 | |
| p-value of lack of fit test | 0.2459 | ns | 0.5126 | ns | 0.3147 | ns | 0.8605 | ns |

ns = not significant for $p > 0.05$; * = significant for $p \leq 0.05$; ** = significant for $p \leq 0.01$; *** = significant for $p \leq 0.001$.

**Table 3.** Concentration of the main phenolic compounds identified in the 50% ethanol extracts from untreated (SLE) and PEF treated potato peels after 240 min extraction at different temperatures.

| Phenolic Acid | T = 20˚C | | T = 35˚C | | T = 50˚C | |
|---|---|---|---|---|---|---|
| | SLE | PEF | SLE | PEF | SLE | PEF |
| PCA | $3.1 \pm 0.2^{a,A}$ | $3.3 \pm 0.2^{a,A}$ | $3.9 \pm 0.2^{a,B}$ | $4.0 \pm 0.2^{a,B}$ | $4.0 \pm 0.2^{a,B}$ | $4.4 \pm 0.2^{a,B}$ |
| CGA | $284.0 \pm 1.0^{a,A}$ | $332.0 \pm 1.0^{b,A}$ | $313.0 \pm 1.0^{a,B}$ | $348.0 \pm 1.0^{b,B}$ | $331.0 \pm 1.0^{a,C}$ | $365.0 \pm 1.0^{b,C}$ |
| CFA | $13.9 \pm 0.9^{a,A}$ | $17.1 \pm 0.9^{b,A}$ | $42.4 \pm 1.0^{a,B}$ | $53.2 \pm 0.9^{b,B}$ | $67.8 \pm 1.1^{a,C}$ | $74.9 \pm 1.0^{b,C}$ |
| SGA | $8.4 \pm 0.3^{a,A}$ | $9.0 \pm 0.2^{b,A}$ | $9.1 \pm 0.2^{a,B}$ | $9.1 \pm 0.3^{a,A}$ | $9.3 \pm 0.3^{a,B}$ | $9.4 \pm 0.2^{a,A}$ |
| CMA | $6.8 \pm 0.2^{a,A}$ | $7.0 \pm 0.2^{a,A}$ | $7.5 \pm 0.3^{a,B}$ | $8.0 \pm 0.2^{a,B}$ | $7.8 \pm 0.2^{a,B}$ | $8.2 \pm 0.2^{a,B}$ |

PCA = protocatechuic acid, CGA = chlorogenic acid, CFA = caffeic acid, SGA = syringic acid, CMA = *p*-coumaric acid.
Results are expressed as mean ± standard deviation (SD) in mg/kg FW PP
PEF treatments were carried out at 1 kV/cm and 5 kJ/kg.
Different lowercase letters in the same line within the same temperature indicate significant differences between the samples ($p \leq 0.05$).
Different uppercase letters in the same line for the same extraction process (SLE or PEF) indicate significant differences among the samples ($p \leq 0.05$).