Effects of ohmic heating on extraction of food-grade phytochemicals from colored potato

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ABSTRACT

The influence of ohmic heating (OH) through the application of moderate electric fields on phytochemical compounds recovery from colored potato (*Solanum tuberosum* L. var. Vitelotte) was studied. A Box–Behnken design was used to simultaneously assess the effects of operational parameters such as electric field strength, temperature and process time on the yields of anthocyanins and total phenolic recovery on pretreatment of potato samples. From the analysis of the model, electric field, temperature and time were shown to have independent and interactive effects on the values of extraction yields. Aqueous extraction of phytochemical compounds after pretreatments can be described by using a two-step model involving simultaneous washing and diffusion of the solutes from the samples. Results shows that electrical fields of low energy levels and thermal effects can be combined and optimized into a single step treatment on extraction of anthocyanins and phenolic compounds from vegetable tissues providing high recovery yields with a reduced treatment time, less energy consumption and with no utilization of organic solvents (green extraction).

1. Introduction

The growing concerns over environmental issues are driving a strong and sustainable demand for "green" processing technology solutions. Maximizing process efficiency, reducing waste or using byproducts and nonconventional sources of raw materials are interesting alternatives and are drawing attention form researchers both in academia and industry (Lin et al., 2013). Food-stuff is an interesting source of biomolecules as proteins, polysaccharides, lipids, as of lower amounts of high added-value substances such as polyphenols, pigments, vitamins, among others (Galanakis, 2012). During food processing significant amounts of this compounds are lost ether by the degradation during processing or discarded along the waste. Most of these compounds, when recovered, may be reintroduced in the food industry or valorized e.g. for energy production, use in pharmaceutical products, among others (Galanakis, 2012; Lin et al., 2013). Together with the increasing interest in this compounds, new recovery methods are being developed. The focus is to maintain the energy input low, reduce the use of chemicals and organic solvents and increase recovery efficiencies (Chemat, Vian, & Cravotto, 2012).

Recently, electro-technologies such as Pulsed Electric Fields (PEF) and Ohmic Heating (OH), also known as Moderate Electric Fields (MEF), gained considerable interest for the processing of foods and in bioprocesses. They have shown to be mild processing technologies preserving nutritional, functional, structural, and sensory properties of products better than conventional technologies (Knirsch, Alves dos Santos, Martins de Oliveira Soares Vicent, & Vessoni Penna, 2010; Vorobiev and Lebovka, 2009). These are also environmentally clean technologies (at least locally), be it by improving the overall energy efficiency of the process or by reducing the use of non-renewable resources, reducing environmental footprint, while reducing processing costs and improving the added-value of the products (Pereira & Vicente, 2010.). In both cases the presence of an electric field causes electroporation of cellular tissues allowing an enhanced extraction of bioactive compounds (El Darra, Grimi, Vorobiev, Louka, & Maroun, 2012; Sensoy & Sastry, 2004; Vorobiev & Lebovka, 2009). PEF is usually presented as an attractive alternative due to its high efficiency, low energy

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requirements and lower heat generation. However, this technology still presents some drawbacks related with electrodes oxidation and erosion, power generator costs and complexity, together with difficulties in controlling e.g. temperature as well as other processing consequences such as electrode fouling, bubbling, among others (Mohamed & Eissa, 2012; Ravishankar, Zhang, & Kempkes, 2008). OH is mostly known for its capacity to provide fast, homogeneous and precise heating wherever direct application of electrical energy to the food ensures a highly efficient energy transfer. For these reasons OH is currently being successfully implemented in food processing industry (Sakr & Liu, 2014; Sastry, 2008). MEF non-thermal effects (e.g. electroporation) were also reported in the literature, nevertheless they were not yet as studied as in PEF nor commercially exploited (Kusnadi & Sastry, 2012; Sastry, 2008). Despite electric effects in MEF being reported to be more effective at low frequencies (Nair et al., 2014; Shynkaryk, Ji, Alvarez, & Sastry, 2010), they have also been observed at high frequencies (i.e. kHz range) and proven effective in other applications such as cancer cell disruption (Kirson, 2004). From the process point of view, working at high frequencies (i.e. >17 kHz) is more advantageous since electrolysis, electrode erosion and leakage of metals to the heating medium are effectively eliminated, ensuring the reliability of the system and the safety of the processed products, even when a low-cost and electrochemically active electrode material like stainless steel is used (Pataro et al., 2014; Sastry, 2008; Shynkaryk et al., 2010).

Heat generation is commonly faced as an issue in studies regarding electric effects, however technologically thermal effects may be advantageous (Allali, Marchal, & Vorobiev, 2008; Lebovka, Kupchik, Sereda, & Vorobiev, 2008) and the synergistic effects of temperature and electric field may bring new perspectives in the processing industry, as in the case of blanching or thermal stabilization of bio-materials.

Potato tissue is commonly used as a model for vegetable and foodstuff cellular material. Several studies report thermal and electric effects in this material, regarding for example stabilization or blanching (González-Martínez, Ahrné, Gekas, & Sjöholm, 2004; Zielinska, Błaszczak, & Devahastin, 2015) and permeabilization (Kulshrestha & Sastry, 2006; Lebovka, Praporscic, Ghnimi, & Vorobiev, 2006). *Solanum tuberosum* L. var. Vitelotte, is a colored potato variety with blue skin and violet flesh widely used for human consumption. The pigments responsible for its color are anthocyanins, which have reported antioxidant and antimicrobial activities (Bontempo et al., 2013). Anthocyanins belong to the class of phenolic compounds and are characterized by their color and stability, which are both affected by physical and (bio)chemical factors - i.e. temperature, light, pH and attacks by specific chemical groups or enzymes (Reyes & Cisneros-Zevallos, 2007). Other phenols are present in this potatoes, also with reported bioactivities and recognized value (Al-Saikhan, Howard, & Miller, 1995; Mulinacci et al., 2008; Nems et al., 2015).

The present study has the objective of evaluating the combined effects of the most relevant OH process parameters (i.e. electric field strength, temperature and time of process), in the pretreatment and further extraction of bioactive compounds from colored potatoes. A Box-Behnken design in Response Surface Methodology (RMS) was used to study the electric, thermal and time effects and to establish the most favorable pretreatment conditions and its influence on anthocyanin and total phenols extraction. The selection of treatment and extraction conditions aimed at achieving a feasible, low-cost and low-environmental impact process.

2. Material and methods

2.1. Sample material

Purple potatoes (*Solanum tuberosum* L. var. Vitelotte) were kindly supplied by a local producer. Potatoes with no apparent defects and approximately the same caliber (Ø 50 mm) were selected and stored (4 °C and ≈ 100% RH) in the dark without the addition of sprouting inhibitors. Before utilization potatoes were allowed to equilibrate at room temperature, then washed and peeled. A potato cylinder was obtained with a tubular cutter (Ø 20 mm) along with the tuber axis, obtaining a sample constituted by medulla and perimedulla tissue. This cylinder was then cut in 5 mm thick discs, from which the end pieces were discarded to ensure maximum homogeneity between samples. The obtained discs were washed with distilled water and gently dried with paper towels. For each individual potato, the samples were divided in two groups and immediately used; one group was used to perform the electro-heating treatments and the other group was used to determine maximum content of anthocyanins and total phenolic content (TPC) of that particular individual. This allowed to normalize all the results as a percentage of the maximum content of these compounds found in each individual potato thus overcoming its natural variability.

2.2. Experimental setup

The electro-heating treatments were performed in a jacketed static ohmic heater as described elsewhere (Pereira et al., 2015; Pereira, Souza, Cerqueira, Teixeira, & Vicente, 2010). The distance between electrodes was kept constant, and the voltage varied in the power source working with a sinusoidal wave at 25 kHz (1 Hz—25 MHz and 1—10 V; Agilent 33220A, Penang, Malaysia). A circulator water system (F25-ED, Julabo, Seelbach, Germany) was used to heat up in the case of conventional thermal treatment (0 V/cm) and to refrigerate when higher electric fields were applied, thus allowing to maintain similar heating rates in all types of treatments. A KCl iso-conductive solution was prepared according with Kulshrestha and Sastry (2006) to ensure a homogeneous current flow and poured to the processing chamber in a solid-liquid ratio of 1:1. Temperature was recorded with a type-K thermocouple (temperature precision of ±1 °C; Omega Engineering, Inc., Stamford, CT, USA), placed at the geometric center of the sample volume and connected to a data logger (USB-9161, National Instruments Corporation, Austin, TX, USA). Measurements of electrical frequency, voltage and current intensity during OH treatments were measured through portable oscilloscope (ScopeMeter® 125/S, Fluke, Everett, WA, USA).

2.3. OH treatments

Electro-heating treatments were performed through three-factor Box—Behnken experimental design in which the combined effect of three independent variables − electric field, holding temperature and treatment time were evaluated. During OH the MEF applied was of 0 V/cm (conventional heat exchange heating), 15 V/cm and 30 V/cm (OH); for each MEF level the temperatures selected were 30 °C (≈ room temperature), 60 °C and 90 °C (corresponding to minimum and maximum blanching temperatures (Abu-Ghannam & Crowley, 2006). The holding time was of 0, 5 and 10 min. The response values were anthocyanins yield and TPC yield. The design consisted of 15 combinations including three replicates of the center point (Table 1). All experiments were made in a randomized order to avoid variability in the independent variables due to the eventual occurrence of systematic errors. The heating

kinetics of the electric treatments was adjusted to simulate the one of heat exchange heating (0 V/cm), as shown in Fig. 1. In order to take maximum advantage of OH potentialities, a pre-treatment based on high temperature short time (HTST) principle was carried out, in which a high temperature 100 °C were combined together in under 1 s of holding time and a MEF of 200 V/cm.

2.4. Aqueous extraction procedure

According with the results from the experimental design the most favorable conditions for OH pre-treatment were selected within the experiment variables limits for further aqueous extraction. After pre-treatments under the defined conditions, potato samples were put in distilled water at 1:5 w/v and placed in an orbital shaker (KL-2 Edmund Buhler, Germany) at 90 rpm (≈ 20 °C). Aliquots were collected periodically from the liquid extract for a pre-determined time, until no significant increment on the concentration of the interest compounds was noticed.

2.5. Extraction kinetics modeling

The aqueous extraction kinetics of phenolic and anthocyanin compounds were mathematically described by using the two-step model or phenomenological model through Equation (1) (So & Macdonald 1986; Milic, Rajkovic, Stamenkovic, & Veljkovic, 2013). This empirical model is well suited for extraction processes involving assisted means such as an electrical treatment (as well as other types of treatments such as microwaves and ultrasound) which are hard to describe theoretically (Chan, Yusoff, & Ngoh, 2014). This model involves two main stages: (1) washing, a fast extraction step during which the solutes located on or near surfaces of tissues are dissolved; and (2) diffusion, a slow extraction step characterized by mass transfer of solutes from the interior of the tissue matrices into the aqueous solution. Both processes were here assumed to be exponential and to occur simultaneously from the beginning:

$$y = y_{\infty}[1 - a \cdot exp \cdot (-k_w \cdot t) - (1 - a) \cdot exp \cdot (-k_d \cdot t)] \tag{1}$$

where y is the extraction yield of the solutes extracted − i.e. anthocyanins or total phenolic compounds; y_{∞} is solute extraction yield at the saturation point; a is the amount of solute extracted into water during the washing step; k_w and k_d are the rate constants for washing and diffusion, respectively.

2.6. Quantitative analyses

Anthocyanins and TPC were immediately determined in the pretreatment medium and in the extraction solvent using fast and reliable spectrophotometric methods. This procedure avoided oxidation and degradation of these compounds which often occur during storage (Sarkis, Jaeschke, Tessaro, & Marczak, 2013). Total anthocyanin content was measured using the pH differential method described by Giusti and Wrolstad (2001) and expressed as cyanidin-3-glucoside equivalents (Equation (2)).

$$Anthocyanin \left(\frac{mg}{kg} \right) = (Abs \times MW \times D \times 1000)/(\varepsilon * L) \tag{2}$$

where Abs is absorbance, MW is the molecular weight of anthocyanin, D is the dilution factor, ε is the cyanindin-3-glucoside molar absorbance and L is the cell path length.

Extracted TPC were determined using Folin-Ciocalteau's colorimetric method as described by Singleton (1965) and expressed as gallic acid equivalents. In both cases results were expressed as a fraction (percentage) relatively to their maximum content in the sample. Maximum anthocyanins and phenolics contents were determined by ensuring full cellular rupture of the samples (by freezing and mechanical mashing with a mortar and pestle) and subsequently suspending the sample through vortexing for 1 min in the same solution used for the electro-heating treatment in a solid-liquid ratio of 1:5. All samples were clarified by centrifugation at 10.000 g for 5 min (MIKRO 120, Hettich, Germany) before determination. The yield of extraction was determined by the following equation (Eq. (3)):

$$Yield(\%) = \frac{Extract\ content}{Sample\ maximum\ content} \times 100 \tag{3}$$

2.7. Chromatographic analyses

2.7.1. Phenolic compounds analysis by ultra-high performance liquid chromatography (UHPLC)

Samples were analysed by Shimatzu Nexpera X2 UHPLC chromatograph equipped with Diode Array Detector (Shimadzu, SPD-M20A). Separation was performed on a reversed-phase Aquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size; from Waters) and a pre-column of the same material at 40 °C. The flow rate was 0.4 mL/min. The HPLC grade solvents used were water/formic acid (0.1%) as solvent A and acetonitrile as solvent B. The elution gradient for solvent B was as follows: from 0.0 to 5.5 min eluent B at 5%, from 5.5 to 17 min a linear increase to 60%, from 17.0 to 18.5 min a linear increase to 100%, then column equilibration from 18.5 to 30.0 min again at 5%. Phenolic compounds were identified by comparing their UV spectra and retention times with that of corresponding standards. Compounds were quantified and identified at different wavelengths: chlorogenic acid and ferulic acid at 320 nm, ellagic acid at 250 nm, catechin at 280 nm and rutin at 350 nm.

2.7.2. Anthocyanin analysis by HPLC and mass spectrometry (LC-MS-MS)

For chromatographic separation we used a HPLC (Elite Lachrom system, L-2130) on a 250 × 4.6 mm i.d. reversed-phase C18 column (Merck, Darmstadt, Germany) and a diode array detector (L-2455) as described elsewhere (Fernandes, de Freitas, Reis, & Mateus, 2012). The mobile phase consisted of Solvent A: 10% formic acid in water, and Solvent B: 30% acetonitrile and 10% formic acid in water. The applied elution gradient was: 0−70 min, 80%−20% A; 70−80 min, 100% B; 80−90 min, 80% A and 20% B. The flow rate was of 0.5 ml/min, and the injection volume of 20 μL. The quantification was performed using calibration curves of delphinin-3-glucoside ($r^2 > 0.98$), and the results were expressed as delphinin-3-glucoside equivalents. Mass analysis was performed on a liquid chromatograph (Hewlett-Packard 1100 series) with an AQUATM (Phenomenex, Torance, CA, USA) C18 reversed-phase column C18 (150 × 4.6 mm, 5 mm), stabilized at temperature of 35 °C. The mass detector (Finnigan LCQ, Finnigan Corporation, San Jose, USA) was equipped with an API source, using an electrospray ionization (ESI) interface and spectra were recorded in positive ion mode between m/z 120 and 1500. The mass spectrometer was programmed to do: 1) full mass; 2) zoom scan of the most intense ion of the first scan; and MS-MS of the most intense ion using relative collision energies of 30% and 60%, as described elsewhere (Fernandes et al., 2012).

2.8. Energy input

The electrical energy consumption (E_c) of the OH treatment was

Table 1
Box-Behnken design including independent variables and the respective responses of dependent variables — i.e. extraction of anthocyanin and total phenolic content (TPC).

| Run	Independent variables			Dependent variables	
	Electric field (V/cm)	Temperature (°C)	Time (min)	Anthocyanin yield (%)	TPC yield (%)
1	0	30	5	0.284	10.392
2	30	30	5	4.577	16.618
3	0	90	5	29.134	39.252
4	30	90	5	35.155	58.900
5	0	60	0	1.015	22.984
6	30	60	0	8.972	17.024
7	0	60	10	15.560	25.769
8	30	60	10	31.055	46.942
9	15	30	0	0.429	10.979
10	15	90	0	23.607	28.055
11	15	30	10	3.612	11.955
12	15	90	10	53.066	63.814
13	15	60	5	18.691	29.580
14	15	60	5	20.088	34.225
15	15	60	5	25.472	32.663
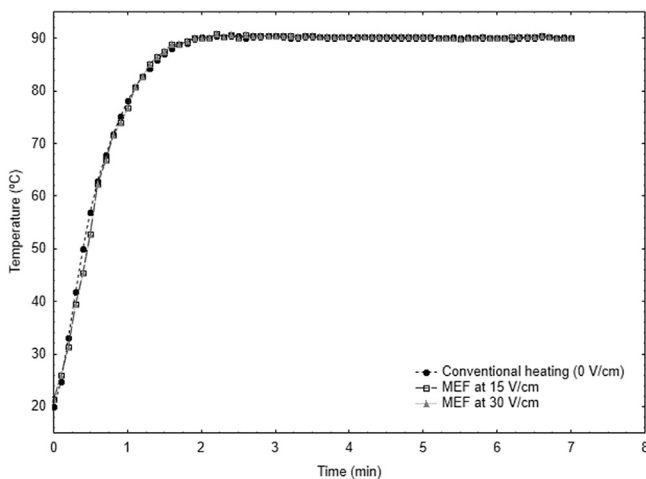
Fig. 1. Examples of identical thermal profiles of conventional heating (0 V/cm) and OH with a MEF of 15 V/cm and 30 V/cm, both performed at holding temperature of 90 °C for 5 min.

expressed in kJ/kg and calculated as described elsewhere (El Darra et al., 2012):

$$E_c = \frac{\int_0^t U \cdot I \cdot dt_{OH}}{m} \tag{4}$$

where U is the voltage applied (V), I is the current intensity (A), t is time of OH treatment (s), and m is the mass of treated product (kg).

2.9. Statistical analysis

For statistical analysis of experimental data, the Statistica package software version 10.0.228.8 (StatSoft Inc.) was used applying an analysis of variance to estimate any statistically significant differences at a confidence level of 95%. Fitting quality of the models used on the experimental data was evaluated by means of statistical tests: linear regression coefficient (R^2) and mean relative percent deviation (MRPD). Unless otherwise stated, all determinations were run at least in triplicate and all the data were reported as mean ± SD (standard deviation).

3. Results and discussion

3.1. Pre-treatment yields

Potato samples presented a maximum content of anthocyanins (as cyanidin-3-glucoside equivalents) and TPC (as gallic acid equivalents) of 182.6 ± 4.5 mg kg^{-1} and 582.8 ± 23.3 mg kg[1], respectively. These values agree with those reported in the literature for this species (Jansen & Flamme, 2006; Nems et al., 2015). Authors emphasize the importance of anthocyanins stability during food processing and discuss changes in the overall antioxidant activity due to thermal processing of potato tissue and preservation procedures (Nems et al., 2015). When anthocyanins undergo structural changes their specific properties are affected, degrading into their constituent phenolic acids (Woodward, Kroon, Cassidy, & Kay, 2009). Therefore, independent determination of anthocyanins and TPC was performed in this study in order to eventually evidence selective extraction or degradation of these compounds.

The yields of extraction as response variables for the experimental design are shown in Table 1. Maximum recovery yields of anthocyanins and TPC were 53.1% (out of the total amount of anthocyanins) and 63.8% (out of the total amount of TPC) respectively, both at the same experimental conditions (see Table 1). In general the extraction yield of TPC was 10—20% higher when compared to anthocyanins extraction yield, for the same experimental conditions. This suggests that other phenolic compounds are possibly more easily extracted than anthocyanins under the experimental conditions tested.

3.2. Effects of temperature, time and electric field

Statistical analyses (summarized in Table 2) showed that the adjusted model to the Box—Behnken design was adequate and a good representation of the behavior of the system, with R^2 values for anthocyanins and TPC yields of 0.978 and 0.987, respectively.

According to the fitted model (see Table 2), the linear terms of all independent variables tested — electric field, temperature and time — presented statistical significance for both dependent variables ($p < 0.05$). The quadratic term of the electric field has shown statistical significance ($p < 0.05$) for anthocyanins yield only. The linear interactions between temperature and time for anthocyanins and TPC yields, as well as the linear interactions between electric field and time for TPC yield, have also reached statistical significance ($p < 0.05$). It is thus clear that changes in the above factors significantly affect the extraction yields of anthocyanins and TPC.

By analyzing the surface plots the optimum conditions for extraction of anthocyanins and TPC can be accessed. As expected, the "time" and "temperature" linear terms, as well as their interaction, appear as the main factors affecting the extraction of solutes from potato tissue; from Figs. 2a and 3a it is possible to observe that extraction yields increase almost linearly with increasing of both treatment time" and "temperature". Interestingly, together with "time" and "temperature", the electric filed applied had also a significant effect on extraction of anthocyanin's and TPC ($p < 0.05$) from potato tissue. Through Fig. 2b it is possible to observe the relationship between "electric field" and "temperature", with a maximum yield of anthocyanins being observed at 20 V/cm and 90 °C. However, this increase in extraction yield of anthocyanins seems to be reversed at electric fields strength above 20 V/cm. In accordance, Fig. 2c shows that the anthocyanins yield increases with treatment time but not always with the electric field; anthocyanin yield increases with treatment time until reach a plateau for electric fields higher that 20 V/cm. In respect to TPC, a synergistic interaction between time and electric field can be observed through Fig. 3c, thus resulting in a higher extraction yield with their increase. Moreover, when an electric field strength above 20 V/cm is combined with a treatment temperature above 70 °C, a significant increase in extraction is also noticed (Fig. 3b). Curiously this increase matches at electrical field conditions at which apparently anthocyanin yield starts to decrease, suggesting a possible degradation of anthocyanins to its constituent phenolic acids. These results highlight non-thermal effects of OH eventually on the permeabilization of plant tissues already addressed elsewhere (Kulshrestha & Sastry, 2003; Sensoy & Sastry, 2004), but more important than that synergistic effects on the extraction of solutes when MEF is combined with other treatment variables such as treatment time and temperature. OH ability to provide fast, homogeneous and precise internal heating always with an MEF associated provide opportunities to development of novel experimental designs for solutes extraction from plant tissues aiming, for example a selective extraction when temperature conditions are properly controlled.

3.3. Kinetic modeling of aqueous extraction

After the pre-treatments, the iso-conductive solution was recovered for further analysis and the potato samples used for aqueous extraction (see section 2.3). The potato samples pre-treated at 90 °C for 5 min using an MEF of 15 V/cm were chosen for aqueous extraction. Within the experimental design limits, these were the optimal treatment conditions regarding the extraction yields previously obtained taking into consideration anthocyanins extraction. However, aqueous extraction of this pre-treatment was also benchmarked with an identical pre-treatment but without the presence of electric fields (90 °C, 5 min at 0 V/cm) and with OH pre-treatment at MEF of 200 V/cm, aforementioned. This allowed a direct comparison of pre-treatments in similar thermal conditions and evaluation of non-thermal effects promoted by MEF, as well as to take maximum advantage of OH potentialities (HTST processing) coupled with electric fields of relatively high moderate intensity. Experimental results from the extraction of the total phenolic and anthocyanins from pre-treated potato samples are depicted in Fig. 4. At a constant room temperature of approximately of 20 °C, and independently of the treatment applied, the extraction kinetics curves had similar trends to classical mass transfer processes, reaching equilibrium after a certain extraction time. The extraction mechanism was also shown to be characterized by two steps − 1) fast washing and 2) slow diffusion.

Kinetic parameters resulting from of Eq. (1) are shown in

Table 3. The adjusted model was statistically significant ($p < 0.0001$) for all extraction kinetics and well fitted to the experimental data (see Fig. 4). This was also indicated by the value of R^2 which ranged between 0.98 and 0.99 and by mean relative percentage deviation (MPRD) values ranging from 0.6 to 3.2% (Table 3); MPRD values below 10% are considered to be indicative of a reasonably good fit for most practical purposes (García-Pascual, Sanjuán, Melis, & Mulet, 2006). Both anthocyanins and TPC extraction yield increased with extraction time in aqueous medium at room temperature, being the highest values obtained ($p < 0.05$) in the case of OH pre-treated samples. For instance, with conventional pre-treatment it was possible to get a saturation extraction yield - y_∞ - of 73% and 78% of the total anthocyanins and TPC, respectively, while with OH pre-treatment y_∞ values were of 84% and 92%. This extraction enhancement can be attributed to the reported effects on permeabilization of tissues (Asavasanti, Ristenpart, Stroeve, & Barrett, 2011; Lebovka et al., 2008; Pereira, Galindo, Vicente, & Dejmek, 2009). The fraction of extractable solutes during washing step - a — was dependent on the type of solute and pre-treatment applied, but did not follow any particular trend. The washable fraction of anthocyanins was higher ($p < 0.05$) for OH pre-treatment when compared with the conventional treatment, but the opposite was true in case of TPC extraction. The rate constants (k_w and k_d) increase, both for anthocyanins and TPC extraction, with application of OH pre-treatments. In particular kw increased about 210% with application of OH pre-treatment at MEF of 200 V/cm, when compared with conventional heating. Independently of thermal conditions applied, OH pre-treatments also enhanced solute diffusion - k_d - through solid tissue. Combination of high temperature with a mild electric field, or the effect of a mild electric field alone, may have contributed to disruption of cells walls located at the external surfaces and deeper in tissues, or simply to an intensification of mass transfer, liberating solutes that became more easily accessible for dissolution into water (Milic et al., 2013).

3.4. Quality assessment of extracted phytochemicals

As shown in Figs. 5 and 6, UHPLC and HPLC analysis allowed the identification of the major phenolic compounds (including anthocyanins) from the extracts that resulted from harsher treatment conditions − i.e. high electric field (200 V/cm) and high temperature (~100 °C). Table 4 lists phenolic compounds that were consistently identified in untreated and treated samples. Apart from flavonoids (i.e. anthocyanins) it was possible to identify other phenol classes such as phenolic acids (i.e. chlorogeinc, ferulic and ellagic) and flavonols (i.e. catechin and rutin). MEF at 200 V/cm enhanced considerably the extraction of chlorogenic acid from (already present in untreated samples) but also allowed to extract ellagic and ferulic acids, catechin and rutin in a very comparable way to a freeze/thawing treatment. These compounds are commonly found in purple potatoes, in particularly the chlorogenic acid that can range from 76 to 181 mg/100 g fresh product (Reyes, Miller, & Cisneros-Zevallos, 2005), which is well in the concentration range determined in this study (see Table 4).

Through LC-MS-MS it was possible to confirm the presence and identify different types of anthocyanins in the extracts resulting from thermal treatments at 200 V/cm. Anthocyanins are one the most important groups of phenolic compounds in food, attracting much interest due to their health-related functionality (Lila, 2004). But they are also the most instable polyphenols being easily oxidized or degraded during thermal processing at high temperatures (Hou, Qin, Zhang, Cui, & Ren, 2013). Table 5 shows the MS fragmentation pattern of the individual anthocyanins identified by LC−MS-MS, as well as the retention time recorded from the HPLC

Table 2
Effects linear (L) and quadratic (Q) estimates obtained from analysis of variance (ANOVA) from the response surface linear model for anthocyanin and total phenolics content (TPC) yields.

|	Anthocyanin yield R^2 = 0.978 Adj: 0.399 MS residual 14.198				TPC yield R^2 = 0.987 Adj: 0.965 MS residual 9.837			
	Effect	Std. Error	t(5)	p	Effect	Std. Error	t(5)	p
Mean/Interc	17.206	1.088	15.818	<0.0001	28.841	0.905	31.855	<0.0001
(1)MEF(V/cm)(L)	8.441	2.664	3.168	0.025	10.272	2.218	4.632	0.006
MEF(V/cm)(Q)	5.079	1.961	2.590	0.049	−0.131	1.632	−0.080	0.939
(2)T (°C)(L)	33.015	2.664	12.391	0.000	33.371	2.218	15.047	<0.0001
T (°C)(Q)	−0.949	1.961	−0.484	0.649	0.997	1.632	0.611	0.568
(3)time (min)(L)	17.318	2.664	6.500	0.001	19.008	2.218	8.571	<0.0001
time (min)(Q)	2.188	1.961	1.116	0.315	4.107	1.632	2.517	0.053
1L by 2L	0.864	3.768	0.229	0.828	6.711	3.136	2.140	0.085
1L by 3L	3.769	3.768	1.000	0.363	13.567	3.136	4.326	0.008
2L by 3L	13.137	3.768	3.487	0.018	20.689	3.136	6.597	0.001

MS, mean square.
Adj, adjusted.
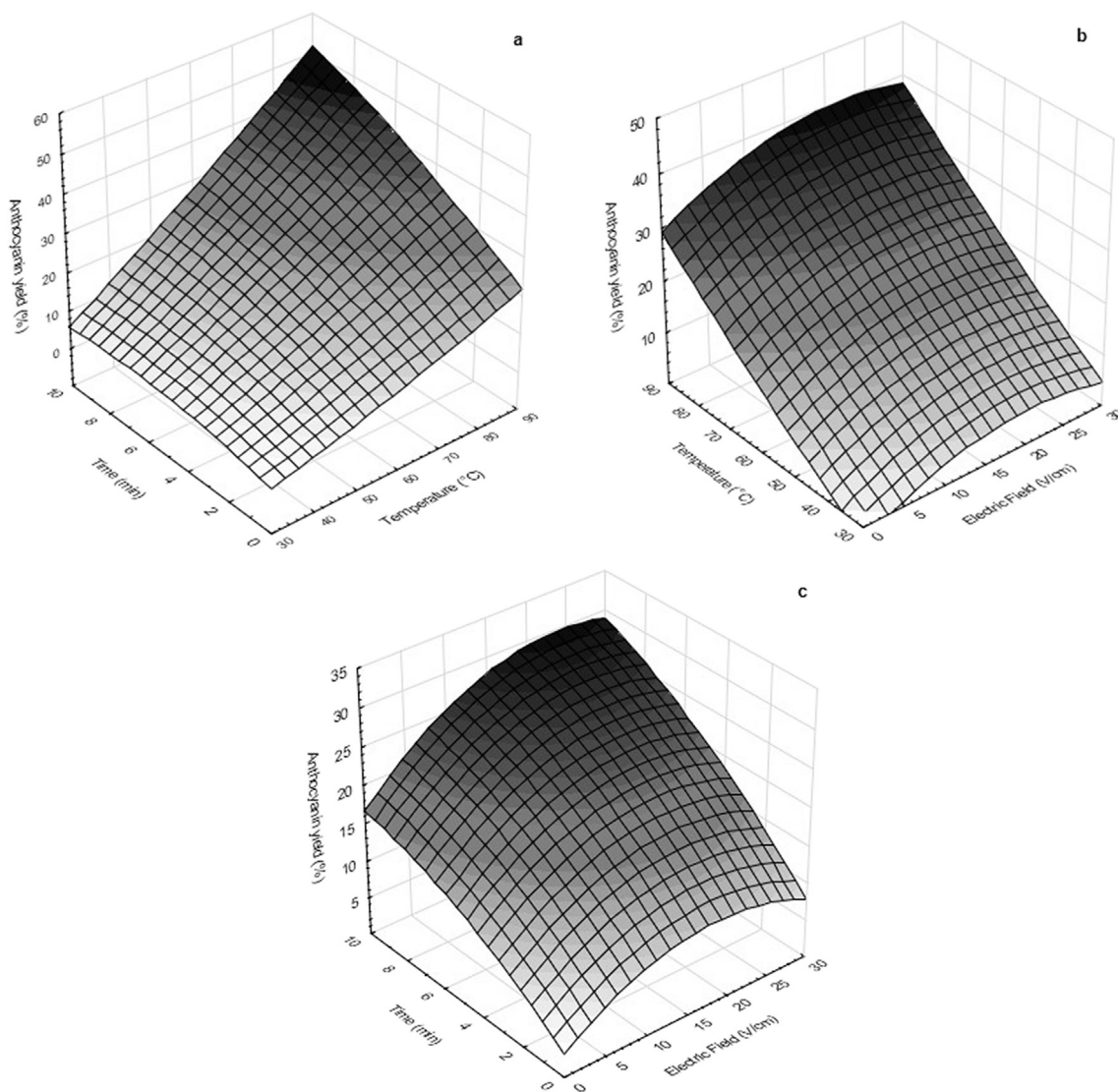
Fig. 2. Surface plots for the effects on anthocyanin extraction yield of temperature and time at a constant electric field of 15 V/cm (a), temperature and electric field at a constant time of 5 min (b) and electric field and time at a constant temperature of 60 °C (c).

detector and concentration range. Petunidin-glucoside-cumaroylglucoside and petunidin-glucoside-cumglc-vinylguaiacol represented about 20% and 60% of the total anthocyanins in all extracts, respectively. Petunidin-based is the dominant anthocyanins in potatoes with purple-colored flesh as described elsewhere (Brown, Wrolstad, Durst, Yang, & Clevidence, 2003). Malvindin and
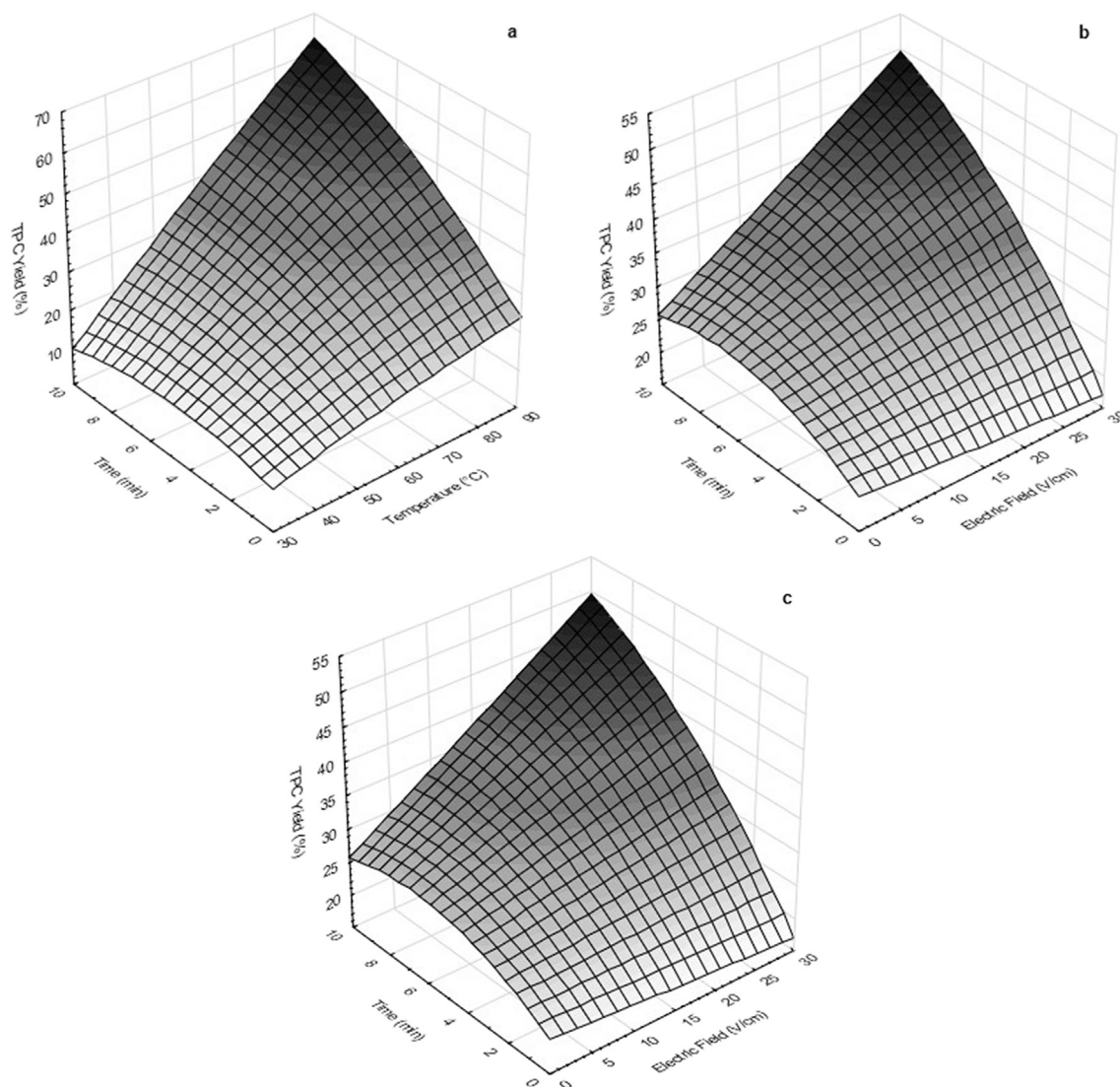
Fig. 3. Surface plots for the effects on TPC yield of temperature and time at a constant electric field of 15 V/cm (a), temperature and electric field at a constant time of 5 min (b) and electric field and time at a constant temperature of 60 °C (c).

delphinidin, also responsible for purple color, accounted together for about 8% of the total anthocyanins in the extracts. Total content of anthocyanins determined by HPLC varied from approximately 20 to 70 mg/100 g of fresh weight. MEF treatments allowed to extract a total content of anthocyanins within this range i.e. from 8 to 35 mg/100 g. It is important to note that these contents may be underestimated due to self-association and aggregation phenomena of these kind of anthocyanins that are known to occur in relatively concentrated solutions (Fernandes, Bras, Mateus, & de Freitas, 2015; Han & Xu, 2015) impairing an accurate chromatographic separation, which in turn can help to explain the wide range of concentrations observed for each determination (see Table 5). But in a general way these results are in accordance with previous reports where total content of anthocyanins in purple potato vary widely from 10 up to 300 mg/100 g of fresh weight product, depending on the type of potato cultivar (Ahmed, Akter, & Eun, 2011; Brown, 2005; Ezekiel, Singh, Sharma, & Kaur, 2013; He, Li, Lv, & He, 2015; Lewis, Walker, Lancaster, & Sutton, 1998).

The effects of ohmic heating and electric fields intensity on anthocyanins in several food products have been evaluated by different authors (Loypimai, Moongngarm, Chottanom, & Moontree, 2015; Pereira, Pereira, Teixeira, & Vicente, 2007; Sarkis et al., 2013) but were still inconclusive. The inability to eliminate temperature differences between thermal and electro-thermal treatments, proper control of electrochemical reactions, diversity of food matrices as well different thermal protocols applied between these studies do not allow drawing a general conclusion about the influence of negative electrical effects on anthocyanin degradation. Results from this study show at least that working at relatively high electric field strength values (200 V/cm) and high temperature for a very short period (HTST principle) offers a good extraction yield of anthocyanins (up to 85% - see Table 3) without affecting significantly their quality profile (see Table 5).

3.5. OH as an alternative to assisted extraction

OH treatments have determined increase of extraction yields when compared with conventional heating treatments (without presence of electric fields), even under identical thermal profiles. The increase of extraction yields with application of OH corroborates the hypothesis of non-thermal effects on permeabilization of vegetable tissues, through electrical disturbances in cells'
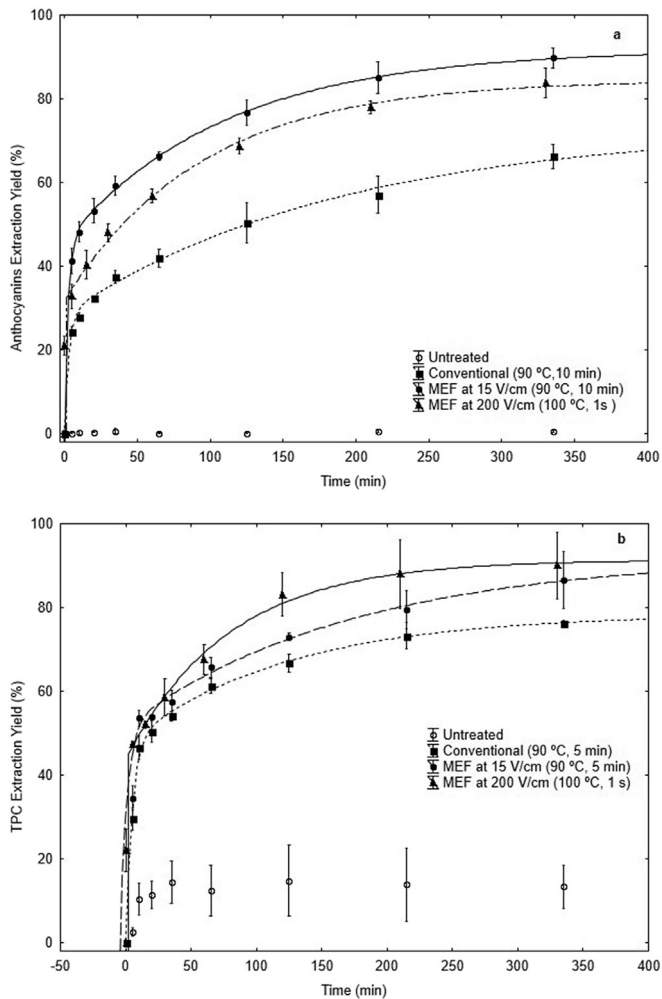
Fig. 5. Example UHPLC chromatogram showing identification of the chlorogenic acid at UV wavelength of 320 nm.

Fig. 4. Kinetic of anthocyanin a) and TPC b) extraction from purple potato in water after different pre-treatments using two-step model (Eq. (1)): conventional heating (- - - -); MEF of 15 V/cm at 90 °C (——) and MEF of 200 V/cm at 100 °C, 1 s (— —).

extraction of solutes of interest, eventually promoted through electrically-induced permeabilization. It is worth mentioning that the conditions under which this work was carried out — i.e. high frequency (25 kHz) and low electric field ($\leq$30 V/cm) — are usually reported as being well suited for industrial applications (cheaper equipment and feasible power input) (Pataro et al., 2014; Sakr & Liu, 2014) At these electrical conditions the extraction yields of anthocyanins and TPC in potato tissue are enhanced by OH. Furthermore, extraction yields can be further improved at higher temperatures (i.e. > 90 °C), provided that such high temperatures will not interfere with the product being processed (e.g. through excessive thermal degradation).

The type of solvent and solid:liquid ratio were not considered as variables in this study for that was not among the objectives. The choice upon water as a solvent and a solid:liquid ratio of 1:1 were simply to test the selected variables in conditions that assured the lowest cost and environmental impact of the process. In typical extraction studies several constraints can be found such as: employment of high amounts of organic solvents at solid:liquid ratios of one to several tens; high temperatures are often applied; long treatment times, taking from one to several hours; and complex steps of processing, as freezing, mashing, pulverization among others, are often used (Bontempo et al., 2013; Chirinos, Rogez, Campos, Pedreschi, & Larondelle, 2007; Fan, Han, Gu, & Chen, 2008; Kita, Bąkowska-Barczak, Lisińska, Hamouz, & Kułakowska, 2014). Heating and freeze-thawing are reported to be the most

membranes or eventually electroporation effects (Pereira et al., 2009), as reported in the results of other authors (Kulshrestha & Sastry, 2010; Lebovka et al., 2008; Lebovka et al., 2006). Electric treatments (i.e. permeabilization) are often used as a fundamental study of electric effects and/or as an alternative to thermal treatments. The present work shows that electrical and thermal effects during OH can be conjugated, yielding a synergistic effect on
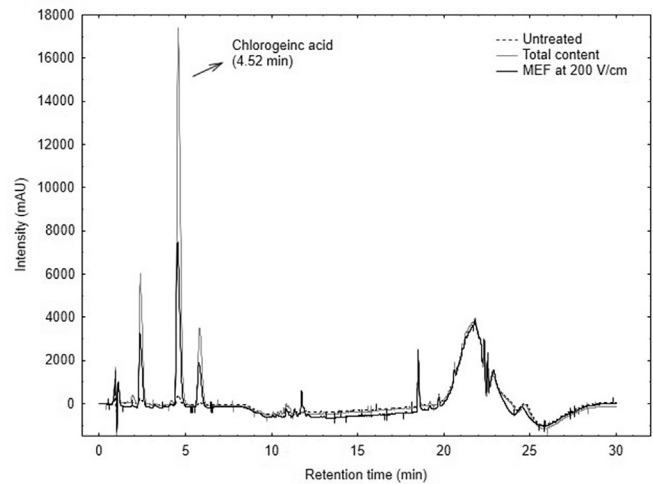
Table 3
Kinetic model parameters of anthocyanins and total phenolic contents (TPC) extraction in water after conventional heating (CH) and OH pre-treatments.

Parameters	y_∞ (%)	a	k_w (min^{-1})	k_d (min^{-1})	R^2	MRPD (%)
Anthocyanin:						
CH	73,0 ± 5,7[a]	0,39 ± 0,02 [a]	0,315 ± 0,076[a]	0,0053 ± 0,0015[a]	0,987	2,6
MEF[1]	91,8 ± 2,2[b]	0,50 ± 0,02[b]	0,382 ± 0,062[a]	0,0091 ± 0,0014[b]	0,995	0,57
MEF[2]	84,5 ± 1,8[c]	0,38 ± 0,02[a]	65,2 ± 10,1[b]	0,0106 ± 0,0012[c]	0,994	1,8
TPC						
CHl	78,4 ± 2,5[a]	0,61 ± 0,02[a]	0,201 ± 0,021[a]	0,0081 ± 0,0020[a]	0,991	2,4
MEF[1]	92,4 ± 7,4[b]	0,56 ± 0,04[b]	0,234 ± 0,039[a]	0,0057 ± 0,0025[b]	0,985	3,2
MEF[2]	91,4 ± 2,8[b]	0,48 ± 0,03[c]	42,1 ± 8,8[b]	0,0127 ± 0,0027[c]	0,984	1,0

[1] 15 V/cm at 90 °C for 5 min.
[2] MEF 200 V/cm at 100 °C for 1 s.
MRPD, mean relative percentage deviation.
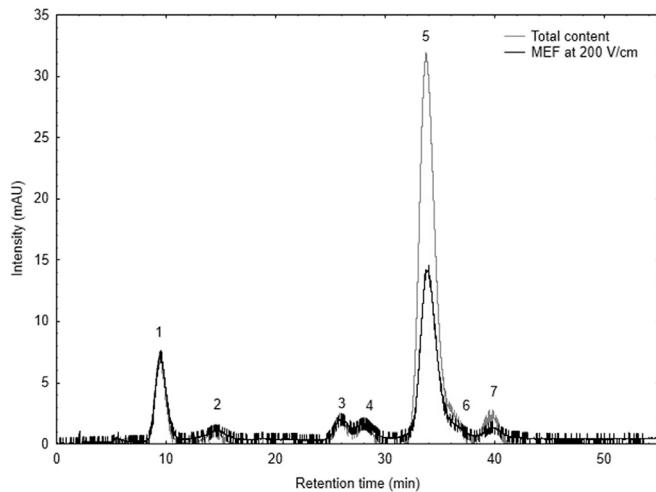For each column different letters in superscripts are significantly different ($p < 0.05$).

Fig. 6. Example of HPLC chromatogram of extracted anthocyanins. The peak numbers correspond to the compounds indicated in Table 5.

energy-consuming methods for cell disintegration in potato tissue, with an energy consumption over 250 kJ/kg (Toepfl, Siemer, Saldaña-Navarro, & Heinz, 2014). Fig. 7 shows total energy consumption of conventional and OH pre-treatments applied in this study. As expected conventional heating resulted in the highest energy consumption of approximately 300 kJ/kg, which is well comparable with reported values for cell disintegration of potato tissue when heating is applied (Eugene & Nikolai, 2011; Toepfl et al., 2014). Despite OH being a thermal process, levels of energy input were significantly lower ($p < 0.05$) when compared with conventional heating under the same thermal conditions (i.e. heating rate, temperature and treatment time); energy consumption of OH pre-treatments increased with increasing MEF intensity applied and was of 95.9 ± 26.5 kJ/kg and 157.0 ± 19.5 kJ/kg for MEFs of 15 V/cm and 30 V/cm, respectively. Energy consumption was substantially reduced in the case of OH pre-treatment based in the

HTST principle, in which power input was of 27.7 ± 5.0 kJ/kg despite a MEF intensity of 200 V/cm. Pulsed electric fields (PEF) still is an ideal method to induce damaging in potato tissue as compared to other methods due to its low energy consumption ranging from 6.4 to 16.2 kJ/kg (Eugene & Nikolai, 2011). However, bacterial inactivation by PEF requires the application of high electric field strengths thus increasing drastically the power consumption. Results from this study show that OH by combining HTST principle (suitable for bacterial inactivation) together with MEF non-thermal effects, allows a good extraction performance. Further, OH require low power consumption well comparable with mechanical (20–40 kJ/kg) and enzymatic (60–100 kJ/kg) methods or less than the ones required for PEF treatments intended for bacterial inactivation that can be in the range of 40–1000 kJ/kg (Vorobiev & Lebovka, 2011; Vorobiev & Lebovka, 2013).

Thus, regarding the conditions tested in this study significant amounts of anthocyanins and phenolic compounds were recovered from the samples and unlike typical extraction studies a) no organic solvents were employed, b) the solid:liquid ratio was very low, and c) the processing steps were maintained at a minimum, as for treatment time and energy input, resulting in a so-called green process (Chemat et al., 2012).

4. Conclusions

It was demonstrated that OH treatments significantly affect phytochemical extraction from potato tissue at several process temperatures and times. This was effective at conditions aiming minimization of costs and impact of the process, while ensuring industrial applicability. Particularly the conjugation of electric treatment with elevated temperatures presents an interesting alternative for extraction processes, particularly as a first step or pretreatment, since in a short period of time it was possible to stabilize the sample (using pasteurization time-temperature binomial) and extract a significant part of the valuable compounds even in a water-based solvent and a low solid:liquid ratio. Through a two-step model, used for modeling the extraction kinetics process after pre-treatments of potato tissues, it was demonstrated that OH

Table 4
Concentration levels of the phenolic compounds identified in the extracts.

Phenolic classes	Phenolic compounds	Untreated (mg/100 g of product)	Total content (mg/100 g of product)	MEF at 200 V/cm (mg/100 g of product)
Phenolic acids	Chlorogenic	35.2 ± 3.7	91.85 ± 41.6	68.6 ± 7.3
	Ferulic	nd	5.5 ± 0.9	4.4 ± 1.0
	Ellagic	nd	nd	11.0 ± 3.1
Flavonols	Catechin	nd	20.7 ± 14.1	10.8 ± 1.4
	Rutin	nd	7.5 ± 2.0	6.5 ± 0.3

Table 5
Mass spectrometric data and concentration range of the anthocyanins in purple potato extracts detected by HPLC and LC–MS-MS.

| Retention time (min)	Peak	Anthocyanins	Mass (m/z)	MS^2 (m/z)	Total content (mg/100 g of product)	MEF at 200 v/cm (mg/100 g of product)
9.43 ± 0.03	1	Petunidin-glucoside-cumglc	787.20	624.99; 479.09; 317.23	3.9–7.4	1.2–7.6
14.55 ± 0.09	2	Unknown	–	–	0.7–1.1	0.1–0.9
25.95 ± 0.06	3	Unknown	949.01	–	1.3–3.4	0.6–2.2
28.09 ± 0.10	4	Delphinidin-glucoside-cumglc-vynilguaiacol	919.17	756.99; 465.13; 303.27	1.1–2.9	0.3–1.9
33.79 ± 0.06	5	Petunidin-glucoside-cumglc-vinylguaiacol	933.07	770.93; 478.73; 317.20	12.4–49.4	5.8–21.3
35.86 ± 0.16	6	Petunidin-glucoside-cumglc-vinylsyringol	963.00	801.00; 478.93; 317.20	0.7–2.1	0.0–0.9
39.67 ± 0.11	7	Malvidin-glucoside-cumglc-vinylguaiacol	947.00	784.93; 492.93; 331.13	0.6–3.0	0.4–1.0
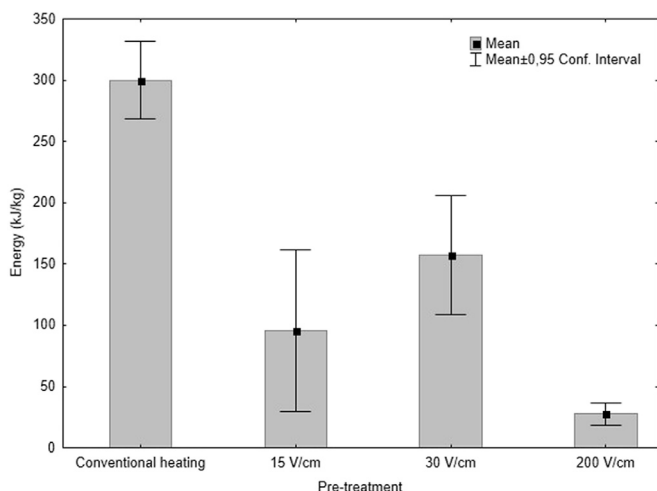
Fig. 7. Total energy consumption of conventional and MEF pre-treatments; conventional heating, MEF at 15 and 30 V/cm were performed at 90 °C for 5 min, while MEF at 200 V/cm was performed at 100 °C for 1 s. Column spreads correspond to confidence interval of 95%.

can influence both washing and diffusion of extractable anthocyanins and TPC. Through combination of UHPLC and LC-MS-MS techniques it was possible to verify and identify several classes of phenolic compounds in the extracts obtained, such as case of anthocyanins. It is particularly significant the demonstration of the positive impact of OH on the process at the operational parameters tested, since the low voltage and high frequency favor energy efficiency and process reliability. Despite the need of a detailed techno-economic analysis to prove the industrial advantage of using OH as a pre-treatment over conventional techniques, this work demonstrates that electro-heating treatments based in OH technology is a suitable processing tool for recovering of food-grade value added compounds from purple potatoes (*Solanum tuberosum* L. var. Vitelotte) in a much shorter time and with a lower consumption of energy than a conventional heating method.

Acknowledgments

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