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# **Improving Polyphenol Extraction from Lemon Residues by Pulsed Electric Fields**

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**Abstract** In this contribution, the influence of Pulsed Electric Fields (PEF) of different intensities (3–9 kV/cm and 0–300 μs) on the extraction of Total Phenols from lemon peel residues by pressing was investigated. According to the cellular disintegration index, the optimum treatment time for the increase in permeability was determined as 30 pulses of 30 μs. It was determined that the effect of PEF was independent of lemon residue size. The effectiveness of pressing-assisted extraction was evaluated by measuring the Total Phenol Content (TPC), the real antioxidant capacity and the concentrations of the main lemon polyphenols, the flavonones hesperidin and eriocitrin. The variables studied in the extraction were time, pressure applied and intensity of the electric fields. This study concludes that electric field intensity of 7 kV/cm increased the efficiency of polyphenol extraction by 300%, giving maximum values of 84 mg of hesperidin in 100 g FW and 176 mg of eriocitrin in 100 g FW. Thus, it was concluded that PEF provides a new methodology to improve polyphenol extraction with a non-thermal, environment-friendly technology, and this represents a method for increasing economic benefits of industrial processes.

**Keywords** Lemon waste · Phenols · Antioxidant · Pulsed electric fields (PEF) · Pressuring · Extraction

# **Introduction**

The worldwide production of lemons in 2010 was 4,200,000 tons. Approximately half of that production was destined to be industrially processed, especially in the juice industry [\[1](#page-7-0)]. This industry thus generates a large amount of agro-food waste material, which is mostly used as a source of cattle feed, or as fertilizer. Current studies have found other uses for this abundant agro-food waste, for example using it as a source of biomass to produce bioethanol [[2\]](#page-7-1). However, a most valuable option would be to obtain "low volume high price" products such as polyphenols [\[3](#page-7-2)].

Polyphenols are described as natural compounds with antioxidant activity due to their free radical scavenging activity [\[4](#page-7-3)]. They are widely studied as natural antioxidants with possible uses as additives in the food industry, or as substitutes for synthetic ones  $[1, 5]$  $[1, 5]$  $[1, 5]$  $[1, 5]$ . The world market for polyphenols is significant. For example, Leatherhead Food Research (2009) estimated that the current market is worth approximately \$200 million. The majority of polyphenols are extracted for sale as nutraceuticals or for use in functional foods [[6\]](#page-7-5).

Traditionally, polyphenol extraction was completed by solvent (solid–liquid) extraction commonly assisted by pressing. Widely used solvents in the extraction procedures were hexane, ether, chloroform, acetonitrile, benzene and ethanol. All of them, excluding ethanol, are toxic for humans and dangerous for the environment. Nowadays, other extraction systems that produce food-grade extracts with water or other Generally Recognized as Safe (GRAS) solvents are used [[7\]](#page-7-6). Some of the most popular extraction methods

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are pressure liquid extraction, supercritical extraction [[8](#page-7-7)], microwave-assisted extraction [\[9](#page-7-8)], ultrasonic-assisted extraction [[10\]](#page-7-9) and one of the latest innovative technology used for this purpose, namely Pulsed Electric Field-assisted extraction (PEF) [\[11](#page-7-10)].

PEF is an emerging technology based on the application of microsecond pulses of high-strength electric fields that induce a non-reversible electroporation in cell membranes [\[12](#page-7-11), [13](#page-7-12)]. Key advantages of PEF-assisted extraction are that is a non-thermal treatment that does not affect the quality of the extracted products and the treatment might be able to be applied in continuous flow  $[14]$  $[14]$ , in pilot plants or even at an industrial scale [[15\]](#page-7-14).

Based on the electroporation process of biological membranes, different applications of PEF for the food industry have been investigated. The area to which most efforts have been initially dedicated is inactivation [[16](#page-7-15)] of microorganisms for enhancing food safety and stability, since it is a technique that doesn't affect the nutritional and sensorial characteristics of foods [[17\]](#page-7-16). Several studies have explored the positive effect of samples pre-treated with PEF in the improvement of industrial extraction processes such as juice production [\[18](#page-7-17), [19](#page-7-18)], olive oil extraction [\[20](#page-7-19)] or in the improvement of the extraction of valuable compound in winery production [\[14](#page-7-13), [21\]](#page-7-20). Other publications are focused on the extraction of phenolic compounds directly form food source [[22](#page-7-21)] but, despite of these studies there are only a reduced number of publications focused on the use of PEF in re-valorization of agro-food waste to extract phenolic compounds [\[23](#page-7-22)–[25\]](#page-7-23).

The main aim of this contribution is to evaluate the potential benefits of the application of a PEF treatment on lemon waste with the purpose of obtaining an extract which is rich in polyphenols using an environment-friendly pressing liquid extraction.

# **Materials and Methods**

#### **Raw Materials**

The lemon waste residues were obtained from fresh lemons (*Citrus limon*) from fresh crops grown in Murcia, Spain purchased at a local supermarket and stored at 4 °C until needed. The citrus pulp was removed from residues and only albedo and flavelo were used. Residues were chopped with a cork borer into pieces of 1, 2 and 3 cm size diameter.

# **PEF Treatment Conditions**

The PEF equipment used (Modulator PG, Scandinova, Uppsala, Sweden) generates square waveform pulses of a width of 3 µs, with a frequency of up to 200 Hz. The maximum output used was 30 kV and the maximum current was 200 A. The equipment operation and constituents were described in detail in [[24\]](#page-7-24). A parallel plate treatment chamber with an electrode gap of 2 cm and a diameter of 5 cm was used. The chamber works at non-pressurized conditions and at room temperature and allows treatment of  $30 \pm 0.5$  g of lemon residues. The real voltage and current intensity applied in the treatment chamber were measured with a 100 MHz digital oscilloscope (TDS 3012, Tektronix, Oregon, USA). For signal conditioning a 75 MHz high voltage (P6015A, Tektronix, Wilsonville, OR) and a 100 MHz current probe (Stangenes Industries, Inc., Palo Alto, California, USA) connected to an amplifier system were used. PEF treatments were tested in a range of  $0-100$  pulses of 3 μs  $(0-300 \text{ }\mu\text{s})$ of total treatment time) and were set also at electric field strengths ranging from 1 to 9 kV/cm. The specific energy of these treatments ranged from 0 to 7.6 kJ/kg. A pulse frequency of 1 Hz was used. All assays were achieved at room temperature conditions.

# **Cell Disintegration Index**

Cell disintegration index (Z*p*) was used to identify the appropriate PEF treatment conditions for the PEF pre-treatment of the lemon peels before the extraction of polyphenols by pressing. This index characterizes the proportion of permeabilized cells based on the frequency dependence of conductivity of intact and permeabilized plant tissue [\[26](#page-7-25)]. The measuring cell was the same treatment chamber used for application of the PEF treatments with the electrodes separated at a distance of 2 cm.  $30 \pm 0.5$  g of chopped lemon peels were introduced into the cell for Z*p* determination. Z*p* analysis was carried out using impedance measurement equipment (DIL, Quakenbrück, Germany) and calculated by the Eq. [1](#page-1-0), previously described by [[24](#page-7-24)].

<span id="page-1-0"></span>
$$
Zp = 1 - \left(\frac{K_h}{K'_h}\right) \times \frac{\left(K'_h - K'_1\right)}{\left(K_h - K_1\right)}; 0 \le Zp \le 1
$$
 (1)

where  $K_1$  and  $K_1'$  are the electrical conductivity of untreated and treated samples respectively, at a low frequency field and  $K<sub>h</sub>$  and  $K<sub>h</sub>$ ' are the electrical conductivities of untreated and treated material, respectively at a high-frequency field.

#### **Polyphenol Pressing Assisted Extraction**

A laboratory press designed and assembling in Zaragoza University laboratory with press chamber volume of 400 mL and 7.5 cm diameter piston activated by compressed air was used. Two pressure treatments were applied: 2.5 and 5 bars. Cycles of 5 min of pressure were applied. After each pressing cycle, samples of 1.5 mL were removed.

#### **Determination of the Total Polyphenols Content (TPC)**

TPC in each extract was quantified by means of the Folin–Ciocalteu method according to [\[27\]](#page-7-26). Samples were incubated for 2 h in darkness. The absorbance at 725 nm was read with a spectrophotometer (Unicam, Cambridge, UK) against blank containing bidistilled water. Results were calculated through a calibration curve and were expressed as mg of Gallic Acid Equivalents (GAE) in 100 g Fresh Weight (FW).

# **Determination of the Main Polyphenol by HPLC**

The main polyphenols contained on lemon residues were characterized by HPLC. Alliance Waters equipment (Barcelona, Spain) with a C18 reverse phase column (Kinetex Phoenix, USA), diode-array detector (Waters, Barcelona, Spain) was used. The method described by [[1\]](#page-7-0) with modifications was applied. The eluents used, in a constant flow rate of 0.5 mL/min, were A: Acidified bidistillated water (0.1% of glacial acetic acid) and B: Acidified acetonitrile (0.1% of glacial acetic acid). Initial conditions were A: 90% and, then, a gradient starting at minute 2 and continuing for 10 min with a decrease to 70% A. Afterwards, a return to the initial conditions continued from minute 12 to minute 17.

#### **Oxygen Radical Absorbance Capacity (ORAC)**

The ORAC method was adapted from [[28\]](#page-7-27). The assay was performed with an automated plate reader of 96-wells at 37  $\degree$ C and the method was reported in detail in [\[5\]](#page-7-4).

#### **Kinetics of Total Phenol Extractions**

The experimental data were fitted to the following equation commonly used to describe solid–liquid extraction of different intracellular compounds [[29\]](#page-7-28).

$$
Y_t = Y_{\text{max}} \left( 1 - e^{-kt} \right) \tag{2}
$$

where  $Y_t$  is the polyphenols extraction efficiency at time t (min),  $Y_{\text{max}}$  is the extraction efficiency at equilibrium  $(t = \infty)$ , and k is the rate constant depending on the extraction parameters  $(\text{min}^{-1})$ . k takes into account the diffusion coefficient of the extracted Phenols, the total surface area, the volume of solvent and the size and geometry of solid particles. Since the total surface area, the volume of solvent and the size and geometry of solid particles are constants, the k value easily enables determination of the influence of each treatment condition on the extraction efficiency.

#### **Statistical Analysis**

All the results reported are the average of at least two measurements found in two independent assays. To determine the  $Y_{\text{max}}$  and k-values from Eq. [2,](#page-2-0) the least-squares criterion by the Solver function of the Excel 5.0 package (Microsoft, Seattle, Washington, USA) and the GraphPad PRISM (GraphPad Software, Inc., San Diego, California, USA) was used. To describe the relationships between  $Y_{\text{max}}$  and k-values versus field strength and pressure, a multiple regression starting from a second-order polynomial model using the software Statgraphics Plus 5.1. (Statistical Graphics Corporation, USA). The response surface representation was determined with the Software Minitab version 16 (Minitab Inc., USA). Figures and graphics were drawn with Microsoft Excel (Microsoft Corporation, USA). A backward regression was used to determine the parameters of the model. The effect of associations which were not significantly associated  $(P>0.05)$  with the response was automatically removed by this procedure.

# **Results and Discussion**

# **Adjustment of PEF Conditions**

Z*p* was used to determine the appropriate PEF conditions to permeabilize the lemon peels. Figure [1](#page-2-1) shows the influence of PEF treatment time and Z*p* at electric field strength of 3, 5, 7 and 9 kV/cm. The increase in electric field strength and treatment time resulted in an increase of the Z*p* values to a highest value of 0.55 for the most intense treatment conditions tested. Independently of the applied electric field strength, the Z*p* values increased significantly with treatment time (30 pulses of 3 µs). Above these values, an increment in the treatment time barely affected the Z*p* value. Results

<span id="page-2-0"></span>

<span id="page-2-1"></span>**Fig. 1** Influence of electric field strength and number of pulses on the cellular disintegration index (Z*p*) of lemon waste material (filled circle) 3 kV/cm, (filled square) 5 kV/cm, (filled triangle) 7 kV/cm (filled diamond) 9 kV/cm

show two general trends of the influence of electric field strength and the treatment time on the Z*p* values observed. On one hand, in samples treated at 5 kV/cm or lower, nearly no cellular damage was caused  $(Zp < 0.2)$ . In samples treated at 7 kV/cm or higher, Z*p* values around 0.5 were observed. No statistically significant differences between treatments of 7 and 9 kV/cm were detected.

Z*p* values observed are significantly higher than values obtained by [[24\]](#page-7-24) in orange peels at equal treatment time and 7 kV/cm, where Z*p* values reached over only 0.3. In contrast, in soft tissues such as potato slices [[30\]](#page-7-29), the values of Z*p* reached 0.57 under treatment with lower electric field strength ( $\leq$ 3 kV/cm) and could increase to 0.75 in very soft tissues such as tomato pulp waste [\[25\]](#page-7-23).

After characterization of the cell damage, it is possible to fix the optimal PEF working conditions at 30 pulses of 3 µs with a maximum electric field of 7 kV/cm. The application of more pulses or a higher electric field does not produce significantly higher Z*p* values.

TPC extracted from lemon peels cut into different sizes of 1, 2 and 3 cm-diameter was evaluated. Samples were pre-treated with PEF for 30 pulses of 7 kV/cm, and then were subjected to a pressurized water solvent extraction (fixed at 5 bar) process for 30 min. Afterwards, TPC was analyzed and compared with samples not treated with PEF (control samples). Results at 45 min of extraction (Fig. [2\)](#page-3-0) showed remarkable differences between control and PEF treated samples with control samples showing an effect of size (1 cm:  $98.63 \pm 5.32$ ; 2 cm:  $75.84 \pm 2.5$ , 3 cm:  $64.56 \pm 10.33$  mg GAE/100 g FW). This effect in control samples can be explained by differences in mass transfer caused by different size material [\[31](#page-7-30), [32](#page-7-31)].

However, no statistically significant differences between samples cut into different sizes in PEF treated samples



<span id="page-3-0"></span>**Fig. 2** Effect of the size of lemon waste material (1, 2 and 3 cm) in Total Polyphenol Content extraction at 150 µs under PEF treatment of 7 kV/cm

were detected (1 cm:  $163.89 \pm 4.38$ ; 2 cm:  $163.56 \pm 3.91$ ; 3 cm:  $161.46 \pm 2.82$  mg GAE/100 g FW). Based on these results, the highest size tested, 3 cm-diameter materials were selected as optimal. This size show the lower values of Gallic Acid in control samples due to allows the detection of differences between treatments with maximum efficacy. In addition, these tests showed that in PEF-assisted extraction the different size materials have equally positive outcomes in polyphenol extraction suggesting that the use of PEF effectiveness is independent of lemon residue size could avoid the use of blending or cutting procedures in industrial processes of residues re-valorization.

# **Total Phenolic Content, Kinetic Analysis**

To evaluate the effect of PEF in the extraction of polyphenols from lemon peels, residues cut at 3 cm-diameter were used, specimens were treated at 0, 2.5 and 5 bars of pressure with a PEF pre-treatment of 30 pulses of 0, 3.5 and 7 kV/cm. TPC was determined every 5 min during all extraction time period (45 min). With the aim to have a global vision of the electric fields effect applied on the extraction procedure, some of the findings obtained are plotted on Fig. [3](#page-4-0). The effect of electric field is illustrate on Fig. [3a](#page-4-0) and the effect of the pressure on Fig. [3](#page-4-0)b. Total phenolic content was improved significantly by increasing the pressing time and electric field strength. For instance, after 45 min of extraction (the highest time applied) the TPC extracted from samples pre-treated with PEF 7 and 3.5 kV/cm (Fig. [3](#page-4-0)a) rose respectively to 292 and to 144% in comparison with control samples.

Furthermore, the extraction curves obtained, Fig. [3b](#page-4-0), show an increment of TPC liberation with increase in the pressure applied. TPC obtained as a result of applying different pressure conditions (2.5 and 5 bar) showed an increase of 156 and 247%, respectively in extraction in comparison with samples extracted without pressing. The same tendency (not represented) was also observed with the other pulse electric field treatments tested.

These results show the increment of total polyphenol extraction when the pressure and energy applied for extraction are higher [[24](#page-7-24)] found comparable results: the TPC increased with the intensity of the electric fields. In the literature report, polyphenols increased linearly and with independence of the pressing time. The positive outcomes obtained by the application of PEF before pressing extraction agree with the results obtained by [[33\]](#page-7-32), where polyphenol extraction from mushrooms increased by nearly 100%.

In order to analyze if the extraction model matches with the exponential extraction model reported in [\[29\]](#page-7-28), (Eq. [2\)](#page-2-0) Ymax and k-values were calculated with their corresponding confidence limit intervals (Table [1](#page-4-1)). In addition, the fitting to the equation was also calculated. In general,  $R^2$  obtained



<span id="page-4-0"></span>**Fig. 3 a** Kinetic analysis of TPC obtained with pressing assisted extraction at 2.5 bars and with different PEF pretreatments: (filled square) no PEF pre-treated sample (control), (filled diamond) 3.5 kV/ cm and (filled triangle) 7 kV/cm. **b** Kinetic analysis of TPC obtained with a pre-treatment of 3.5 kV/cm and different pressure assisted extractions. (filled square) no pressure extraction, (filled diamond) 2.5 bars (filled triangle) 5 bars

show a good adjustment to the equation in all conditions tested. The highest extraction values  $(Y_{max})$  were produced in cases where stronger pulse electric fields were applied. These results agree with those of other works, such as [[34,](#page-7-33) [35](#page-8-0)].

The accurate analysis of  $Y_{\text{max}}$  values obtained with different pressing conditions show that in samples without pressing treatment, there are no significant differences between Ymax values and samples treated with different pulse electric field strength (6.2, 8.5 and 9 in samples treated with 0, 3.5 and 7 kV/cm, respectively). This shows the necessity of using a combined treatment of PEF simultaneously with pressing extraction in order to maximize the effective extraction of bioactive compounds from lemon waste material.

At 2.5 bars of pressure a linear relationship between  $Y_{\text{max}}$ (obtained in the adjustment) and the intensity of the pulse electric field applied was observed. The highest values were obtained at 7 kV/cm. In samples treated at 5 bars of pressure the influence of electric field became non-linear, with 3.5 kV/cm, the extraction process at equilibrium  $(Y_{max})$  produces the maximum value suggesting this treatment as the most effective.

Furthermore, an accurate analysis of the relationship of the electric field and k-values shows that there are no significant differences in any pressure treatments with PEF pretreatments of 3.5 and 0 kV/cm. In control samples (0 bars) and in samples extracted with 2.5 bars, k-values showed significant differences from values obtained by pre-treatment with 7 kV/cm. These results entails that the application of 7 kV/cm significantly increases the extraction velocity.

# **Correlation Between TPC and Real Scavenging Activity**

Oxygen Radical Absorbance Capacity (ORAC) allows the evaluation of the real scavenging activity of the extracts. The ORAC analysis can also be used as a tool to determine if there is any decay in the antioxidant activity of the sample due to the PEF treatment [[36](#page-8-1)] suggested that some possible

<span id="page-4-1"></span>



*CL* confidence limit

changes in molecules can occur when substances are under PEF treatment.

The results obtained suggest that no oxidant effect due to PEF was produced. Samples with higher phenol content rate showed higher antioxidant activity. The antioxidant activity of the samples followed a good correlation (Fig. [4](#page-5-0)) with ORAC (expressed as mg TE/100 g FW) and the TPC [\[37,](#page-8-2) [38](#page-8-3)] found similar results. Furthermore, the statistical analysis conducted showed that there are no statistically significant differences in any of the treatments tested for ORAC and phenol analysis.

In addition, the good correlation between TPC and ORAC is consistent with the hypothesis that most of the antioxidant compounds in the extracts are phenols; thus, the antioxidant effect produced by other compounds such as ascorbic acid or citric acid can be dismissed.

# **HPLC Analysis of the Main Polyphenols Contained in the Extracts**

The previous analysis obtained determined that extracts obtained are a rich source of phenols, between all phenolic compounds; polyphenols have a special industrial interest. The main polyphenols contained in lemons are the flavonones eriocitrin and hesperidin [[39](#page-8-4)]. Both flavonones were analyzed by HPLC in lemon waste extracts obtained from PEF pressing-assisted extraction (Table [2](#page-5-1)) and conditions required to obtain the maximum extraction were studied. The highest eriocitrin and hesperidin contents were found in samples extracted with the highest electric field energy applied, obtaining an increment of nearly 300% giving maximum experimental values of 84 mg of hesperidin in 100 g of FW and 176 mg of eriocitrin in 100 g of FW.

The experimental data obtained were analyzed by a multiple regression ANOVA (Table [3](#page-5-2)), resulting in a second order polynomial equation for each polyphenol analyzed Eq. [3](#page-6-0) for

<span id="page-5-1"></span>**Table 2** Results of eriocitrin (E) and hesperidin (H) obtained by HPLC analysis

Electric field strength (kV/cm)	Pressure applied (kg/cm <sup>2</sup> )	Time	mg E/100 g FW	mg H/100 g FW
$\mathbf{0}$	$\overline{0}$	5	$7.76 \pm 0.35$	$3.42 \pm 0.46$
		25	$9.78 \pm 0.35$	$5.00 \pm 0.57$
		45	$30.39 \pm 2.12$	$15.9 \pm 2.12$
$\overline{0}$	2.5	5	$8.43 \pm 2.57$	$3.74 \pm 1.51$
		25	$10.73 \pm 3.76$	$4.77 \pm 1.35$
		45	$34.37 \pm 3.66$	$17.5 \pm 2.19$
0	5	5	$10.01 \pm 3.44$	$4.47 \pm 1.21$
		25	$16.72 \pm 2.01$	$7.89 \pm 1.99$
		45	$44.2 \pm 1.09$	$27.92 \pm 1.42$
3.5	$\overline{0}$	5	$9.53 \pm 0.61$	$4.68 \pm 0.55$
		25	$10.9 \pm 0.34$	$5.35 \pm 0.15$
		45	$36.29 \pm 5.15$	$20.64 \pm 6.01$
3.5	2.5	5	$13.12 \pm 0.05$	$6.19 \pm 0.67$
		25	$25.94 \pm 3.85$	$12.87 \pm 1.9$
		45	$79.52 \pm 7.33$	$38.16 \pm 6.23$
3.5	5	5	$23.55 \pm 6.41$	$14.14 \pm 3.8$
		25	$59.59 \pm 0.86$	$30.63 \pm 1.03$
		45	$150.06 \pm 10.33$	$72.43 \pm 6.71$
7	$\overline{0}$	5	$12.92 \pm 1.76$	$7.73 \pm 0.57$
		25	$20.17 \pm 2.76$	$11.71 \pm 0.34$
		45	$30.14 \pm 2.01$	$17.04 \pm 4.28$
7	2.5	5	$26.16 \pm 1.66$	$13.42 \pm 1.28$
		25	$49.09 \pm 10.4$	$22.47 \pm 3.79$
		45	$176.35 \pm 15.39$	$84.44 \pm 8.35$
7	5	5	$59.13 \pm 7.98$	$36.85 \pm 6.39$
		25	$90.69 \pm 0.75$	$52.57 \pm 5.38$
		45	$167.84 \pm 3.52$	$91.87 \pm 3.82$

Results are expressed as mean value  $\pm$  standard deviation



<span id="page-5-0"></span>**Fig. 4** Correlation between index of total phenol content and ORAC analysis from all the extracts obtained

<span id="page-5-2"></span>**Table 3** F- and p-values of the ANOVA analysis for models developed (Eqs. [2](#page-2-0) and [3\)](#page-6-0) to describe the influence of extraction time (t), extracting pressure (P) and electric field (E) on polyphenol extraction (mg GAE/100 g FW) from lemon waste peels

	Eirocitrin		Hesperidin	
	F value	p value	F value	p value
Model	23.71	< 0.0001	17.42	< 0.0001
Time(t)	56.26	< 0.0001	24.97	< 0.0001
Electric field (E)	35.58	< 0.0001	26.53	< 0.0001
Extracting pressure (P)	34.36	< 0.0001	35.91	< 0.0001
$t^2$	6.2	0.0222	4.5	0.0472
$t \times E$	9.43	0.0063	11.35	0.0032
$t \times P$	10.37	0.0045	7.6	0.0126
$E \times P$	13.5	0.0016	11.1	0.0035

 $p < 0.05$  is significant

Hesperidin and Eq. [4](#page-6-1) for eriocitrin after removing statistically insignificant terms  $(P>0.05)$ .

$$
Y = 21.87 - 1.47t - 2.93E - 2.32P + 0.023t2
$$
  
+ 0.14804txE + 0.17txP + 1.17ExP (3)

$$
Y = 31.09 - 2.35t - 3.96E - 6.08P + 0.046t2
$$
  
+ 0.23txE + 0.338TxP + 2.20ExP (4)

where t is the extraction time, E is the electric field strength and P is the pressure applied.

In eriocitrin analysis, a coefficient of determination  $(R^2)$ of 0.89 was obtained. Model significance was assessed by  $R^2$ , the lack of fit (p > 0.05) and the F value (17.42) which indicate that the model was significant. Therefore, it can be used to predict the response. In hesperidin analysis,  $R^2$  obtained was 0.865 and the F value 23.73, which implied that the model is significant. There is only 0.01% chance that a Model F Value this large could occur due to noise. All values for p value were less than  $0.005$ ,  $p < 0.05$ . These results show that the terms of the model are significant. According to the F values for the model, pressing time and electric field strength were the most significant parameters.

In Fig. [5](#page-6-2) the response surface obtained in the extraction of eriocitrin and hesperidin at the maximum pressing extraction time (45 min) is graphically represented. The extraction is shown with respect to the electric field and pressure applied. Results showed an increasing concentration of both polyphenolic compounds in relation to the pressure and electric field. These results show a different behavior from that of total phenol extraction (Fig. [3\)](#page-4-0). The total polyphenol extraction rate at 5 bars of pressure did not increase when the electric strength increased from 5 to 7 kV/cm. In addition, with the maximum electric field applied, 7 kV/cm, TPC had similar values at 2.5 and 5 bars of pressure. However, in the case of the concentration of these two target compounds, the concentration extracted increased with the electric field and pressure. These results suggest that the low increment in concentration of these two compounds did not have a significant impact on the increment of the antioxidant capacity of the extracts.

# **Conclusions**

From the conducted experiments it can be concluded that according with cellular disintegration index (Zp), and different extractions assessed with residues chopped at different size, the adequate PEF treatment time is defined in 90 µs with independence of residue size. Results achieved with different PEF and pressing

<span id="page-6-1"></span><span id="page-6-0"></span>

<span id="page-6-2"></span>**Fig. 5** Response surface obtained from the analysis of eriocitrin (**a**) and hesperidin (**b**)

treatments (3.5–7 kV/cm and 0–5 bars) determine that the TPC improves with a combined treatment of pressing and PEF at 7 kV/cm. The extraction kinetic analysis concludes that despite of maximum k-values and Ymax values are obtained at 7 kV/cm, the most efficient extraction is determined at 5 bars and 3.5 kV/cm. In addition it is demonstrated that in all extracts obtained there are a high correlation between TPC and ORAC. The analysis of the main polyphenols contained in lemon residues, hesperidin and eriocitrin reveals a an increasing concentration of both polyphenolic compounds in relation to the pressure and electric field obtaining an increment of nearly 300% giving maximum experimental values of 84 mg of hesperidin in 100 g of FW and 176 mg of eriocitrin in 100 g of FW.

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