EFFECTS OF PULSED ELECTRIC FIELD ON YIELD EXTRACTION AND QUALITY OF OLIVE OIL

Running head: PEF on yield and quality of olive oil

M. Abenoza
M. Benito
G. Saldaña
I. Álvarez
J. Raso
A.C. Sánchez- Gimeno*

Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, C/ Miguel Servet 177, CP 50013, Zaragoza, Spain

* Dr. Ana Cristina Sánchez- Gimeno. Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, C/ Miguel Servet 177, CP 50013, Zaragoza, Spain.
TEL.: 0034 976 761000 ext 4149
FAX: 0034 976 76 15 90
E-mail: anacris@unizar.es

Source of support: Department of Science, Technology and University of the Aragon Government (Grants to M. Abenoza and M. Benito)

Abstract

The effect on oil yield extraction and quality parameters of the application of pulsed electric field treatments of different intensities (0-2 kV/cm) to Arbequina olive paste at different malaxation times (0, 15 and 30 min) and temperatures (15°C and 26°C) has been investigated.

The extraction yield improved by 54% when the olive paste was treated with PEF (2 kV/cm) without malaxation. When the olive paste was malaxated for 30 minutes at 26ºC, the application of a PEF treatment scarcely increased the extraction yield as compared with the control. However, at 15ºC, a PEF treatment of 2 kV/cm improved the extraction yield by 14.1%, which corresponded with an enhancement of 1.7 kg of oil per 100 kg of olive fruits.

Parameters legally established to measure the level of quality of the virgin olive oil were not affected by the PEF treatments. A sensory analysis revealed that the application of a PEF treatment did not generate any bad flavor or taste in the oil.

Keywords: Pulsed electric fields; Olive oil; Yield extraction; Malaxation

1. Introduction

Pulsed electric fields is a treatment that involves the application of direct current high voltage pulses for very short periods of time, in the range between microseconds to milliseconds, through a material placed between two electrodes. This technology has been proven as an effective method for irreversible permeabilization of cell membranes in plant and animal tissues without increasing temperature or requiring high cost operation (Toepfl et al., 2006). Applying PEF to enhance the extraction yield of juices from fruits and vegetables, reducing the drying times or improving the extraction of intracellular valuable compounds such as colorants, sucrose or polyphenols have all been investigated in studies conducted in laboratories and in pilot scale tests (Donsi et al., 2010; Knorr et al., 2011; Vorebiev & Lebovka, 2008).

Olive oil is a high-value, edible oil due to its appreciable flavor characteristics and health properties. The high nutrition value of olive oils mainly arises from its high oleic acid content and its high levels of natural antioxidants (phenols and tocopherol) (Visioli & Galli, 1998).

Virgin olive oil is extracted from the fruit of *Olea europaea L*. by means of mechanical or physical procedures (Uceda et al., 2006). In the olive fruit, the oil lies in the cells of the pulp, that is, the mesocarp of the fruit (Ranalli et al., 2001). The oil within the cells is partly located in the vacuole (approximately 76%), where it is free, and the other portion lies within the cytoplasm (approximately 24%), where it is dispersed in the form of minute droplets bound to colloids. The extraction of virgin olive oil begins by crushing the olive fruits with the purpose of breaking down the cell envelopes of the mesocarp cells and releasing the oil. Then, the olive paste that is obtained by crushing has to be malaxed to facilitate the small oil drops to group together

into larger droplets. These droplets can then be separated easily from the paste through centrifugation, which is currently the most common system used.

A fundamental phase of the extraction process for olive oil is the malaxation of the olive paste, because malaxation improves the successive separation steps and increases the yield of the oil extraction. Furthermore, time and temperature of malaxation have a very important influence in the oil yield and the chemical and sensory characteristics of the final product (Kalua et al., 2006; Boselli et al., 2009). It has been reported that that oil yield improves when extending the malaxation time and increasing the temperature (Angerosa et al., 2001; Ranalli et al., 2001; Aguilera et al., 2010). However, the rate and extension of chemical and enzymatic reactions, which can markedly affect the quality of the oil, also increase with time and temperature during malaxation (Morales & Aparicio, 1999). Thus, a balance between oil yield and quality must be achieved (Servilli et al., 2003).

A few previous studies have investigated using a PEF pre-treatment to improve the extraction of different vegetable oils such as maize, soybeans or rapeseeds (Guderjan et al., 2005; Guderjan et al., 2007). However, only one experiment has been reported on the application of PEF to improve olive oil yield. In this study, oil was extracted after the PEF treatment through centrifugation without the malaxation of the olive paste **(**Guderjan et al., 2005).

This study has evaluated the potential beneficial effects of the applications of PEF on improving the actual extraction process of olive oil. It has accomplished this through investigating the influence of the application of PEF of different intensities (0-2 kV/cm) to the olive paste on the oil yield extraction and quality parameters at different malaxation times (0, 15 and 30 min) and two malaxation temperatures (15ºC and 26ºC).

2. Material and Methods

2.1 Olive fruits

The study was conducted with olive fruits of the Arbequina variety from intensive orchards located in Zaragoza (Aragón, Spain). The orchard had an irrigation system and a frame of 7 x 3.5 m, reaching a density of 200-300 trees/ha. Olive fruits were harvested in the first days of November and immediately transported to the laboratory for olive oil extraction. Maturation index assessed on 100 olive samples following the procedure of Hermoso et al., (1991) was 3.82.

2.2 Oil extraction system

Oil from the olives was obtained using the Abencor laboratory scale equipment (MC2 System, Sevilla, Spain) according to the method described by Martínez et al., (1975). The equipment consists of three units: a hammer mill, a thermo-malaxer and a centrifuge. After grinding the olive fruits with the mill, 650 g of the olive paste was placed into a stainless-steel mixing container for malaxation. The malaxation was conducted at 15±0.2ºC and 26±0.2ºC for 0, 15 or 30 min. When the effect of PEF was investigated, the olive paste was treated before malaxation. After malaxation, the olive paste was centrifuged at 3000 rpm for 2 min, and then the oil was collected. The oil was filtered for chemical analysis.

The oil extraction yield was calculated as the percentage of olive oil extracted from the olive paste, expressed in terms of weight on a fresh matter.

114
115 *2.3 PEF equipment*

116 The PEF equipment used in this investigation (Modulator PG, ScandiNova, Uppsala,
117 Sweden) generates square waveform pulses of a width of 3 µs with a frequency of up to
118 300 Hz. The maximum output voltage and current were 30 kV and 200 A, respectively.

119 The actual voltage and the current intensity applied were measured with a high
120 voltage probe (Tektronix, P6015A, Wilsonville, Oregon, USA) and a current probe,
121 respectively (Stangenes Industries Inc. Palo Alto, California, USA). These probes were
122 connected to an oscilloscope (Tektronix, TDS 220, Wilsonville, Oregon, USA).

123 A colinear treatment chamber was used in this investigation. The colinear design
124 defines two treatment zones of 2 cm between the electrodes with an inner diameter of 2
125 cm. Using this design, the applied electric field strength in the treatment zones was not
126 uniform. In order to know its distribution, the electric field strength was numerically
127 simulated via the finite elements method by using the Comsol Metaphysics software
128 (Comsol Inc., Stockholm, Sweden). To standardize the results, the electric field strength
129 used to characterize the PEF treatments corresponded to the electric field strength in the
130 mid-position of the central axis of the treatment zone (Toepfl et al., 2007).

131 A progressive cavity pump (Rotor-MT, Bominox, Gerona, Spain) was used to pump
132 the olive paste into the treatment chamber. The mass flow rate was 120 kg/h. This flow
133 corresponds with a medium residence time in the treatment zone of 0.41s.

134 *2.4 PEF treatments*

135 After milling, the olive paste was PEF treated. The PEF treatment consisted of fifty
136 pulses at electric field strength of 1 kV/cm (1.47 kJ/kg) and 2 kV/cm (5.22 kJ/kg) and
137 frequency of 125 Hz. Preliminary experiments showed that longer treatments or more
138 intense electric field strengths did not increase the oil extraction yield (Sánchez-Gimeno
139 et al., 2010). The temperature was measured both on the entry and on the ending of the

treatment chamber. The initial temperature of the mass was around 20 ºC. In all experiments, the increment of the temperature due to the treatment never exceeded 2 ºC.

2.5 Olive oil analysis

2.5.1 Physicochemical parameters

An analysis of free acidity, peroxide value and UV absorption characteristics at 232 and 270 nm (K_{232} and K_{270} respectively) were carried out following the analytical methods described in Regulation EEC/2568/91 of the European Union Commission.

Oxidation stability was evaluated with the Rancimat apparatus (Mod. 743, Metrohm, Switzerland) using an oil sample of 3 g warmed to 120 °C and an air flow of 20l/h. Stability was expressed as the oxidation induction time in hours.

Determination of carotenoids (mg lutein per Kg of oil) and chlorophylls (mg pheophitin per Kg of oil) were evaluated by measuring directly the adsorption at 470 nm and 670 nm respectively, according to the method of Mínguez-Mosquera et al., (1991).

The CIELAB color coordinates of the oils were determined from the spectra in the range of 380 to 780 nm. Illuminant 65 and CIE64 were chosen. The oil color was measured without dilution in a 1 cm transmission optical cell made of clear optical glass, using hexane as reference.

Bitterness index *(K_{225})* was determined by solid phase extraction with octadecyl (C_{18}) packing of bitter compounds (Gutiérrez-Rosales et al., 1992). Oil dissolved in n-hexane, was added to the SPE cartridge, and the bitter compounds were eluted with methanol: water (1:1). Then, absorbance at 225 nm was measured.

2.5.2 Nutritional parameters

The total phenols content were measured with a modification of the method described by Favati et al., (1994). The phenols were extracted with SPE by using Isolute

C18 columns. The extract was dried in a rotary evaporator and the residue was dissolved in 5 ml methanol. For the colorimetric determination of total phenols, 2.5 ml of extract was mixed with 1.25 ml of Folin-Ciocalteau reagent, and after 3 min, 2.5 ml of sodium carbonate was added. The absorption of the solution was measured at 725 nm. Results were expressed as mg of gallic acid per Kg of oil.

Concentration of individual phenols was measured with the HPLC, HP 1100 series (Hewlett Packard, Palo Alto, CA), which was equipped with a Zorbac SB-C_{18} (3.5 µm, 150 mm x 4.6 mm i.d., Agilent Technologies) column. Phenolic compounds were extracted from the olive oil according to the method described by Gutfinger, (1981). HPLC analysis was performed following the procedure described by Montedoro et al., (1992). The eluents were a 0.2% aqueous acetic acid (pH 3.1) and methanol, the flow rate was 1.5 ml/min and the inject volume was 20 µl. The total run time was 60 min, the initial composition was 95% aqueous acetic acid and 5% methanol. The gradient changed as follows: the concentration of methanol was maintained for 2 min; then, it was increased to 25% at 8 min, and finally, the methanol percentage was increased to 40, 50 and 100% in subsequent 10 min intervals. Initial conditions were reached in 15 min. Retention times were compared with the standards: Tyrosol, Hydrotyrosol and Oleuropein, which were purchased from Extrasynthese (Geney, France); vanillic acid, vanillin, and p-cumaric acid, which were purchased from Sigma-Aldrich (Steinheim, Germany); and Luteolin an apigenin, which were purchased from Alfa-aesar (Ward Hill, USA). Individual phenols were quantified at 280 nm; luteolin and apigenin were identified and quantified at 339 nm. Then, the study calculated 4-(acetoxyethyl)-1,2-dihydroxybenzene (3,4-DHPEA-AC), a dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), a dialdehydic form of elenolic acid linked to tyrosol (p-HPEA-EDA), lignans and oleuropein aglycone (3,4-DHPEA-EA) at 280 nm

using oleuropein as the standard. The results were expressed as mg per Kg of oil, except in the case of 3,4-DHPEA-AC, 3,4-DHPEA-EDA, p-HPEA-EDA, lignans and 3,4-DHPEA-EA, which were expressed as mg oleuropein equivalents per Kg of oil.

The concentration of α-tocopherol was measured in a solution of hexane (1 g oil/10 mL hexane) by HPLC using a reverse phase column Zorbax SB-C_{18} (particle size 3.5 µm, 150 mm x 4.6 mm i.d.; Agilent Technologies) and a photodiode array detector (DAD) (G1315 B, Serie 1100). The injection volume was 20 µl and the elution was conducted with acetonitrile: water (99:1) at a flow rate of 1 ml/min. The chromatograms were registered at 295 nm. The results were expressed as mg of α-tocopherol per Kg of oil.

The fatty acid methyl esters (FAMEs) were prepared as described by Frega & Bocci, (2001). FAMEs were prepared after saponification by vigorous shaking of a solution of oil in hexane (2 drops olive oil in 2 mL) with 6 drops of 2N methanolic potassium hydroxide and a spatula tip of sodium sulfate anhydrous. Then, the FAMEs were analyzed with a gas chromatograph (Hewlett-Packard 5890 GC) equipped with an injector split/spiltless and a flame ionization detector (FID). A column DB-225 (30 m length x 0.25 mm i.d.), a 0.15-µm particle size (J&W Scientific, Agilent) and an injection volume of 0.4 µl were used. The injector and detector temperatures were maintained at 250 ºC. The oven temperature was programmed to rise from 190 ºC (1 min) to 210 ºC at a rate of 4 ºC/min and maintained for 5 min, then heated to 215 ºC at 3 ºC/min and finally, it was maintained as an isotherm for 18 minutes; the carrier gas was nitrogen. Fatty acids were identified by comparing retention times with those of standard compounds. The relative composition of the fatty acids in the oils was determined as percentage of total fatty acids.

2.5.3 Sensory analysis

Sensory analysis was performed by the panel test procedure according to EU Regulations EEC/2568/91 and EEC/640/2008. Oil samples were evaluated by 10 trained and selected panelists of the certification of origin Bajo Aragon. Panelists evaluated samples by ascribing them positive (fruity, bitter and pungent) and negative (fusty, winey/vinegary, musty, muddy, rancid, metallic and other) attributes.

2.6 Statistical analysis

A response surface methodology was used to study the possible advantages of PEF application to improve oil extraction yield. A central composite design was constructed to investigate the influence of the electric field strength (from 0 to 2 kV/cm) and malaxation time (from 0 to 30 min) at the two malaxation temperatures investigated (15 and 26 ºC). A backward regression procedure was used to determine the parameters of the models. This procedure systematically removes the effects that were not significantly associated ($P > 0.05$) with the response until a model with only a significant effect was obtained.

The central composite design, the surface response function and the corresponding analysis of the data were carried out using the software package Design-Expert 6.0.6 (Stat-Ease Inc., Minneapolis, MN, USA).

3. Results and discussion

3.1 Effect of the application of PEF treatments on oil yield and quality indices

The oil extraction yield resulting from the experimental conditions investigated for the two malaxation temperatures are shown in Table 1. The highest malaxation temperature used in this study was 26 ºC, because the indication “cold extraction” can only be used for olive oil obtained at temperatures below 27 ºC, according to the EC (European Commission regulation Nº 1019/2002). The extraction yield ranged from less

than 5% when the oil was extracted without malaxation to 13.6-14.1% when the olive paste treated at 1 or 2 kV/cm was malaxed for 30 min before centrifugation. Oil extraction yield depends not only on the operation conditions used for obtaining the oil but also on the characteristics of the olive fruit, such as variety and maturity. Values of oil extraction yield obtained in this study were lower than others reported in the literature for the same variety. However, those studies generally used higher temperatures and longer malaxation times (Torres & Maestri, 2006; Cruz et al., 2007; Espínola et al., 2009).

The application of a PEF treatment to the olive paste resulted generally in an improvement in the extraction yield as compared with the control. This enhancement was higher when the oil was extracted from the paste without malaxation. Under these conditions, the extraction yield improved by 54% when the olive paste was treated with PEF (2 kV/cm). However, the extraction yield obtained was around the 50% of the values obtained when the paste was malaxed for 30 minutes. When malaxation was performed for 30 minutes at 26ºC, the application of a PEF treatment scarcely increased the extraction yield as compared with the control. However, at 15ºC, the permeabilization of the olive cells by a PEF treatment of 2 kV/cm improved the extraction yield by 14.1%, which corresponded with and enhancement of 1.7 kg of oil per 100 kg of olive fruits. As it is estimated that no more that 80-90% of the oil contained in the fruit is extracted using the current industrial systems for olive processing, so different strategies has been proposed to improve the extraction of olive oil (Chiaccherini et al., 2007). Improvements obtained by these strategies are in the order of those obtained in this study when the oil was extracted at 15ºC. For example, a yield increase in the range of 1.02-1.35 kg of oil per 100 kg of olive fruits was reported using natural enzymatic complexes as coadjuvants (Ranalli et al., 2003b). The use of

NaCl, or calcium carbonate has been proposed more recently as physical-acting coadjuvants alternative to talc. Talc is the only coadjuvant allowed by European regulations due to its exclusively physical action. A maximum increment of 2.64 kg of oil per 100 kg of olive fruit was reported by Cruz et al., (2007) when they used NaCl, and Espínola et al., (2009) reported 2.56 kg of oil per 100 kg olive fruits of the Arbequina variety when they used calcium carbonate as a coadjuvant.

Extraction yield obtained when the olive paste treated by PEF (2 kV/cm) was subsequently malaxated at 15 ºC for 30 min was similar to the highest extraction yield obtained when the paste was malaxated at the more intense extraction conditions investigated (30 minutes at 26 ºC). Therefore, the application of a PEF treatment could permit reductions in the malaxation temperature from 26 to 15 ºC without impairing the extraction yield. This reduction is advantageous, for decreasing malaxation temperature in order to preserve oil quality has been recommended (Ranalli et al., 2001). It has been reported that malaxation temperature has a significant influence on oil quality, particularly on the organoleptic quality of the olive oil (Kalua et al., 2006). Furthermore, the increment of the temperature of the paste during malaxation is one of the main energy costs of olive oil extraction. Therefore, reducing this temperature could present energy savings for the olive oil extraction industry.

To demonstrate whether the application of a PEF treatment to the oil paste affected oil quality, values of the analytical parameters established by EEC Nº 2568/1991 are also shown in Table 1. Values of these analytical parameters for both the control oil and the oil obtained from olive paste treated by PEF were similar, and they did not exceed the established limits for “extra virgin olive oil.” Therefore, the PEF treatments did not exceed the legally established parameters for measuring the level of the quality of the virgin olive oil.

3.2 Response surface modeling of oil extraction yield as a function of process parameters

Response surface methodology enables to evaluate the effect of several factors and their interactions on response variables. This technique has been successfully used recently for studying the influence on the extraction yield of several processing parameters used for obtaining olive oil (Aliakbarian et al., 2008; Espínola et al., 2009; Meziane et al., 2009; Najafian et al., 2009).

The application of a multiple regression analysis to the experimental data corresponding to the oil extraction yield (Table 2) resulted in the following second order polynomial equations for malaxation temperatures of 15 (equation 1) and 26ºC (equation 2) after removing the statistically insignificant terms (P>0.05):

$$Y= 5.10 + 0.87E + 0.50t - 0.008t^2 \text{ (Equation 1)}$$

$$Y= 4.83 + 1.14E + 0.55t – 0.008t^2 – 0.040\ Et \text{ (Equation 2)}$$

Where Y represents the oil extraction yield (g oil per 100 g oil paste), E represents the electric field strength (kV/cm) and t represents the malaxation time (min).

Table 2 shows the results of the analysis of variance for the significant terms of the model. The determination coefficient (R^2) for each model was higher than 0.98, which means that less than 2% of the total response variation remained unexplained by the models obtained. The adjusted-R^2 values that corrected the R^2 according to the number of responses and terms in the model were very similar to R^2 for both equations. The model *F*-values were 163.2 and 199. for malaxation temperatures of 15 ºC and 26 ºC respectively, indicating that both models were significant (P<0.0001).

The *F*-values for the model's parameters are very useful to indicate the significance of the effects of the variables and their interactions. For both malaxation temperatures, the most significant effect on oil yield extraction was the malaxation time. This means

315 that the changes in this factor have the most significant influence on the oil yield. The
316 square of malaxation time was also a significant term for both temperatures
317 investigated. The presence of these square terms in the equation means that when the
318 malaxation time changes, their effect on oil yield extraction was non-linear. From a
319 practical point of view, it could indicate an optimum value for malaxation time; above
320 this value, the increment of the treatment time will not substantially increase the
321 extraction yield. The linear term of the electric field strength was a significant term for
322 both malaxation temperatures. Finally, in the model obtained when the malaxation
323 temperature was 26 ºC, the interaction of malaxation time and electric field strength was
324 also significant but with the lowest *F*-value.

325 To illustrate the influence of the malaxation time and electric field strength on
326 extraction yield at 15 and 26ºC, response surface plots were obtained using the
327 corresponding regression models for each temperature (Eq. 1 and 2). As Figures 1 A
328 and B show, the extraction yield was more influenced by the malaxation time than the
329 electric field strength, but the increment of the extraction yield by increasing the
330 malaxation time tended to plateau at longer times. On the other hand, in the range of the
331 experimental conditions investigated, the oil extraction yield increased linearly when
332 the electric field strength increased. However, the extraction yield was more influenced
333 by the application of a PEF treatment at 15ºC. At this temperature, a positive effect on
334 the extraction yield was observed at any malaxation time. However, at a malaxation
335 temperature of 26ºC, the influence of the electric field strength on the extraction yield
336 tended to disappear as the malaxation time increased. As it is shown in Figure 1B, after
337 30 min of malaxation, no differences in extraction yield were observed between the
338 control and the sample treated by PEF at 2 kV/cm. Therefore, while malaxation
339 temperatures of 26ºC with the application of a PEF treatment could be an effective

approach to increase the yield extraction when malaxation times are lower than 30 minutes, at 15ºC, the application of a PEF treatment would increase the oil extraction yield at any extraction time.

3.3 Effect of PEF treatment on the physicochemical, nutritional and sensory properties of olive oil

In order to evaluate the effect of the application of PEF treatments to the olive paste on the physicochemical, nutritional and sensory properties of the olive oil, 10 kg of olive paste was processed at 2 kV/cm, and them oil was obtained by centrifugation after malaxation at 15ºC for 30 minutes. Table 3 compares the results of the analysis for the sample treated by PEF with an untreated sample (malaxated at 26ºC for 30 min).

The values for the analytical parameters (acidity, peroxide value, K_{232} and K_{270}) did not exceed the limits for "extra virgin olive oil" established by EEC/2568/1991.

It was observed that the concentration of main pigments in virgin olive oils (chlorophylls and carotenoids) was somewhat higher for the control. This higher pigment concentration could explain because the luminosity value (L*) of the control oil was lower than those obtained from olive paste treated by PEF. According to several authors, luminosity values (L*) usually increase with the reduction in the pigment content of the oils, because pigments capture part of the light instead of transmitting it (Tovar de Dios 2001; Criado et al., 2007; Criado et al., 2008).

The most important differences observed between the control oil and the oil obtained from an olive paste treated by PEF was the amount of phenolic compounds recovered from the oils. The concentration of phenolic compounds that are related to the oxidative stability of olive oils was 24% higher for the control than for the PEF sample. It was observed that the concentration of all individual phenols analyzed was higher for the control oil being the greater differences for 3,4 DHPEA-AC and 3,4 DHPEA-EDA, that

365 they are the predominant phenols in olive oil. It has been reported that the application of
366 PEF improves the extraction of polyphenols from grape skins along the fermentation-
367 maceration step of winemaking (Puértolas et al., 2010). The lack of effect of PEF
368 treatment on the improvement of polyphenol extraction from olive fruits could be due to
369 the low malaxation temperature used for the paste treated by PEF. A large increase in
370 total phenolic content of oil was observed by Stefanoudaki et al., (2011) by increasing
371 the malaxation temperature between 15 and 42ºC. Therefore, higher malaxation
372 temperatures (26ºC) seem to be more effective in terms of polyphenol extraction than
373 the application of a PEF treatment combined with a decrease of the malaxation
374 temperatures (15ºC).

375 Although the phenolic content was higher for the control oil, the α-tocopherol
376 content was slightly higher for the sample treated by PEF. Because both phenol and α-
377 tocopherol content contribute to the oxidative stability of olive oil, resistance to the
378 oxidation was slightly lower in the sample treated by PEF.

379 In relation to the effect of PEF on fatty acid composition, the analysis performed
380 showed no significant differences in the content of saturated, unsaturated and
381 polyunsaturated fatty acids. Similar results were obtained for the oleic acid, which is
382 noteworthy, because olive oil is greatly appreciated from a nutritional point of view.

383 Results of the sensory analysis of PEF revealed that the application of a PEF
384 treatment to the olive paste before malaxation did not generate any bad flavor or taste in
385 the oil. No sensorial defects were detected by a panel test in the PEF-treated olive oil,
386 either. Results of the descriptive sensory analysis indicated that the oil obtained from
387 PEF treated olive paste was more fruity, less bitter and less pungent than the control
388 oil. The higher fruity flavor could be due to the lower malaxation temperature used
389 when the paste was treated by PEF, and the lower bitter and pungent flavors could be

explained by the lower concentration of phenols. The sensory attribute of bitter and the measure of bitterness (K_{225}) were correlated also, obtaining values higher for the control oil.

4. Conclusions

Results obtained in this study show that PEF could be an appropriate technology for the production of virgin olive oil. The application of a PEF treatment to the olive paste in continuous conditions after milling led to an increase in the oil extraction yield, depending on the treatment conditions used for obtaining the oil. According to our results, PEF could permit the reduction in the current malaxation temperature used in the olive oil extraction industry. The decrease of malaxation temperature without impairing the oil extraction yield may be advantageous in increasing olive oil quality and saving energy.

The low energy requirements and the short processing times required for PEF process and the fact that the treatment did not deviate from the regulated parameters established to evaluate the quality of olive oil are key advantages to implementing this technology in the olive oil extraction industry.

References

- Aguilera, M.P., Beltrán, G., Sánchez-Villasclaras, S., Uceda, M., & Jimenez, A. (2010). Kneading olive paste from unripe “Picual” fruits: I. Effect on oil process yield. Journal of Food Engineering, 97, 533-538.
- Aliakbarian, B., Faveri, D., Converti, A., & Perego, P. (2008). Optimisation of olive oil extraction by means of enzyme processing aids using response surface methodology. Biochemical Engineering Journal, 42, 34-40.

- Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2001). Influence of malaxation temperature and time on the quality of virgin olive oils. Food Chemistry, 72, 19-28.
- Boselli, E., Di Lecce, G., Strabbioli, R., Pieralisi, G., & Frega, N. G. (2009). Are virgin olive oils obtained below 27°C better than those produced at higher temperatures? LWT-Food Science and Technology, 42, 748-757.
- Chiacchierini, E., Mele, G., Restuccia, D., & Vinci, G. (2007). Impact evaluation of innovative and sustainable extraction technologies on olive oil quality. Trends in Food Science & Technology, 18, 299-305.
- Criado, M.N., Motilva, M.J., Goñi, M., & Romero, M.P. (2007). Comparative study of the effect of the maduration process of the olive fruit on the chlorophyll and carotenoid fractions of drupes and virgin oils from Arbequina and Farga cultivars. Food Chemistry, 100, 748-755.
- Criado, M.N., Romero, M.P., Casanovas, M., & Motilva, M.J. (2008). Pigment profile and colour of monovarietal virgin olive oils from Arbequina cultivar obtained during two consecutive crop seasons. Food Chemistry, 110, 873-880.
- Cruz, S., Yousfi, K., Pérez, A.G., Mariscal, C., & García, J.M. (2007). Salt improves physical extraction of olive oil. European Food Research and Technology, 225, 359-365.
- Donsì, F., Ferrari, G., & Pataro, G. (2010). Application of pulsed electric fields treatments for the enhancement of mass transfer from vegetable tissue. *Food* Engineering Reviews, 2, 109-130.
- Espínola, F., Moya, M., Fernández, D., & Castro, E. (2009). Improved extraction of virgin olive oil using calcium carbonate as coadjuvant extractant. Journal of Food Engineering, 92, 112-118.

- European Comission Regulation (EEC) Nº 2568/1991 of 1 july of 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Official Journal of European communities. L248/ 1-114.
- European Comission Regulation (EEC) Nº 1019/2002 of 13 june of 2002 on marketing standards for olive oil.
- European Comission Regulation (EEC) Nº 640/2008 of 4 july of 2008 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis.
- Favati, F., Caporale, G., & Bertuccioli, M. (1994). Radip determination of phenol content in extra virgin olive oil. Grasas y Aceites, 45(1-2), 68-70.
- Frega, N., & Bocci, F. (2001). L´analisi rápida dell´olio di oliva (3) (28). Laboratorio 2000, Italy.
- Guderjan, M., Töpfl, S., Angersbach, A., & Knorr, D. (2005). Impact of pulsed electric field treatment on the recovery and quality of plant oils. Journal of Food Engineering, 67, 281-287.
- Guderjan, M., Elez-Martínez, P., & Knorr, D. (2007). Application of pulsed electric fields at oil yield and content of functional food ingredients at the production of rapeseed oil. Innovative Food Science and Emerging Technologies, 8, 55-62.
- Gutfinger, T. (1981). Phenols in olive oils. Journal of the American Oil Chemist´s Society, 58, 966-968.
- Gutiérrez-Rosales, F., Perdiguero, S., Gutiérrez, R., & Ollas, J.M. (1992). Evaluation of the bitter taste in virgin olive oil. Journal of the American Oil Chemist´ Society, 69(4), 394-395.

- Hermoso, M., Uceda, M., García, A., Morales, B., Frías, M.L., & Fernández, A. (1991). Elaboración del aceite de oliva de calidad. Consejería de Agricultura y Pesca, Serie Apuntes 5/92. Sevilla, España.
- Kalua, C.M., Bedgood, D.R., Bishop, A.G., & Prenzler, P.D. (2006). Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. Journal of Agricultural and Food Chemistry, 54(20), 7641-7651.
- Knorr, D., Froehling, A., Jaeger, H., Reineke, K., Schlueter, O., & Schoessler, K. (2011). Emerging technologies in food processing. Annual Review of Food Science and Technology, 2, 203-235.
- Martínez, J.M., Muñoz, E., Alba, J., & Lanzón, A. (1975). Informe sobre la utilización del analizador de rendimientos "Abencor". Grasas y aceites, 26(6), 379-385.
- Meziane, S., Kadi, H., Daoud, K., & Hannane, F. (2009). Application of experimental design method to the oil extraction from olive cake. Journal of Food Processing and Preservation, 33(2), 176-185.
- Mínguez-Mosquera, M.I., Rejano-Navarro, L., Gandul-Rojas, B., Sánchez-Gómez, A.H., & Garrido-Fernández, J. (1991). Color pigment correlation in virgin olive oil. Journal of the American Oil Chemist´Society, 68(5), 332-336.
- Montedoro, G., Servilli, M., Baldioli, M., & Miniati, E. (1992). Simple and hydrosable phenolic compounds in virgin olive oil. 1. Their extraction, separation and quantitative and semiquantitative evaluation by HPLC. Journal of Agricultural and Food Chemistry, 40, 1571-1576.
- Morales, M.T., & Aparicio, R. (1999). Effect of extraction conditions on sensory quality of virgin olive oil. Journal of the American Oil Chemist´ Society, 76, 295-300.

- Najafian, L., Ghodsvali, A., Haddad Khodaparast, M.H., & Diosady, L.L. (2009). Aqueous extraction of virgin olive oil using industrial enzymes. Food Research International, 42, 171-175.
- Puértolas, E. López, N., Condón, S., Álvarez, I., & Raso, J. (2010). Potencial applications of PEF to improve red wine quality. Trends in Food Science and Technology, 21, 247-255.
- Ranalli, A., Contento S., Schiavone, C., & Simone, N. (2001). Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. European Journal of Lipid Science and Technology, 103(4), 228-238.
- Ranalli, A., Malfatti, A., Pollastri, L., Contento, S., & Lucera, L. (2003a). Analytical quality and genuineness of enzyme-extracted virgin olive oil. Journal of Food Quality, 26, 149-164.
- Ranalli, A., Pollastri, L., Contento, S., Lucera, L., & Del Re, P. (2003b). Enhacing the quality of virgin olive oil by use of a new vegetable enzyme extract during processing. European Food Research and Technology, 216, 109-115.
- Sánchez-Gimeno, A.C., Benito, M., Abenoza, M., Puértolas, E., Álvarez, I., & Raso, J. (2010). Improving the extraction of virgin olive oil by pulsed electric fields. IFT Annual Meeting & Food Expo (Chicago).
- Servilli, M., Selvaggini, R., Taticchi, A., Esposto, S., & Montedoro, G. F. (2003). Volatile compounds and phenolic composition of virgin olive oil: optimization of temperature and time of exposure of olive paste to air contact during the mechanical extracction process. Journal of Agricultural and Food Chemistry, 51, 7980-7988.
- Stefanoudaki, E., Koutsaftakis, A., & Harwood, J.L. (2011) Influence of malaxation conditions on characteristic qualities of olive oil. Food Chemistry, 127, 1481-1486.

- Toepfl, S., Heinz, V., & Knorr, D. (2006). Application of pulsed electric field technology for the food industry. Pulsed electric field technology for the food industry: Fundamentals and applications. In J. Raso (Ed.), V Heinz, (pp. 197-221). New York: Springer.
- Toepfl, S., Heinz, V., & Knorr, D. (2007). High intensity pulsed electric fields applied for food preservation. Chemical Engineering and Processing, 46(6), 537-546.
- Torres, M., & Maestri, D. (2006). Chemical composition of Arbequina virgin olive oil in relation to extraction and storage conditions. Journal of the Science and Agriculture, 86, 2311-2317.
- Tovar de Dios, M.J. (2001). Estudio del efecto de la aplicación de diferentes estrategias de riego al olivo (Olea europea L.) de la variedad Arbequina sobre la composición del aceite. Tesis Doctoral. Universidad de Lleida. Escola Tècnica Superior d´Enginyeria Agrària.
- Visioli, F., & Galli C. (1998). Olive oil phenols and their potential effects on human health. Journal of Agriculture and Food Chemistry, 46, 4292-4296
- Vorebiev, E., & Lebovka, N. (2008). Electrotechnologies for extraction from plants and biomaterials. In E. Vorobiev, N. Leboouka, & F.D. Ovcharenko (Eds.), Industrial-Scale Treatment of Biological Tissues with Pulsed Electric Fields (pp. 237-270). New York: Springer
- Uceda, M., Jiménez, A., & Beltrán, G. (2006). Olive oil extraction and quality. Grasas y Aceites, 57(1), 25-31

Figure and table captions

Figure 1. Response surface plots of the influence of electric field strength and malaxation time on oil yield extraction at 15ºC (A) and 26ºC (B).

Table 1. Effect of application of PEF treatments to the olive paste on oil yield and quality indices of Arbequina olive oil.

Table 2. Result of the analysis of variance for the significant terms of the models.

Table 3. Quality and nutritional parameters of control oil (malaxated at 26ºC for 30 min) and oil obtained from olive paste treated by PEF (2 kV/cm, malaxated at 15ºC for 30 min).

549 **Table 1**.
550

Malaxation temperature (ºC)	Malaxation time (min)	Electric field strength (kV/cm)	Oil yield (%)	Acidity (%oleic acid)	Peroxide value (meq O_2 Kg^{-1})	K_{232}	K_{270}
15°C	0	0	4.75±0.30	0.10±0.00	2.33±0.00	1.22±0.00	0.05±0.00
	15	0	11.24±0.42	0.08±0.00	4.24±0.14	1.25±0.02	0.06±0.01
	30	0	12.37±0.47	0.10±0.00	4.98±0.02	1.27±0.01	0.05±0.00
	0	1	5.84±0.50	0.10±0.00	3.32±0.00	1.19±0.00	0.06±0.00
	15	1	11.50±0.46	0.10±0.00	4.99±0.00	1.26±0.00	0.08±0.00
	30	1	13.80±0.19	0.11±0.00	4.31±0.01	1.25±0.00	0.07±0.00
	0	2	7.34±0.30	0.10±0.00	3.32±0.01	1.19±0.01	0.04±0.00
	15	2	12.15±0.47	0.10±0.00	4.66±0.00	1.25±0.01	0.07±0.00
	30	2	14.10±0.10	0.11±0.00	5.65±0.01	1.32±0.01	0.06±0.00
26°C	0	0	4.75±0.30	0.11±0.01	2.67±0.00	1.21±0.01	0.04±0.00
	15	0	11.36±0.33	0.10±0.00	2.75±0.12	1.12±0.02	0.03±0.00
	30	0	13.30±0.40	0.11±0.00	4.66±0.00	1.15±0.03	0.05±0.00
	0	1	5.84±0.50	0.10±0.00	2.91±0.11	1.25±0.01	0.08±0.01
	15	1	11.90±1.10	0.10±0.00	3.31±0.00	1.22±0.03	0.06±0.00
	30	1	13.60±0.50	0.11±0.00	4.23±0.12	1.24±0.01	0.08±0.01
	0	2	7.34±0.30	0.12±0.00	3.06±0.00	1.21±0.04	0.02±0.00
	15	2	11.90±0.36	0.12±0.01	3.15±0.24	1.21±0.01	0.06±0.00
	30	2	13.77±0.39	0.11±0.00	3.97±0.00	1.17±0.01	0.03±0.01

551 Each value represents mean ± standard deviation of two replicates

Table 2.

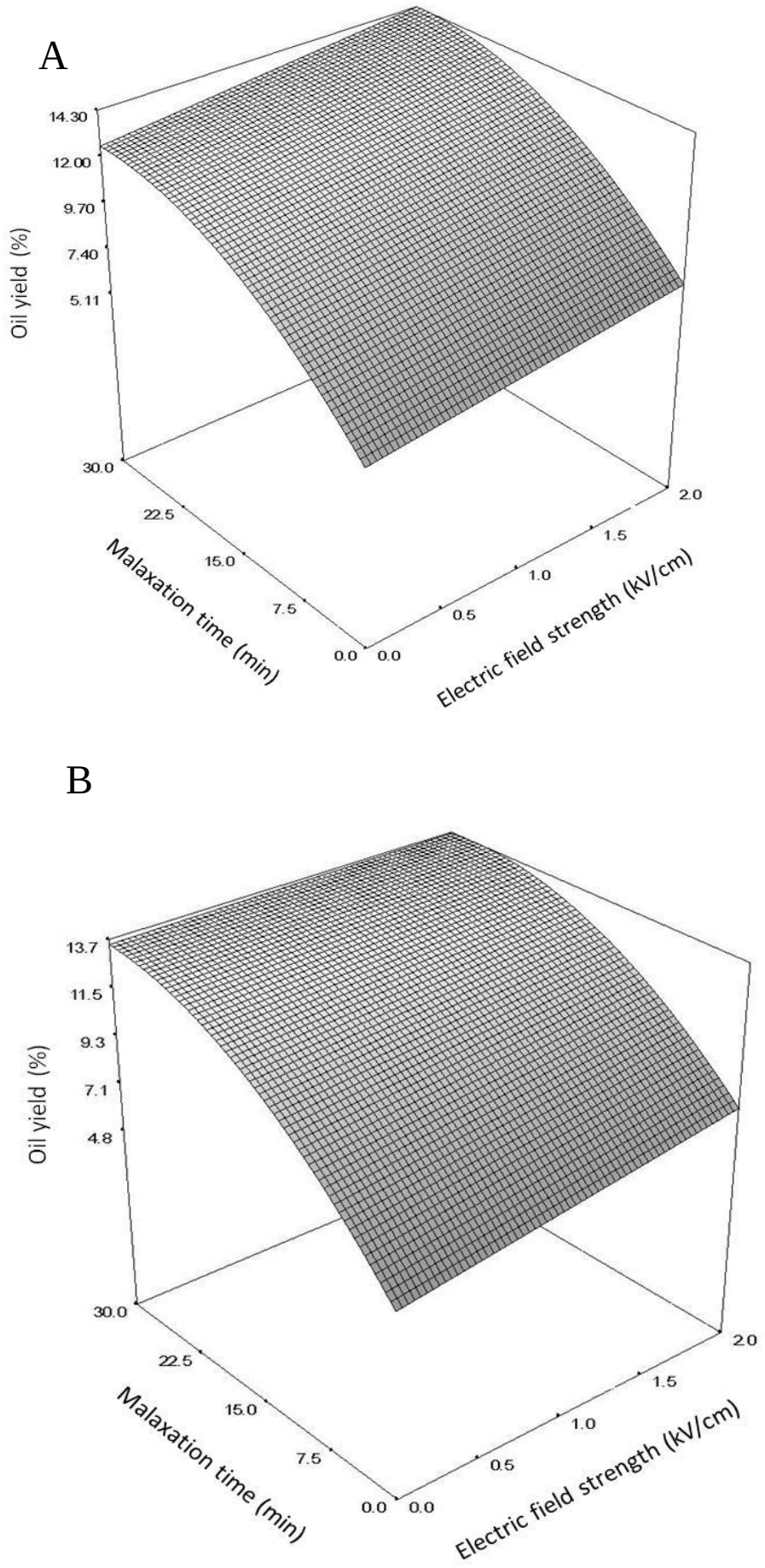
	Malaxation temperature 15 ºC (Equation 1)		**Malaxation temperature 26 ºC (Equation 2)**	
	F value	*Prob> F	F value	*Prob> F
E	23.45	0.0047	25.67	0.0071
t	427.91	<0.0001	985.58	<0.0001
t^2	38.30	0.0016	89.36	0.0007
E*t	-	-	12.85	0.0231
R^2	0.99		0.99	
R^2_{adj}	0.98		0.98	
F-value	163.23		199.17	

Table 3.

Parameter	Control oil	PEF treated
Acidity (% oleic acid)	0.12±0.00[a]	0.12±0.01[a]
Peroxide value (meq O_2 Kg^{-1})	1.99±0.01[a]	2.33±0.01[b]
K_{232}	1.27±0.01[a]	1.40±0.02[b]
K_{270}	0.06±0.01[a]	0.08±0.01[a]
Cholorophyll content (mg pheophitin Kg^{-1})	4.38±0.01[a]	3.19±0.03[b]
Carotenoids content (mg lutein Kg^{-1})	4.36±0.01[a]	3.94±0.14[b]
Colour parameters:		
L* (lightness)	92.24±0.07[a]	94.24±0.16[b]
a* (redness-greenness)	-13.11± 0.04[a]	-11.16±0.04[b]
b* (yellowness-blueness)	80.17±0.13[a]	76.39±0.14[b]
h* (hue angle)	99.29±0.03[a]	98.31±0.03[b]
C* (chroma)	81.24±0.13[a]	77.20±0.14[b]
a/b	-0.16±0.14[a]	-0.15±0.00[b]
Total phenols (mg gallic acid/kg)	148.94±2.73[a]	112.22±2.22[b]
Individual phenols:		
Hydroxytyrosol	0.62±0.02[a]	0.40±0.01[b]
Tyrosol	1.98±0.03[a]	1.45±0.06[b]
Vanillic acid	0.71±0.01[a]	0.69±0.01[a]
Vanillin	1.31±0.01[a]	1.29±0.01[a]
Cumaric acid	0.64±0.01[a]	0.55±0.01[b]
3.4-DHPEA-AC	72.21±0.05[a]	51.29±0.44[b]
3.4-DHPEA-EDA	150.79±0.35[a]	91.96±0.48[b]
p-HPEA-EDA	23.60±0.24[a]	20.37±0.18[b]
Lignans	46.79±0.02[a]	41.13±0.08[b]
3.4-DHPEA-EA	24.50±0.31[a]	18.40±0.62[b]
Luteolin	3.53±0.02[a]	2.88±0.04[b]
Apigenin	1.67±0.02[a]	1.51±0.02[b]
α-Tocopherol (mg/Kg)	247.84±0.36[a]	252.01±0.53[b]
Oxidative stability (hours)	13.73±0.43[a]	12.44±0.19[a]
Fatty acids:		
Palmitic acid (C16:0)	13.86±0.01[a]	13.82±0.07[a]
Palmitoleic acid (C16:1)	1.57±0.01[a]	1.52 ±0.01[a]
Margaric acid (C17:0)	0.09±0.01[a]	0.09±0.00[a]
Margaroleic acid (C17:1)	0.21±0.00[a]	0.21± 0.00[a]
Stearic acid (C18:0)	1.72±0.01[a]	1.70± 0.01[a]
Oleic acid (C18:1)	71.29±0.03[a]	71.42±0.10[a]
Linoleic acid (C18:2)	10.15±0.05[a]	10.12±0.04[a]
Linolenic acid (C18:3)	0.50±0.00[a]	0.50± 0.00[a]
Arachidic acid (C20:0)	0.34±0.00[a]	0.33±0.01[a]
Gadoleic acid (C20:1)	0.28± 0.01[a]	0.28±0.01[a]
Oleic/Linoleic	7.02±0.03[a]	7.06±0.04[a]
SFA (saturated fatty acids)	16.01±0.00[a]	15.95±0.06[a]
MUFAS (monounsaturated fatty acids)	73.34± 0.04[a]	73.43±0.10[a]
PUFAS (polyunsaturated fatty acids)	10.65± 0.05[a]	10.62±0.04[a]
MUFAS/PUFAS	6.89± 0.03[a]	6.91±0.02[a]
Bitterness (K_{225})	0.17±0.00[a]	0.13±0.00[b]

Each value represents mean±standard deviation of three replicates. For each parameter, values followed by different small letter are significantly different according tot-test.
3.4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene;
3.4-DHPEA-EDA, dialdehydric form of elenolic acid linked to hydroxytyrosol;
p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol;
3.4-DHPEA-EA, oleuropein aglycone

Figure 1


A
14.30
12.00
9.70
7.40
5.11
Oil yield (%)
30.0
22.5
15.0
7.5
0.0
Malaxation time (min)
0.0
0.5
1.0
1.5
2.0
Electric field strength (kV/cm)
B
13.7
11.5
9.3
7.1
4.8
Oil yield (%)
30.0
22.5
15.0
7.5
0.0
Malaxation time (min)
0.0
0.5
1.0
1.5
2.0
Electric field strength (kV/cm)