Olive oil pilot-production assisted by pulsed electric field: Impact on extraction yield, chemical parameters and sensory properties

Eduardo Puértolas *, Iñigo Martínez de Marañón

AZTI-Tecnalia, Food Research Division, Parque Tecnológico de Bizkaia, Astondo Bidea Edificio 609, 48160 Derio, Bizkaia, Spain

ABSTRACT

The impact of the use of pulsed electric field (PEF) technology on Arroniz olive oil production in terms of extraction yield and chemical and sensory quality has been studied at pilot scale in an industrial oil mill. The application of a PEF treatment (2 kV/cm; 11.25 kJ/kg) to the olive paste significantly increased the extraction yield by 13.3%, with respect to a control. Furthermore, olive oil obtained by PEF showed total phenolic content, total phytosterols and total tocopherols significantly higher than control (11.5%, 9.9% and 15.0%, respectively). The use of PEF had no negative effects on general chemical and sensory characteristics of the olive oil, maintaining the highest quality according to EU legal standards (EVOO; extra virgin olive oil). Therefore, PEF could be an appropriate technology to improve olive oil yield and produce EVOO enriched in human-health-related compounds, such as polyphenols, phytosterols and tocopherols.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Virgin olive oil (VOO), especially extra virgin olive oil (EVOO), constitutes one of the most appreciated and consumed vegetable oils worldwide because of its renowned organoleptic properties. Furthermore, due to the significant content in bioactive compounds, such as phenols, phytosterols or tocopherols, its regular consumption improves antioxidant status and blood lipid profile, reducing the incidence of some degenerative diseases such as atherosclerosis or cancer (Cicerale, Conlan, Sinclair, & Keast, 2009; Kritchevsky & Chen, 2005; Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2010; Normén et al., 2001; Perona, Cabello-Moruno, & Ruiz-Gutiérrez, 2006).

* Corresponding author. Address: AZTI-Tecnalia, Food Research Division, Parque Tecnológico de Bizkaia, Astondo Bidea Edificio 609, 48160 Derio, Bizkaia, Spain. Tel.: +34 94 657 40 00; fax: +34 94 657 25 55.
E-mail address: epuertolas@azti.es (E. Puértolas).

In order to assure maximum quality, VOO and EVOO are only obtained using mechanical methods (Regulation EC 1513/2001). Industrial extraction of this kind of premium oil essentially involves (1) the crushing of fruit to break plant tissues and allow oil release, (2) the malaxation of the olive paste to induce the oil drops coalescence (typically < 27 °C, <1 h), and lastly (3) the mechanical recovery of the oil by centrifugation (continuous mode) or pressing (discontinuous mode). The olive oil obtained is usually filtered or decanted to remove any possible solid residues prior to bottling.

One of the most important industrial handicaps of VOO and EVOO production is the low efficiency of current extraction techniques. Typically only 80% of the oil present in the fruit is easily released (Aguilera, Beltrán, Sánchez-Villasclaras, Uceda, & Jiménez, 2010; Clodoveo & Hbaieb, 2013). The rest remains inside cells or is emulsified with water, linked to different factors such as olive variety or extraction conditions (Aguilera et al., 2010; Espínola, Moya, Fernández, & Castro, 2009; Moya et al., 2010).

Furthermore, associated with these phenomena, an important amount of the bioactive compounds, such as polyphenols, phytosterols and tocopherols, still remains in the olive paste (Aliakbarian, Casazza, & Perego, 2011; Dermeche, Nadour, Larroche, Moulti-Mati, & Michaud, 2013). Nowadays, the most used solution in oil mills for improve extraction is increasing malaxation time or/and temperature. However, these practices have an important negative effect on the sensorial parameters, so their use is limited to olive oils of low quality (Anegrosa, Mostallino, Basti, & Vito, 2001). For that reason, an important research effort is being devoted to find innovative mild techniques to enhance VOO and EVOO production. The proposed techniques can be divided into in two groups: (1) the addition of chemicals or biochemicals to the olive paste, such as enzymes to degrade cell membranes or chemical coadjutants to avoid oil/water emulsions (e.g. calcium carbonate, natural talc), and (2) the treatment of the olive paste by physical technologies such as microwaves or ultrasound basically to break the cell envelopes (Chiacchierini, Mele, Restuccia, & Vinci, 2007; Clodoveo & Hbaieb, 2013; Espínola et al., 2009; Hadj-Taieb et al., 2012; Jiménez, Beltrán, & Uceda, 2007; Moya et al., 2010; Ranalli, Gomes, Delcuratolo, Contento, & Lucera, 2003).

The application of pulsed electric field (PEF) is an emerging physical technology that has been proposed for improving mass transfer processes in the food industry (Puértolas, Luengo, Álvarez, & Raso, 2012). The method is based on the formation of pores in cell membranes due to their exposure to low-moderate external electric fields of adequate strength (<10 kV/cm) and duration (microseconds). This electroporation mechanism increases the permeability of the vegetable cells, enhancing the diffusion of solutes through their membranes (Vorobiev & Lebovka, 2011). Published data relating to the use of PEF for assisting olive oil extraction are promising. Guderjan, Töpfl, Angersbach, and Knorr (2005) demonstrated firstly at laboratorial preliminary tests, the potential of PEF for increasing oil extraction yield from fresh olives (up to 7.4%). Although these authors did not study the PEF effect on olive oil bioactive compounds recovery, they published improvements on concentration of tocopherols, phytosterols and polyphenols in other vegetable oils, such as maize germ or rapeseed oils (Guderjan, Elez-Martínez, & Knorr, 2007; Guderjan et al., 2005). Recently, Abenoza et al. (2013) remarked upon the benefits of PEF on olive oil extraction yield and also studied its impact on product quality, using a laboratory-scale olive oil extraction system. However, in order to implement PEF technology in olive oil mills, it is necessary to hold pilot scale extraction studies to confirm the good results obtained in the laboratory.

The main objective of the present study was to demonstrate at pilot scale in an industrial oil mill, the potential benefits of PEF technology in high quality olive oil production (VOO/EVOO), both to increase the extraction yield and to enhance the content of bioactive substances. In order to achieve this objective, a pilot production using a full continuous PEF-assisted extraction system was accomplished at a small olive oil producer. The effect of PEF treatment on olive oil extraction yield, general quality parameters, polyphenol content, tocopherol and phytosterol profiles, and sensory attributes was determined.

2. Materials and methods

2.1. Plant material

Olive fruits (*Olea europaea L.*) from Arroniz variety were harvested during 2012 season in a controlled non-irrigated orchard sited in Moreda de Álava (Basque Country, Spain). Arroniz fruits, one of the most popular olive cultivars in Navarra and Basque Country Spanish Regions, were harvested by hand (rakes) in December at the industrial optimum ripening state under the International Olive Council recommendations (2007) based on the black skin colour. Harvested fruits were transported the same day to the olive oil mill "Trujal Comperativo la Equidad" sited in Moreda de Álava (Spain) and subsequently, were washed, cleaned of leaves, weighed and, finally, processed.

2.2. Pilot PEF-assisted extraction system for olive oil production

Pilot PEF-assisted extraction system was arranged to accomplish olive oil production. This one comprised two units: (1) a commercial olive oil extraction plant (up to 800 kg/h; K30, Oleomio, Granada, Spain), including knives crusher, a batch malaxation container and a horizontal 2-phase centrifuge; and (2) a pilot PEF-system (KEA-smart, KEA-TEC, Waghäusel, Germany). This device is based on a 3-kW generator that produces monopolar exponential-decay electric pulses of 0.3 ms at a maximum peak voltage of 10 kV. KEA-smart is completed by parallel-plate in-line treatment chamber (tube) with a 3-cm gap between the electrodes.

2.3. Olive oil extraction conditions

Once olives had been mechanically crushed (3000 rpm; 3 mm sieve), the obtained paste was malaxated at 24 °C for 60 min in a stainless-steel horizontal container equipped with a helical mixing device (10 rpm) and a double jacket heating system. Following the malaxation step, olive paste was continuously pumped at 520 kg/h using a progressive cavity pump (included in the Oleomio system) firstly to the inline PEF treatment chamber and, subsequently, to the horizontal centrifuge (3200 rpm). In this step, olive oil was physically separated from the olive pomace and then was stored in stainless steel containers. After a natural decantation process to remove water waste and solid impurities (2 months, room temperature), final oils were bottled and, subsequently, analysed.

In order to obtain PEF-assisted olive oil production (PEF oil), pulsed electric fields of 2 kV/cm and 65 J were applied to the olive paste at a frequency of 25 Hz. At the flow rate used in the experiments (520 kg/h), this treatment corresponded to a specific energy of 11.25 kJ/kg. The olive paste temperature at the inlet and outlet of the PEF treatment chamber was controlled by KEA-smart system internal probes (thermocouples). The initial temperature of the mass was around 24 °C and the temperature rise due to the PEF treatment did not exceed 3 °C.

Control olive oil production (Control oil) was also obtained to make comparisons. In this case, olive paste was passed through the inline PEF treatment chamber, but without applying any electric field. Thus, any possible interference of the treatment chamber was avoided and same processing times between malaxation and centrifugation were used.

2.4. Determination of olive oil extraction yield

Olive oil extraction yield was calculated taking into account the flow rate of the olive paste (kg/h), the production time (h) and the final olive oil recovered after natural decantation (kg). The extraction yield was expressed in kg oil/100 kg of olive paste.

2.5. Chemical analysis of oil quality

General chemical parameters, free acidity (% of oleic acid), peroxide value (meq O_2/kg), K_{270} and K_{232}, were determined according to the analytical methods described in the Regulation 2568/1991 of the European Union Commission and later modifications.

The total phenolic content was obtained by triple extraction of a solution of oil in hexane with methanol/water mixture (60:40). Folin–Ciocalteu reagent and sodium molybdate were added to a suitable aliquot of the combined extracts. Absorbance of the

solution was measured at 765 nm (Gutfinger, 1981). Total phenol concentration was expressed as mg of caffeic acid/kg of oil.

Phytosterol composition of the oils was determined by capillary column gas chromatography according to the official method described by Regulation (ECC) 2568/1991 and later modifications. The oil sample, after addition of α-cholestanol as internal standard, was saponified with ethanolic potassium hydroxide solution. The unsaponifiable fraction was removed with diethyl ether. The phytosterol fraction was separated by chromatography on silica gel plates. Separation and quantification of the phytosterols was carried out on an Agilent 6890 chromatograph (Agilent Technologies Inc., Santa Clara, CA,) equipped with a TRB-5 column (30 m; 0.32 mm internal diameter; 0.25 μm film thickness; Teknokroma, Barcelona, Spain). The injected quantity was 0.5 μl at a flow rate of 1.1 ml/min, using helium as carrier gas. The working conditions of the chromatograph were: injector 280 °C, isothermal column 260 °C, and detector 290 °C. Quantification was achieved by addition of an internal standard (α-cholestanol, Sigma–Aldrich, St. Louis, MO). Total and individual phytosterols were expressed in mg of α-cholestanol/kg of oil. Apparent β-sitosterol was calculated as the sum of Δ-5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ-5-avenasterol and Δ-5,24-stigmastadienol.

Tocopherols were evaluated according to IUPAC 2432 method (IUPAC, 1992). Oil samples of 2 g was dissolved in 25 mL hexane, filtered (0.45 μm) and injected (20 μL) into the HPLC system (Agilent 1100, Agilent Technologies) with a Lichrosphere Si60 column (25 cm × 4 mm × 5 μm) (Merck, Darmstadt, Germany). The mobile phase was n-hexane/2-propanol (99.5:0.05, v/v) and the flow rate 1 mL/min. Individual tocopherols were identified at 295 nm and quantified as mg/100 g of oil using the corresponding external standards, α-, β-, γ- and δ-tocopherol (Sigma–Aldrich).

2.6. Sensory analysis

Sensory analysis was performed by panel test according to Regulation (EC) 2568/1991. The oil samples were evaluated by 12 trained panellists from Laboratorios Tello (Jaén, Spain). Panellists smelled and then tasted the oil samples, marking on a 10-cm scale provided on the profile sheet, the intensity of their perception of each positive (fruity, bitter and pungent) and negative descriptor (fusty, musty/humid, winey-vinegary/acid sour, metallic, rancid, heated, hay/wood, rough, greasy, vegetable water, brine, esparto, earthy, grubby, cucumber, wet wood, and other). Then, median values for each attribute were calculated. According to EU legislation, EVOO classification implicates median of the defects of 0 and a median for fruity above 0.

2.7. Statistical analysis

Experiments were conducted in duplicate. All chemical analyses were performed at least in triplicate. Results were expressed as means ± 95% confidence interval (95CI). Statistical differences ($p = 0.05$) were determined by student's t-test using Statgraphics Centurion software (Statpoint Technologies Inc., Warrenton, VA).

3. Results and discussion

3.1. Impact of PEF on oil extraction yield

Extraction yield is considered the main parameter to determine the economic efficiency and global performance of olive oil extraction. In the control oil production conducted in this investigation (24 °C; 60 min malaxation), the extraction yield obtained was 20.00 kg/100 kg (control oil) (Fig. 1). This value is similar to the extraction yields obtained with Arroniz variety in past seasons in
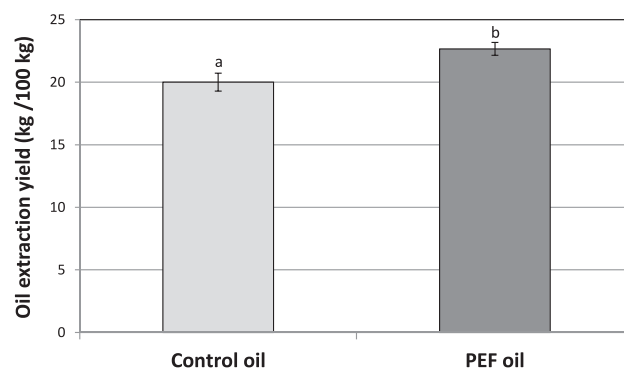

Fig. 1. Extraction yield (kg oil/100 kg of olive paste) obtained in control and PEF olive oil pilot productions. Errors bars indicate 95% confidence interval (95CI). Different letters represent significant differences ($p = 0.05$).

Moreda de Alava with similar weather conditions, and also comparable to extraction yields published for other olive varieties (Espínola et al., 2009; Hadj-Taieb et al., 2012). The application of a PEF treatment (2 kV/cm, 11.25 kJ/kg) to the olive paste after malaxation (24 °C; 60 min) significantly increased ($p < 0.05$) the extraction yield value up to 22.66 kg/100 kg (Fig. 1). According to this, PEF technology yielded an additional 2.66 kg of olive oil per each 100 kg of processed Arroniz olives, improving the oil extraction yield by 13.3%, with respect to the control. The impact of PEF on oil recovery could be explained by the well-known cell membrane electroporation mechanism, and the consequent improving of mass transfer phenomena (Puértolas et al., 2012; Vorobiev & Lebovka, 2011). PEF acts as other technologies, like ultrasound or enzymes, assisting the release of oil from lipo-vacuoles of mesocarp cells that have not been disrupted by crushing (Chiacchierini et al., 2007; Clodoveo, Durante, & La Notte, 2013). However, besides oil remaining in the mesocarp cells, in the conventional olive oil production part of the oil is also emulsified with vegetable water and, consequently, it is lost with olive pomace after centrifugation (Aguilera et al., 2010; Espínola et al., 2009). The difficulty of freeing this bound oil lies mainly in the fact that droplets of emulsified oil are surrounded by a lipoprotein membrane (Espínola et al., 2009). A PEF treatment of olive paste could disrupt this lipoprotein membrane, favouring the release of oil. Furthermore, the application of electric fields has been also described per se as an effective demulsification technique, since electric fields facilitate coalescence processes and the consequent separation of oil from water (Kwon et al., 2010; Rayat & Feyzi, 2011). Therefore, PEF effect on olive oil yield could be explained by a double mechanism: the improvement of oil extraction from olive tissue, and the release of olive oil trapped in oil-vegetable water emulsions.

The improvement found in the current investigation (13.3%) is comparable to the enhancement obtained by Abenoza et al. (2013). These authors reported an increase of a 13.9% in Arbequina oil yield, combining a lower PEF treatment (2 kV/cm; 5.22 kJ/kg) with a subsequent gentle malaxation step of 30 min at 15 °C. However, these authors did not obtain any significant improvement when malaxation temperature was increased up to 26 °C. These results and the present study point out the great influence of olive variety, agronomic practices and extraction conditions on PEF extraction efficiency. Compared with other novel physical methods proposed for olive oil production, like ultrasound or microwave technology, PEF seems to be a more efficient technology for improving olive oil yield. According to laboratory studies, ultrasound treatment (35 kHz; 150 W; 8 min) has the potential to increase the extraction yield by 6% (Clodoveo, Durante, et al., 2013). Nevertheless, when ultrasound process was scaled up, no

increase in the extraction yield was described (Clodoveo & Hbaieb, 2013). In the same report, the application of microwaves to the olive paste (2450 MHz; 800 W; 3 min) did not enhance the extraction yield either (Clodoveo & Hbaieb, 2013). On the other hand, the extra olive oil production obtained in this investigation by PEF technology with respect to the control (2.66 kg/100 kg) is in the same range as results achieved by chemical and biochemical strategies, such as the use of calcium carbonate, talc or enzymes (Chiacchierini et al., 2007; Espínola et al., 2009; Hadj-Taieb et al., 2012; Moya et al., 2010).

In VOO and EVOO production approximately 20% of the oil remains in the olive pomace (Aguilera et al., 2010). The residual oil means a great monetary loss for the olive sector, reaching values up to 4 kg/100 kg of olive processed (Chiacchierini et al., 2007). This oil is normally extracted using organic solvents in specialised industries. Although the oil extracted is still olive oil, it must not be called virgin olive oil (VOO), as it is not obtained by physical methods and is low in quality (Regulation EC 1513/2001), so its monetary value is lower. Taking into account this general basis and according to the results presented in this study, a PEF treatment could potentially recover 50% of the oil that normally remains in the olive pomace. It would mean an increase of the VOO/EVOO recovery percentage from 80% to up to 90%, reducing in consequence the by-product generation and the environmental impact of olive oil production. In a medium-size industrial oil mill of 3000 kg/h (16 h/day), a PEF treatment could potentially increase the VOO/EVOO daily production by 1277 kg (from 9600 to 10,877 kg). This would increase profit margins (by at least 13% according to the yield improvement) and pay back the investment in PEF equipment.

3.2. Impact of PEF on general chemical parameters

Table 1 shows the general chemical characteristics of control and PEF oils. The application of a PEF treatment to the olive paste (2 kV/cm, 11.25 kJ/kg) significantly increased the free acidity of the oil obtained from 0.19% to 0.22% of oleic acid ($p < 0.05$). A similar rise has been described in PEF-assisted extraction of rapeseed oil (Guderjan et al., 2007). However, both control and PEF oil free acidity values remained below the maximum limit for extra virgin olive oil (EVOO) according to EU legislation (Regulation EC 1989/2003). Therefore, this slight increase did not negatively affect the oil quality and had no practical implications. No significant differences were observed for the other parameters tested: K_{232}, K_{270} indices and peroxide value, ($p \geqslant 0.05$). These results are consistent with data published on olive oil extraction assisted by PEF (Abenoza et al., 2013) and are also similar to those that have been published for other chemical, biochemical and physical methods, such as enzymes, ultrasound or microwave (Clodoveo & Hbaieb, 2013; Ranalli et al., 2003).

3.3. Impact of PEF on total phenolic content

Olive oil phenolics have been demonstrated to possess beneficial biological activities, such as altered lipid composition or a reduction in oxidative damage and inflammation (Cicerale et al.,

2009; Perona et al., 2006). Moreover, phenolic content is also related to oxidative stability of olive oil during storage (Lozano-Sánchez et al., 2010). Therefore, maximising its concentration is important to increase the health benefits of VOO/EVOO and preserve its quality during storage. As reported by different authors, the total phenolic content normally ranges between 50 and 1000 mg/kg, depending on various factors, such as olive variety, harvest time, climate location or the oil extraction procedure (Cicerale et al., 2009; Gimeno, Castellote, Lamuela-Raventós, De la Torre, & López-Sabater, 2002; Pardo, Cuesta, & Alvarruiz, 2007). In the present work, a PEF treatment of 11.25 kJ/kg at 2 kV/cm significantly increased ($p < 0.05$) the total phenolic content of Arroniz oil from 404 to 451 mg /kg (Fig. 2), an increment of 11.5%, similar to those reported in other PEF-assisted processes, such as red wine production, rapeseed oil extraction or valorisation of oilseed residues, confirming the increase in phenolic release by the PEF electric disruption of cell envelopes (Boussetta, Soichi, Lanoisellé, & Vorobiev, 2014; Guderjan et al., 2007; Puértolas, López, Condón, Álvarez, & Raso, 2010). However, a decrease in total phenolic content has been recently published regarding PEF-assisted olive oil extraction (Abenoza et al., 2013). As the same authors explained, this behaviour could be due to the low malaxation temperature used for olive paste treated by PEF (15 °C) in comparison with the control (26 °C), one of the main parameters for phenolic extraction (Anegrosa et al., 2001; Ranalli, Malfatti, Lucera, Contento, & Sotiriou, 2005). The present work may confirm this possible explanation, since malaxation temperature was the same for both control and PEF experiments (24 °C), so the positive PEF effect was not influenced by the malaxation temperature.

Regarding other olive oil enhancing techniques, the application of PEF could be a useful alternative for improving phenolic content. Chemical co-adjuvants, such as calcium carbonate and talc, have no effect on phenolic extraction (Moya et al., 2010). Similarly, the application of ultrasound directly to olive paste has no effect or even decreases total phenolic content (Clodoveo, Durante, et al., 2013; Jiménez et al., 2007). A positive relationship was only reported when ultrasound treatment was applied to olives submerged in water (Clodoveo, Durante, et al., 2013). The beneficial impact of PEF on phenols only could be compared with enzymes, where increments from 4% to 48% have been published, depending on diverse factors, such as enzyme formulation, olive variety and extraction conditions (De Faveri, Aliakbarian, Avogadro, Perego, & Converti, 2008; Hadj-Taieb et al., 2012; Ranalli et al., 2003).

3.4. Impact of PEF on phytosterols

Phytosterols have an important role on quality, since they contribute to the nutritional value of olive oil through the exhibition of
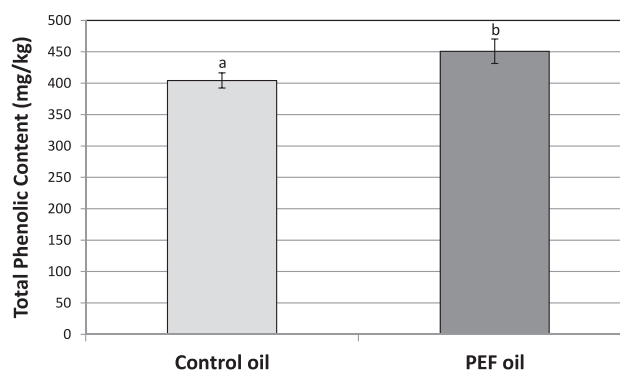
Table 1
General chemical parameters of control and PEF olive oil pilot productions.[*]

	Control oil	PEF oil
Free acidity (% of oleic acid)	0.19 ± 0.01^a	0.22 ± 0.01^b
Peroxide value (meq O_2/kg)	10.85 ± 0.43^a	10.58 ± 0.25^a
K_{232}	1.71 ± 0.09^a	1.68 ± 0.02^a
K_{270}	0.11 ± 0.02^a	0.11 ± 0.01^a

[a,b] Different superscripts in the same row represent significant differences ($p = 0.05$)
[*] Values expressed as mean ± 95% confidence interval.

Fig. 2. Total phenolic content (mg caffeic acid/kg of oil) obtained in control and PEF olive oils. Errors bars indicate 95% confidence interval. Different letters represent significant differences ($p = 0.05$).

certain health benefits, such as blood hypocholesterolaemic effect or cancer prevention (Kritchevsky & Chen, 2005; Normén et al., 2001). Phytosterol composition of control and PEF oils is shown in Table 2. Both oils possessed a total phytosterol concentration fitting to EVOO category ($\geqslant 1000$ mg/kg) (Regulation EC 1989/2003). PEF oil presented a significant higher ($p < 0.05$) total phytosterol content (1520 mg/kg) than control (1382 mg/kg), an increment of 9.9%. This higher value could be associated with the higher free acidity found in PEF oil (Lozano-Sánchez et al., 2010).

Despite of the importance of these compounds, data about the impact of emerging extraction techniques on olive oil phytosterols are scarce. Regarding PEF, the improvement shown in this work was in the same range as other findings published for rapeseed oil (Guderjan et al., 2007). Concerning other novel techniques, no effect has been detected on phytosterols levels in enzyme-assisted olive oils (Chiacchierini et al., 2007; Ranalli et al., 2003).

Regarding phytosterol composition, as expected the main compound present in control and PEF Arroniz oils was β-sitosterol, followed by Δ-5-avenasterol and campesterol. These substances comprised 96% of the total phytosterol content. The remaining 4% included small amounts of cholesterol, 24-methylenecholesterol, campestanol, stigmasterol, Δ-7-campesterol, Δ-5,23-stigmastadienol, clerosterol, sitostanol, Δ-5,24-stigmastadienol, Δ-7-stigmastenol and Δ-7-avenasterol. As shown in Table 2, PEF increased the average value of all the chemical species by 9% to 20% with respect to the control. However, significantly higher contents were only detected in 24-methylenecholesterol, campestanol, β-sitosterol and Δ-5-avenasterol ($p < 0.05$). These increments did not affect the EU legal categorisation of these olive oils (% of cholesterol, campesterol, stigmasterol, Δ-7-stigmastenol and apparent β-sitosterol fell within EVOO EU legal classification (Regulation EC 1989/2003)).

When relative percentage of each compound was studied in detail, slight differences in the phytosterol profile were found. PEF treatment caused a relative decrease in β-sitosterol (from 80.25% to 79.57%), coupled mainly to an increase in Δ-5-avenasterol (from 13.52% to 14.08%). This meant an increase in Δ-5-avenasterol/β-sitosterol ratio from 0.168 to 0.177. It has been published that these two major phytosterols are strongly and negatively correlated according to olive variety and maturity degree (Manai-Djebali et al., 2012; Pardo et al., 2007). Current research suggests that the use of novel extraction systems like PEF technology could slightly modify phytosterol profile. Although this behaviour has low importance from a nutritional and quality point of view (EVOO

Table 2
Phytosterol composition of the control and PEF olive oil samples (mg of α-cholestanol/kg of oil).[*]

	Control oil	PEF oil
Cholesterol	1.88 ± 0.67[a] (0.17%)	2.40 ± 0.63[a] (0.12%)
24-Methylenecholesterol	2.76 ± 0.17[a] (0.20%)	3.04 ± 0.09[b] (0.20%)
Campesterol	32.9 ± 3.21[a] (2.38%)	36.5 ± 2.61[a] (2.40%)
Campestanol	1.45 ± 0.01[a] (0.11%)	1.55 ± 0.01[b] (0.10%)
Stigmasterol	4.87 ± 1.07[a] (0.35%)	5.33 ± 0.97[a] (0.35%)
δ-7-Campesterol	0.69 ± 0.06[a] (0.05%)	0.82 ± 0.13[a] (0.05%)
δ-5,23-Stigmastadienol	1.41 ± 0.07[a] (0.10%)	1.54 ± 0.01[a] (0.10%)
Clerosterol	11.8 ± 1.07[a] (0.85%)	13.3 ± 0.80[a] (0.87%)
β-Sitosterol	1110 ± 68.2[a] (80.25%)	1210 ± 38.0[b] (79.57%)
Sitostanol	6.59 ± 0.97[a] (0.48%)	7.98 ± 0.81[a] (0.52%)
δ-5-Avenasterol	187 ± 12.19[a] (13.52%)	214 ± 6.63[b] (14.08%)
δ-5,24-Stigmastadienol	7.60 ± 0.92[a] (0.55%)	8.75 ± 0.96[a] (0.58%)
δ-7-Stigmastenol	2.76 ± 0.16[a] (0.20%)	3.42 ± 0.79[a] (0.23%)
δ-7-Avenasterol	11.1 ± 0.66[a] (0.80%)	12.5 ± 0.92[a] (0.82%)
Apparent β-sitosterol	1320 ± 78.8[a] (95.71%)	1450 ± 38.5[a] (95.64%)
Total phytosterols	1380 ± 82.3[a] (100%)	1520 ± 42.4[b] (100%)

[a,b] Different superscripts in the same row represent significant differences ($p = 0.05$).
[*] Values expressed as mean ± 95% confidence interval. Percentage of total phytosterols is indicated in brackets.

Table 3
Total and individual tocopherol concentrations (mg/100 g of oil) presented in control and PEF olive oils.[*]

	Control oil	PEF oil
α-Tocopherol	11.4 ± 1.54[a]	14.3 ± 1.28[b]
β-Tocopherol	4.60 ± 1.57[a]	4.53 ± 1.08[a]
γ-Tocopherol	2.82 ± 0.82[a]	2.93 ± 0.46[a]
δ-Tocopherol	0.30 ± 0.11[a]	0.27 ± 0.17[a]
Total tocopherols	19.2 ± 1.61[a]	22.0 ± 0.64[b]

[a,b] Different superscripts in the same row represent significant differences ($p = 0.05$).
[*] Values expressed as mean ± 95% confidence interval.

EU legal classification is not modified), it should be taken into account in the future, since phytosterol profile has been proposed for olive oil authentication purposes (Manai-Djebali et al., 2012).

3.5. Impact of PEF on tocopherols

Tocopherols, together with phenols, play an important role in the antioxidant properties of olive oil, helping to maintain quality during storage (Lozano-Sánchez et al., 2010). Furthermore, a positive relationship between α-tocopherol and anti-inflammatory and anti-endothelial activation properties of olive oil has been reported (Perona et al., 2006). Table 3 contains the values of total tocopherols, α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol in control and PEF Arroniz oils. All values are in the same range as data reported for other monovarietal olive oils (Ballus et al., 2014; Gimeno et al., 2002; Pardo et al., 2007). Control oil presented a total tocopherol concentration of 19.2 mg/100 g. The application of PEF caused a significant increase in this content of 15.0% ($p < 0.05$), reaching a value of 22.0 mg/100 g. Regarding individual isomers, only significant differences were obtained for α-tocopherol ($p < 0.05$), increasing its value by 25%, from 11.4 mg/100 g (control oil) to 14.3 mg/100 g (PEF oil). Abenoza et al. (2013) found a slight PEF-mediated increase of α-tocopherol in Arbequina oil (1.67%). As stated earlier regarding the phenolic content, the effect of PEF could also be blurred by the different malaxation temperature used for control and PEF oils reported by these authors. According to Ranalli et al. (2005), the tocopherol content clearly depends on the malaxation temperature.

The effect of PEF on olive oil tocopherols was similar to the impact of enzyme formulations. Depending on the olive variety, improvements ranging from 13.5% to 30.8% have been published for α-tocopherol + γ-tocopherol content (Ranalli et al., 2003), very close to the 20.9% obtained using PEF in this investigation. The impact of other emerging physical extraction technologies on tocopherols is unclear. Ultrasound did not modify or even slightly decreased the content of total tocopherols in comparison with conventional procedures (Jiménez et al., 2007). However, Clodoveo, Durante, et al. (2013) recently reported an improvement of more than 60%.

3.6. Impact of PEF on sensory properties

Table 4 shows the intensity attributes perceived by testers. Both control and PEF olive oils showed sensory profiles belonging to EVOO category (Regulation EC 640/2008). From a practical point of view, PEF did not affect sensory properties. The median of the defects was 0 for both olive oils, meaning that testers did not perceive any specific off-flavour or taste associated with the PEF treatment. Abenoza et al. (2013) neither found any defect associated with PEF on Arbequina oil production. The impact of PEF on olive oil sensory quality would be similar to other novel extraction

Table 4
Values of the sensory attributes of control and PEF oils.

	Control oil	PEF oil
Median fruity	2.8	2.6
Median bitter	2.9	2.9
Median pungent	3.1	3.2
Median defect	0	0

techniques proposed, such as enzymes or chemical co-adjuvants (Espínola et al., 2009; Moya et al., 2010).

4. Conclusions

According to the results presented in this work, pulsed electric field (PEF) has been shown as an appropriate technology to improve yield of virgin and extra-virgin olive oil (VOO and EVOO). Thus, PEF could potentially help olive oil mills, increasing oil production and consequently their profit margins. From a chemical and sensory point of view, PEF treatment not only has no negative effects, but increases the content of human-health-related compounds, such as polyphenols, phytosterols and tocopherols, maintaining the EU legal standards of highest quality olive oil (EVOO). When results were compared with data published on other chemical, biochemical and physical techniques proposed for enhancing olive oil production PEF revealed great potential, being matched only by results obtained by enzymes. In any case, due to the great influence of external factors on extraction efficiency (olive variety, maturity, malaxation temperature and duration, process variables, etc.) further research must to be devoted to clarify their effect on PEF-assisted extraction and to make comparative studies with other emerging techniques such as enzymes or ultrasound.

Acknowledgements

References

Abenoza, M., Benito, M., Saldaña, G., Álvarez, I., Raso, J., & Sánchez-Gimeno, A. C. (2013). Effects of pulsed electric field on yield extraction and quality of olive oil. *Food and Bioprocess Technology, 6*, 1367–1373.

Aguilera, M. P., Beltrán, G., Sánchez-Villasclaras, S., Uceda, M., & Jiménez, A. (2010). Kneading olive paste from unripe Picual fruits: I. Effect on oil process yield. *Journal of Food Engineering, 97*, 533–538.

Aliakbarian, B., Casazza, A. A., & Perego, P. (2011). Valorization of olive oil solid waste using high pressure-high temperature reactor. *Food Chemistry, 128*, 704–710.

Anegrosa, 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.

Ballus, C. A., Dillenburg-Meinhart, A., de Souza Campos, F. A., de Oliveira da Silva, L. F., de Oliveira, A. F., & Teixeira-Godoy, H. (2014). A quantitative study on the phenolics compound, tocopherol and fatty acid contents of monovarietal virgin olive oils produced in the southeast region of Brazil. *Food Research International, 62*, 74–83.

Boussetta, N., Soichi, E., Lanoisellé, J. L., & Vorobiev, E. (2014). Valorization of oilseed residues: Extraction of polyphenols from flaxseed hulls by pulsed electric fields. *Industrial Crops and Products, 52*, 347–353.

Chiacchierini, E., Mele, G., Restuccia, D., & Vinci, G. (2007). Impact of innovative and sustainable extraction technologies on olive oil quality. *Trends in Food Science & Technology, 18*, 299–305.

Cicerale, S., Conlan, X. A., Sinclair, A. J., & Keast, R. S. J. (2009). Chemistry and health of olive oil phenolics. *Critical Reviews in Food Science and Nutrition, 49*, 218–236.

Clodoveo, M. L., Durante, V., & La Notte, D. (2013). Working towards the development of innovative ultrasound equipment for the extraction of virgin olive oil. *Ultrasonics Sonochemistry, 20*, 1261–1270.

Clodoveo, M. L., & Hbaieb, R. H. (2013). Beyond the traditional virgin olive oil extraction systems: Searching innovative and sustainable plant engineering solutions. *Food Research International, 54*, 1926–1933.

De Faveri, D., Aliakbarian, B., Avogadro, M., Perego, P., & Converti, A. (2008). Improvement of olive oil phenolics content by means of enzyme formulations: Effect of different activities and levels. *Biochemical Engineering Journal, 41*, 149–156.

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochemistry, 48*, 1532–1552.

Espínola, F., Moya, M., Fernández, D. G., & Castro, E. (2009). Improved extraction of virgin olive oil using calcium carbonate as coadjuvant extractant. *Journal of Food Engineering, 92*, 112–118.

European Union Commission (1991). Regulation (ECC) No 2568/91. *Official Journal of the European Communities*, L248.

European Union Commission (2001). Regulation (EC) No 1513/2001. *Official Journal of the European Communities*, L146.

European Union Commission (2003). Regulation (EC) No 1989/2003. *Official Journal of the European Communities*, L295.

European Union Commission (2008). Regulation (EC) No 640/2008. *Official Journal of the European Communities*, L178.

Gimeno, E., Castellote, R. M., Lamuela-Raventós, M. C., De la Torre, M. C., & López-Sabater, M. C. (2002). The effects of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol, and β-carotene) in virgin olive oil. *Food Chemistry, 78*, 207–211.

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.

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.

Gutfinger, T. (1981). Polyphenols in olive virgin oils. *Journal of the American Oil Chemists Society, 58*, 966–968.

Hadj-Taieb, N., Grati, N., Ayadi, M., Attia, I., Bensalem, H., & Gargouri, A. (2012). Optimization of olive oil extraction and minor compounds content of Tunisian olive oil using enzymatic formulations during malaxation. *Biochemical Engineering Journal, 62*, 79–85.

International Olive Council (2007). *Production techniques in olive growing*. Madrid: International Olive Council, pp. 319–327.

IUPAC (1992). International Union of Pure and Applied Chemistry (IUPAC) Regulation N° 2432. In *Standard methods for the analysis of oils, fat and derivatives*. Oxford: Pergamon Press.

Jiménez, A., Beltrán, G., & Uceda, M. (2007). High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin olive oil characteristics. *Ultrasonics Sonochemistry, 14*, 725–731.

Kritchevsky, D., & Chen, S. C. (2005). Phytosterols-health benefits and potential concerns: A review. *Nutrition Research, 25*, 413–428.

Kwon, W. T., Park, K., Han, S. D., Yoon, S. M., Kim, J. Y., Bae, W., et al. (2010). Investigation of water separation from water-in-oil emulsion using electric field. *Journal of Industrial and Engineering Chemistry, 16*, 684–687.

Lozano-Sánchez, J., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Composición del aceite de oliva. In F. Fernández-Gutiérrez & A. Segura-Carretero (Eds.), *El Aceite de Oliva Virgen: Tesoro de Andalucía* (pp. 195–224). Málaga: Fundación Unicaja.

Manai-Djebali, H., Krichène, D., Ouni, Y., Gallardo, L., Sánchez, J., Osorio, E., et al. (2012). Chemical profiles of five minor olive oil varieties grown in central Tunisia. *Journal of Food Composition and Analysis, 27*, 109–119.

Moya, M., Espínola, F., Fernández, D. G., de Torres, A., Marcos, J., Vilar, J., et al. (2010). Industrial trial son coadjuvants for olive oil extraction. *Journal of Food Engineering, 97*, 57–63.

Normén, A. L., Brants, H. A., Voorrips, L. E., Andersson, H. A., Van Den Brandt, P. A., & Goldbohm, R. A. (2001). Plant sterol intakes and colorectal cancer risk in the Netherlands cohort study on diet and cancer. *The American Journal of Clinical Nutrition, 74*, 141–148.

Pardo, J. E., Cuesta, M. A., & Alvarruiz, A. (2007). Evaluation of potential and real quality of virgin olive oil from the designation of origin "Aceite Campo de Montiel" (Ciudad Real, Spain). *Food Chemistry, 100*, 977–984.

Perona, J. S., Cabello-Moruno, R., & Ruiz-Gutiérrez, V. (2006). The role of virgin olive oil components in the modulation of endothelial function. *Journal of Nutritional Biochemistry, 17*, 429–445.

Puértolas, E., López, N., Condón, S., Álvarez, I., & Raso, J. (2010). Potential applications of PEF to improve red wine quality. *Trends in Food Science & Technology, 21*, 247–255.

Puértolas, E., Luengo, E., Álvarez, I., & Raso, J. (2012). Improving mass transfer to soften tissues by pulsed electric fields: fundamentals and applications. *Annual Reviews of Food Science and Technology, 3*, 263–282.

Ranalli, A., Gomes, T., Delcuratolo, D., Contento, S., & Lucera, L. (2003). Improving virgin olive oil quality by means of innovative extracting biotechnologies. *Journal of Agricultural and Food Chemistry, 51*, 2597–2602.

Ranalli, A., Malfatti, A., Lucera, L., Contento, S., & Sotiriou, E. (2005). Effects of processing techniques on the natural colourings and the other functional constituents in virgin olive oil. *Food Research International, 38*, 873–878.

Rayat, K., & Feyzi, F. (2011). Influence of external electric field on the polarity of water droplets in water-in-oil emulsion phase transition. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 375*, 61–67.

Vorobiev, E., & Lebovka, L. (2011). Pulsed electric field-assisted extraction. In L. Lebovka, E. Vorobiev, & F. Chemat (Eds.), *Enhancing extraction processes in the food industry* (pp. 25–84). Boca Ratón: CRC Press.