

Pulsed electric field treatment improves the oil yield, quality, and antioxidant activity of virgin olive oil

Siyuan Yang^a, Sha Li^a, Gang Li^b, Chao Li^b, Wei Li^c, Yang Bi^{a,*}, Juan Wei^{a,*}

^a College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, China

^b Gansu Time Oil Olive Technology Co., Ltd, Longnan 746000, China

^c Longnan Olive Green Agricultural Development Co., Ltd, Longnan 746000, China

ARTICLE INFO

Keywords:

Virgin olive oil
Pulsed electric field
Oil yield
Quality
Antioxidant activity

ABSTRACT

Pulsed electric field (PEF) is an innovative technique used to assist in the extraction of vegetable oils. There has been no research on the effects of PEF on virgin olive oil (VOO) quality and antioxidant activity to date. The present study aimed to analyze the effects of PEF on oil yield, quality, and *in vitro* antioxidant activity of “Koroneiki” extra virgin olive oil. The results show that the PEF treatment increased the oil yield by 5.6%, but had no significant effect on the saponification value, K232, K270, and ΔK value of the VOO. PEF treatment reduced the oleic acid content by 3.12%, but had no significant effect on the content of palmitic acid, linoleic acid, linolenic acid, arachidonic acid, stearic acid, oleic acid, and palmitic acid. After PEF treatment, the levels of total phenolics, total flavonoids, and oleuropein increased by 7.6%, 18.3% and 76%, respectively. There was no significant effect on the levels of 4 phenolic acids (vanillic acid, *p*-coumaric acid, ferulic acid and cinnamic acid), 2 lignans (lignans and apigenin), hydroxytyrosol, and 3 pigments (lutein, demagnetized chlorophyll, and carotenoids). In addition, PEF treatment significantly increased the content of tocopherols, with α , β , γ , and δ tocopherols increasing by 9.8%, 10.7%, 13.6% and 38.4%, respectively. The free radical scavenging ability of DPPH and ABTS was also improved. In conclusion, the use of PEF significantly increased the yield of VOO oil as well as the levels of total phenolics, total flavonoids, oleuropein, tocopherol, and *in vitro* antioxidant activity.

1. Introduction

Virgin olive oil (VOO) is a vegetable oil obtained through the process of cold pressing fresh olives. It contains a high concentration of unsaturated fatty acids, vitamins, polyphenols, and other components (Ghanbari, Anwar, Alkharfy, Gilani, & Saari, 2012). Pulsed electric field (PEF) is a novel extraction technology that can effectively increase the oil yield of plant oils by disrupting cell walls (Wang, Li, Sun, & Zhu, 2018). PEF increases the permeability and rupture of cell membranes, which positively affects the extractability and quality of olive oil (Veniziani et al., 2019). There are reports that PEF treatment can significantly increase the oil yield of sesame oil and sunflower oil (Sarkis, Boussetta, Tessaro, Ferreira Marczak & Vorobiev, 2015; Shorstkii, Mirshekarloo & Koshevoi, 2015). Implementation of this approach could potentially result in higher oil production, increased acidity levels, and increased phenolic composition in virgin olive oil and rapeseed oil (Martínez-Navarro, Cebrián-Tarancón, Oliva, Salinas and Alonso, 2021; Andreou et al., 2022; Eduardo & Martínez, D.M.I., 2015). In addition,

PEF could increase the acidity and total phenolic content of Niger seed oil and hemp seed oil (Mohseni, Mirzaei, & Moghimi, 2020a, 2020b; Haji-Moradkhani, Rezaei, & Moghimi, 2019). The acidity and peroxide value of peanut oil were found to be significantly increased by PEF treatment as reported by Zeng, Han, and Zi (2010). Furthermore, as reported by Ćurko et al. (2023), the application of PEF treatment increased the total phenolic content as well as the oxygen radical absorbance capacity (ORAC) of grape seed oil. In addition, Rábago-Panduro, Romero-Fabregat, Martín-Belloso and Welti-Chanes (2021) found that PEF significantly improved the ability of walnut oil to scavenge free radicals such as DPPH and increased oil yield. Few studies have investigated the possible effects of PEF on the total flavonoid, polyphenol, and pigment content, as well as the *in vitro* antioxidant capacity of VOO, despite the fact that prior research has shown the positive effects of PEF on the oil yield, acidity, and phenolic compounds amount in VOO.

In this study, the olive fruit pulp was pulped using PEF technology. The goal of the current study was to determine how PEF treatment

* Corresponding authors.

E-mail address: biyang@gsau.edu.cn (Y. Bi).

<https://doi.org/10.1016/j.fochx.2024.101372>

Received 25 January 2024; Received in revised form 21 March 2024; Accepted 8 April 2024

Available online 9 April 2024

2590-1575/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

affected the following characteristics of oil: total phenolics, total flavonoids, polyphenolic fractions, pigment content, fatty acid composition, and oil yield. Additionally, the treated samples' *in vitro* antioxidant activity was assessed. To develop a theoretical framework for the use of PEF in VOO processing is the overall aim of this work.

2. Material and methods

2.1. Fruits

The olive fruits (*Olea europaea* L. cv. Koroneiki) were collected in October 2022 at the Longnan Research Institute of Economic Forestry, located at 104.85566 longitude and 33.42094 latitude. The harvested fruits were immediately crushed and processed.

2.2. Fruit crushing and homogenization

After harvesting, the fruits are washed and crushed using a high-speed multifunctional grinder (Model 304, Yongkang Bio Hardware Co., Ltd., China). They were then malaxated at approximately 30 °C for 30 min. The samples were then centrifuged using horizontal (RI6400-06, Beares International Trading Ltd., Italy) and butterfly centrifuges (RRD3504-05, Beares International Trading Ltd., Italy). The oil phase was collected and stored at 4 °C in the dark.

2.3. PEF treatment

The fruits were washed and crushed as described in section 2.2. PEF treatment was then carried out before malaxation. The method of PEF treatment was based on the method of Leone, Tamborrino, Esposto, Berardi, and Servili (2022) and Veneziani et al. (2019), with some modifications according to the actual conditions of the factory production line and the pulsed electric field equipment. The pulp was treated with PEF using a PEF device (EPULSUS-LPM1A-10 China Putten Food Equipment Co. Ltd) with a fixed pulse width of 15 s and a frequency of 20 Hz. The treatment involved an energy input of 4.6 KJ/kg, an electric field strength of 1.6 KV/cm, and an operating temperature of 25 °C. At the end of the treatment, the pulp was malaxated and centrifuged as described in section 2.2. The oil phase collected without PEF treatment was used as the control. To felicitate measurement, the samples were then stored in a dark at 4 °C.

2.4. Oil yield determination

The oil yield was determined using the method of Veneziani et al. (2019). The extraction rate was determined by calculating the percentage of the oil phase's mass relative to the mass of the olive pulp.

2.5. Determination of acidity, peroxide value, saponification value and UV absorbance value

Acidity was determined using the European Commission method EEC/2568/91. A two-gram sample was combined in a 3:2 ratio with 25 ml of acetic acid/chloroform for the analysis. The combination was then exposed to 1 ml of a concentrated iodine and potassium iodide solution, and it was left in a low-light setting for two minutes to undergo a chemical reaction. Then, 1 ml of a solution of starch and 75 ml of filtered water were added. A 0.01 M solution of sodium thiosulfate was used to neutralize the issue till the blue-violet color disappeared. The ultimate outcome was measured in micrograms (µg/g) per gram.

The method outlined by Francesco et al. (2021) was applied with the aim to figure out the peroxide value. A 0.1 g specimen was mixed with 1 ml of a 0.5% hydrochloric acid-ethanol solution, 0.10 ml of a concentrated potassium iodide solution, and 4 ml of water. The absorbance of the sample was taken at 350 nm, and the results for the peroxide value were expressed in mol/kg.

The determination of the saponification value followed the European Commission method EEC/2568/91. A 2 g sample was combined with 25 ml of a solution containing potassium hydroxide and ethanol, and subsequently subjected to boiling for 60 min. Next, a volume of 0.5 ml was utilized, to which 1 ml of an indicator made of phenolphthalein was added. Following this, a standard hydrochloric acid solution was titrated until the pink color of the indicator was eliminated. The outcome was denoted in milligrams per gram (mg/g).

The examination of K232, K270, and ΔK was carried out following the European Commission procedure EEC/2568/91. The sample was dissolved in isobutane, with a volume of 25 ml for both. The measurement of absorbance was conducted at wavelengths of 232, 270, 264, 268, and 272 nm, followed by the determination of the ΔK value. The outcome was denoted as $K^{1\%}_{1cm}$.

2.6. Determination of three saturated fatty acids and five unsaturated fatty acids

The sample was combined with 4 ml of isooctane and 0.2 ml of a 2 mol/L KOH-methanol solution. The mixture is agitated and left undisturbed until it achieves clarity. NaHSO₄ is introduced into the mixture and subsequently agitated once more. The supernatant is gathered. The analysis is conducted using gas chromatography–mass spectrometry (GC–MS). Chromatographic conditions: The chromatographic conditions involved the use of a DB-WAX column measuring 30 m × 0.25 mm × 0.25 m. The flow rate was set to 1.0 ml/min. Shunt injection was conducted with a shunt ratio of 25:1. The initial temperature was initially set to 50 °C and held for 1 min. The temperature was then increased to 200 °C and then to 230 °C and held for a duration of 12 min. The inlet temperature was kept at 250 °C. Palmitic acid (C16:0), palmitoleic acid (C16:2), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), and arachidonic acid (C20:1), with the results presented as the percentage of total fatty acids for each specific acid (Demirag and Konuskan, 2021).

2.7. Determination of β-carotene, lutein, and demagnesium chlorophyll content

A solvent mixture containing methanol, ethyl acetate, and ethanol in a volumetric ratio of 5:3:2 was used to dissolve 1.0 g. The solution was then mixed vigorously to achieve complete homogenization. Finally, the oil underwent full crystallization through freezing at –40 °C for 18 h. Subsequently, the oil was then removed through rapid filtration. After evaporating the filtrate to dryness under vacuum, the resulting residue was dissolved in an acetate solvent. It was subsequently passed through a 0.45 µm. Chromatographic conditions: ODS-BP column, with a column temperature of 25 °C and a flow rate of 1.0 ml/min. The mobile phases consist of mobile phase A, which is a 1:1 (v/v) ratio of methanol and ethyl acetate, and mobile phase B, a mixture of methanol, water, and 1 M ammonium acetate in a ratio of 8:1:1 (v/v). Gradient elution was used throughout the analysis. Gradient elution was employed for the duration of the analysis. Detection was carried out at wavelengths of 450 nm and 670 nm using an injection volume of 20 µl was used. Concentrations of lutein, β-carotene, and demagnesium chlorophyll were quantified in micrograms per gram (µg/g) as per the method described by Huang, Lu, & DI (2022).

2.8. Determination of total phenolic and total flavonoid content

Take 4 ml of the sample and add hexane and an 80% methanol solution. Centrifuge for 10 min and then collect the bottom phase. Repeat this process three times, followed by the addition of hexane to ensure thorough mixing and complete removal of any residual fat. Finally, collect the methanol phase as a sample. In a 10 ml volumetric flask, the experimental procedure involves adding 0.5 ml of folinol reagent, 1 ml of polyphenol extract, and 2 ml of a 7.5% Na₂CO₃ solution were added.

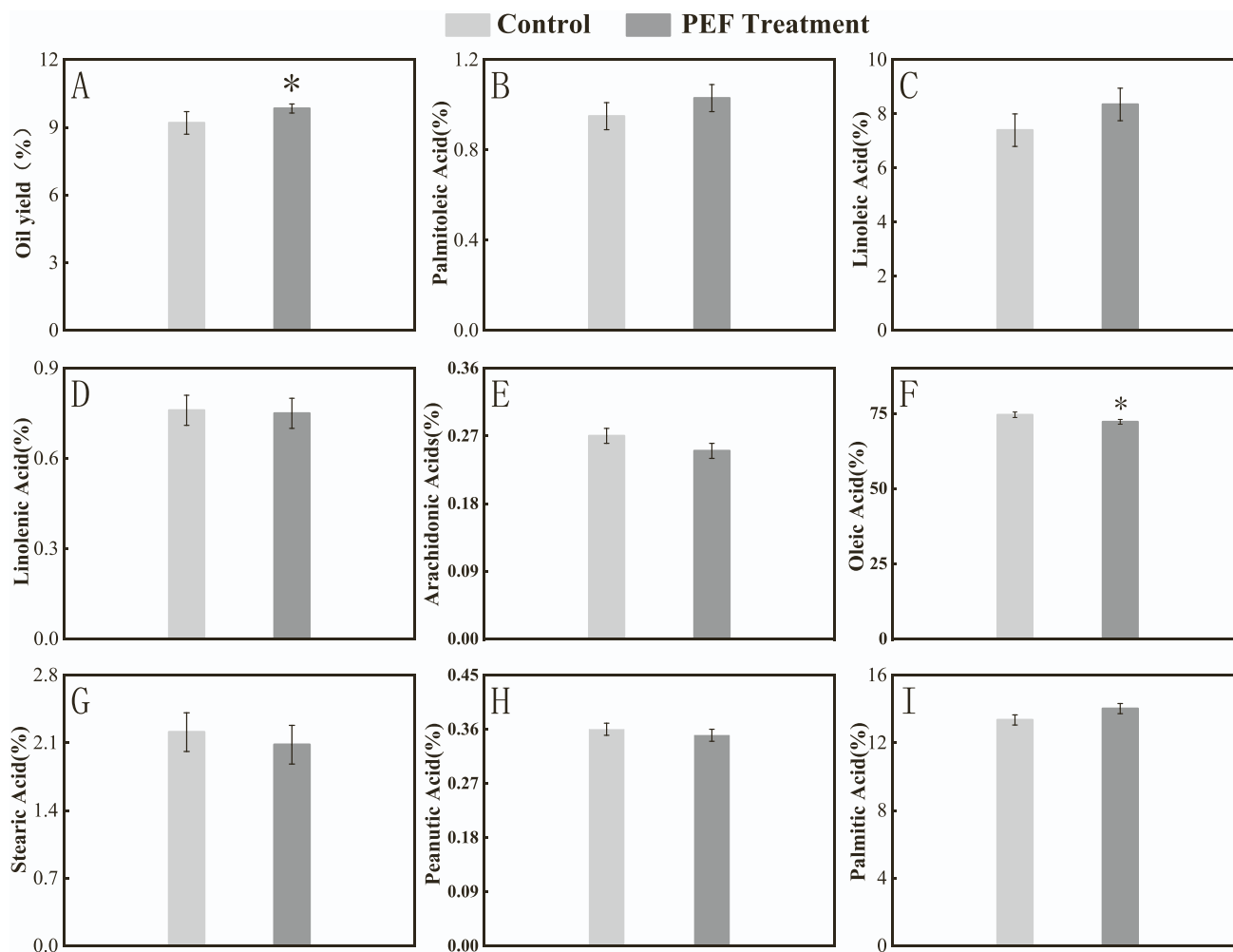


Fig. 1. Effects of PEF treatment on VOO oil yield (A) and on the content of palmitoleic acid (B), linoleic acid (C), linolenic acid (D), arachidonic acid (E), oleic acid (F), stearic acid (G), peanitic acid (H), palmitic acid (I). Bars indicate standard errors. Asterisks indicate significant differences ($P < 0.05$).

The flask was then filled with deionized water. The reaction was protected from light for 2 h. A suitable quantity of the sample stock solution was collected and the absorbance measurement was conducted at a wavelength of 765 nm. The concentration of polyphenols was indicated in micrograms per gram. The determination of flavonoids was performed using the aluminum trichloride color development method. In a 10 ml volumetric flask, 3 ml of a flavonoid extract and 4 ml of a 0.1 mol/L aluminum trichloride methanol solution were added. The mixture was shaken well and then fixed with methanol. The determination of the absorbance value was determined at 410 nm to quantify the flavonoid content expressed in $\mu\text{g/g}$ (Ikbel et al., 2023).

2.9. Determination of polyphenol monomer content

Take off 1.5 g of the material and put it in a brown volumetric container to dissolve it in hexane. After giving the mixture a good shake, the mixture was passed over a membrane with a hole size of 0.22 μm . The polyphenolic monomer content was quantified using high-performance liquid chromatography (HPLC). The separation utilized a Supelcosil silica gel column. In addition, a chromatographic column of dimensions 30 m \times 0.25 mm, packed with a 0.25 μm DB-5 stationary phase, was employed. The mobile phase was composed of a mixture of n-hexane and isopropanol in a volumetric ratio of 98.5:1.5, and it flowed at a rate of 0.8 ml/min. The column temperature was held constant at 30 $^{\circ}\text{C}$. The elution conditions were as follows: 90% to 60% (Phase A) for 0 to 40 min, and 50% to 60% (Phase A), 50 to 60 min. 0% (Phase A), 63

min, 90% (Phase A). At 295 nm, a 20 μl injection was used for the detection. The polyphenol standards were utilized in the construction of a standard curve for quantification using the external standard method. The concentrations of hydroxytyrosol, vanillin, p-coumaric acid, ferulic acid, oleuropein, lignocerosol, apigenin, and cinnamic acid are reported in mg/kg (Mateos, Espartero, & Trujillo, 2001).

2.10. Determination of tocopherols

The AOCS Ce 8–89, 1993 method is followed in the analysis of tocopherols, with high-performance liquid chromatography (HPLC) being used. Here, a fluorescent indicator is combined with a chromatographic column, SI 60–5 (LiChrosorb), that has a diameter of 250 \times 4.5 mm and a particle size of 5 μm . A combination of 95% acetonitrile and 5% distilled water is used as the mobile phase, and the flow rate is set to 0.6 $\mu\text{l}/\text{min}$. The chromatogram that is produced from the injected oil sample and the retention period of the tocopherols determine their quantity.

2.11. Determination of antioxidant activity in vitro

The DPPH radical scavenging ability was evaluated using the method outlined by Brand-Williams, Cuvelier, & Berset (1995). A sample weighing 0.1 g was combined with 1 ml of an 80% methanol solution. The mixture was stirred thoroughly and then transferred to an ice bath for 10 min to aid in extraction. The liquid above the sediment was then collected. In the reaction system, 400 μl of supernatant with a

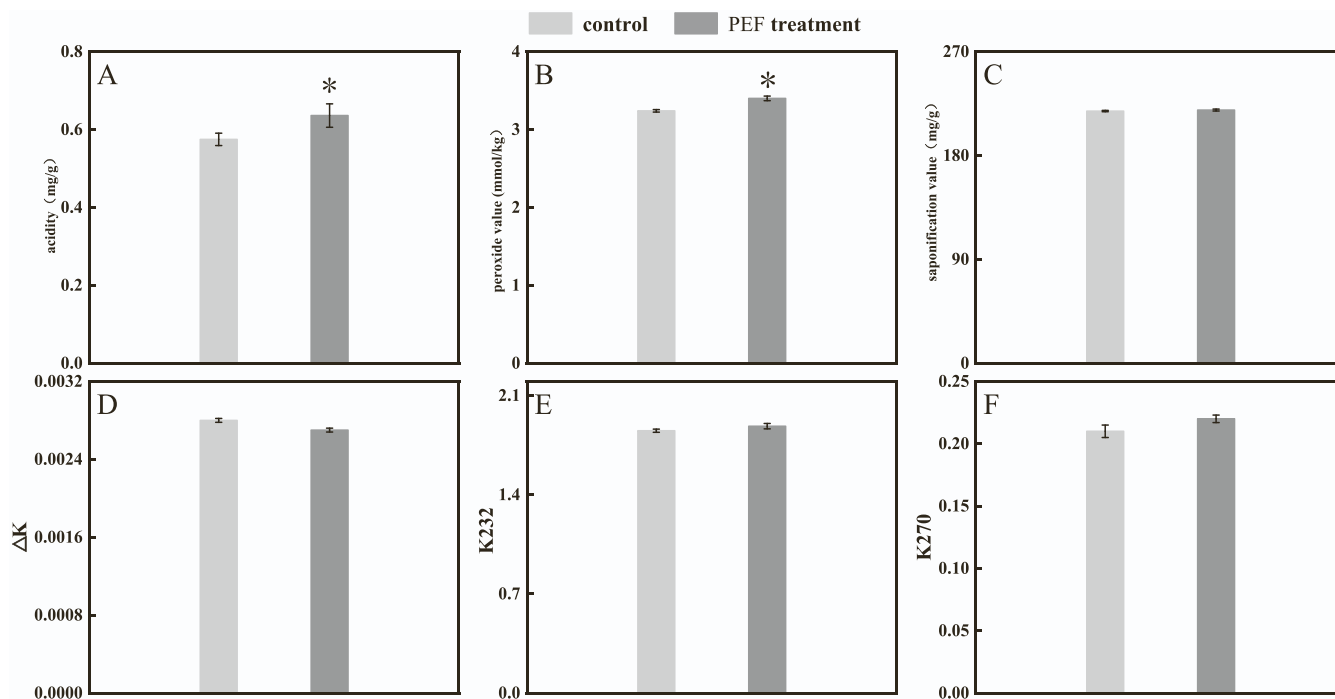


Fig. 2. Effect of PEF treatment on VOO acid value (A), peroxide value (B), saponification value (C), ΔK (D), K232 (E), and K270 (F). Bars indicate standard errors. Asterisks indicate significant differences ($P < 0.05$).

concentration of 1 mg per milliliter were combined with 600 μl of a 0.1 millimolar DPPH solution (an 80% methanol solution served as a reference). Following the blending process, the mixture was left undisturbed at a temperature of 25 $^{\circ}\text{C}$ for a period of 30 min. Subsequently, the absorbance reading was measured at 517 nm. A standard curve was generated using Trolox, and the findings were reported in terms of Trolox equivalents ($\mu\text{mol TE/g DW}$).

The free radical scavenging capacity of ABTS was assessed using the method described by Re, Pellegrini, Proteggente, and Rice-Evans (1999). 20 μl of the sample was combined with 180 μl of the ABTS reaction solution for 1 min, followed by the measurement of absorbance at 732 nm. The results were expressed in terms of Trolox equivalents ($\mu\text{mol TE/g DW}$).

The ferric reducing antioxidant power (FRAP) was assessed using the method of Jones et al. (2017). A 10 μl aliquot was combined with 0.3 ml of the FRAP reagent. The FRAP solution consisted of 40 millimolar hydrochloric acid in 300 millimolar acetate, with a pH of 3.6 in a glacial acetic acid buffer. It also contained 20 millimolar ferric chloride and 10 millimolar TPTZ, all in a ratio of 10 parts hydrochloric acid to 1 part acetate to 1 part ferric chloride to 1 part TPTZ, by volume. Afterwards, the combination was placed in an incubator set at a temperature of 37 $^{\circ}\text{C}$ for a duration of 4 min, and the measurement of absorbance was conducted at a wavelength of 593 nm. The findings were measured in relation to Trolox equivalents ($\mu\text{mol TE/g DW}$).

The oxygen radical absorbance capacity (ORAC) was assessed according to the procedure outlined by Stübler et al. (2019). Samples and various concentrations of the Trolox standard were added to each well. After incubation for 10 min, 200 μl of a 6.0 M sodium fluorescein working solution was introduced into each well. Furthermore, 20 μl of a recently prepared 119 mmol/L AAPH solution was added to each well following agitation. The fluorescence value of each well was measured every 5 min for 31 cycles using a wavelength for stimulation of 485 nm and an emission band of 538 nm. Subsequently, the ORAC value of each sample was computed. The findings were quantified in terms of Trolox equivalents ($\mu\text{mol TE/g DW}$).

2.12. Data statistics and analysis

All of the above determinations were repeated at least three times. All data were calculated using Microsoft Excel 2021 to determine the mean and standard error (\pm SE). The data were then plotted using OriginLab OriginPro 8.5 (Northampton, USA) and analyzed for significance using Duncan's test ($P < 0.05$) with SPSS 24.0.

3. Results and discussion

3.1. Effect of PEF treatment on oil yield and three saturated fatty acid and five unsaturated fatty acid content of VOO

Compared to the control (Fig. 1A), the use of PEF treatment led to a significant rise of 5.6% in the oil production of Virgin Olive Oil (VOO). This outcome aligns with the results of PEF in enhancing the oil yield of VOO (Eduardo & Martínez, 2015). PEF can cause structural changes to cell walls and membranes, which increases the possibility of distinction between the inside as well as the outside of olive pulp cells (Angersbach, Heinz and Knorr, 2000). This process ultimately accelerates the extraction of oil (Puértolas et al., 2015). The PEF intervention did not result in a notable effect on the levels of palmitoleic acid, linoleic acid, linolenic acid, arachidonic acid, stearic acid, peanutic acid, and palmitic acid (Fig. B-I). However, it reduced the oleic acid content by 3.12% (Fig. 1 F). This outcome aligns with prior research indicating that PEF treatment reduces the oleic acid concentration in peanut oil (Zeng et al., 2010). Due to its monounsaturated nature, oleic acid is prone to cleavage when subjected to PEF treatment, leading to a decrease in its content (Leone et al., 2022).

3.2. Effect of PEF treatment on VOO acid value, peroxide value, saponification value, ΔK , K232, and K270

PEF treatment significantly increased the acidity of VOO. Compared to the control group, the acidity value showed an increase of 12.5% (Fig. 2A). This result is consistent with the research of Zeng et al., (2010) and Mazroei Seydani, Gharachorloo and Asadi (2022), who

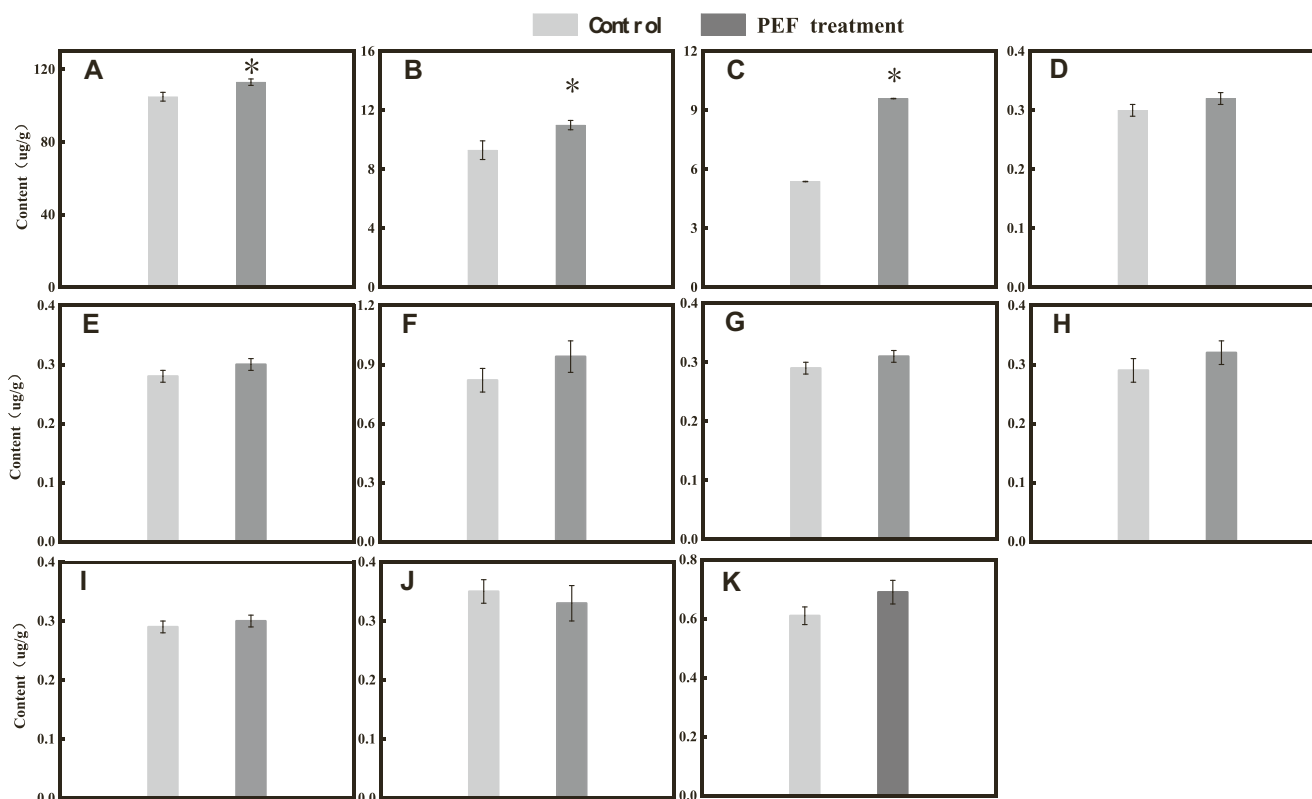


Fig. 3. Effects of PEF treatment on the content of total phenolics (A), total flavonoids (B), oleuropein (C), hydroxytyrosol (D), vanillic acid (E), luteolin (F), vanillic aldehyde (G), *p*-coumaric acid (H), ferulic acid (I), cinnamic acid (J) and apigenin (H) in VOO. Bars indicate standard errors. Asterisks indicate significant differences ($P < 0.05$).

demonstrated that PEF treatment resulted in increased acidity levels in rapeseed oil. As the electric field intensity increased, there was a gradual increase in the cell wall, thickness, which was accompanied by an increase in lipase activity. Hydrolysis of triglycerides occurred, increasing the acid concentration (Mazroei Seydani, Gharachorloo and Asadi, 2022; Guderjan, Elez-Martínez, & Knorr, 2006). In addition, an increase in the polyphenol content of the beef tallow can lead to an increase in acidity. This is because some phenols are acidic in solution and will consume NaOH during titration, resulting in an increased acidity measurement (Li, Zhang, & Wang, 2022). The peroxide value of our results were also increased by 4.49% after PEF treatment (Fig. 2B), which is consistent with the result of Zeng et al., (2010). As the process of lipid oxidation intensifies, it leads to the formation of oxidative by-products such as carbonyls, aldehydes, and conjugated double bonds. This process is also associated with increased peroxide level (Mazroei Seydani et al., 2022). Although acidity and peroxide values and were increased after PEF treatment, they were still within the limits set by EEC/2568/91. In addition, PEF treatment did not significantly affect the saponification value, K232, K270, and ΔK values of VOO (Fig. 2C-F).

3.3. PEF treatment increased the total phenolic, total flavonoid, and oleuropein content of VOO

Phenolic compounds represent a significant category of secondary metabolites found in olive oil, exhibiting antioxidant activity (Tian, Sun, Ch, Yang, & Wang, 2019). The total phenolic and flavonoid content in VOO was significantly improved by the use of PEF. The total phenolic content showed an increase of 7.6% compared to the control, while the total flavonoid content showed a remarkable increase of 18.3% compared to the control (Fig. 3A, B). A similar conclusion about the effect of PEF on the increase in total phenolic compounds in hemp oil was also reported in a study by Haji-Moradkhani et al. (2019).

According to Rajha, Maria, Khattar, Kantar, and Vorobiev (2019), the main locations for the presence of phenolics and flavonoids are the vesicles and cell walls. The using of PEF treatment results in the disruption of cell walls and cell membranes, thereby enhancing the solubilization of total phenolics and total flavonoids.

Oleuropein is the primary active compound in olive oil, which not only has remarkable free radical quenching activity but is also believed to potentially reduce the incidence of various cancers (Gentile, Uccella, & Sivakumar, 2017). According to Andrejč, Butinar, Knez, Tomazič, and Marevci (2022), Oleuropein has demonstrated properties such as being an antioxidant, having antimicrobial and antiviral effects, as well as being antiatherogenic (Wang et al., 2022). The administration of PEF led to a notable rise in oleuropein concentration, which was 76% higher than that of the control group (Fig. 3C). This was further illustrated by the results of Leone, Tamborrino, Esposto, Berardi and Servili (2022). The use of PEF treatment induces electroporation, thereby facilitating the extravasation of intracellular solutes and increasing the concentration of free oleuropein. In addition, it was discovered that PEF treatment increased β -glucosidase's enzymatic activity, which led to the release of oleuropein from oleuropein glycosides (Martínez-Navarro, Cebrián-Tarancón, Oliva, Salinas, & Alonso, 2021). The research analyzed the affection of PEF treatment on the content of four phenolic acids (vanillic acid, *p*-coumaric acid, ferulic acid, and cinnamic acid), two lignans (lignans and apigenin), and hydroxytyrosol in VOO was investigated. Based on the results, the utilization of PEF treatment did not result in a notable influence on the concentration of these substances (Fig. 3D-K).

3.4. Effect of PEF treatment on lutein, β -carotene, and demagnetized chlorophyll content of VOO

Lutein, demagnetized chlorophyll, and carotenoids were the main pigments in VOO. Furthermore, these compounds are not only

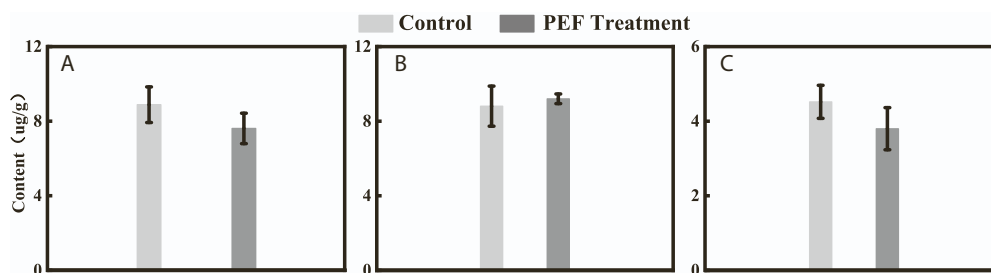


Fig. 4. Effects of PEF treatment on lutein (A), β -carotene (B), and pheophytin (C) content of VOO. Bars indicate standard errors. Asterisks indicate significant differences ($P < 0.05$).

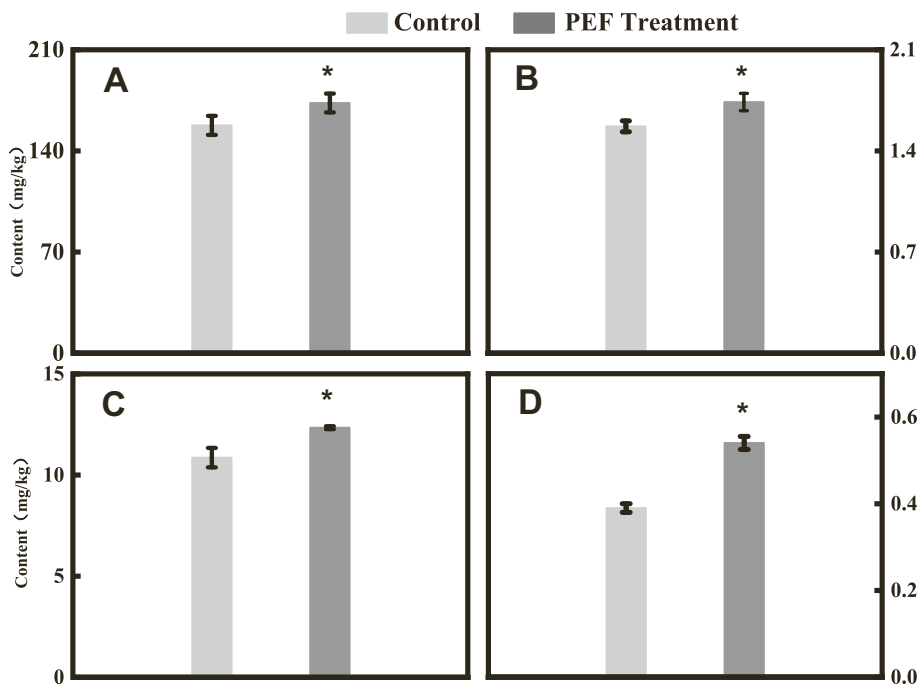


Fig. 5. Effects of PEF treatment on α -tocopherols (A), β -tocopherols (B), γ -tocopherols (C), and δ -tocopherols (D) content of VOO. Bars indicate standard errors. Asterisks indicate significant differences ($P < 0.05$).

accountable for the oil's color but also possess antioxidant and nutritional health attributes. The measurement of the product can be correlated with its quality and genuineness. (Borello & Domenici, 2019). The PEF treatments showed a decrease in lutein and de-epoxidized chlorophyll content (Fig. 4A, C) and an increase in β -carotene content (Fig. 4B). However, none of these differences were statistically significant compared to the control.

3.5. Effect of PEF treatment on α -tocopherol, β -tocopherol, γ -tocopherol, and δ -tocopherol content of VOO

Tocopherols are the primary active compounds in olive oil, known for their anti-inflammatory, antibacterial, and antioxidant properties. The most important component is α -tocopherols. PEF treatment can increase the tocopherol content in olive oil, with α -tocopherol, β -tocopherol, γ -tocopherol, and δ -tocopherol increasing by 9.8%, 10.43%, 13.63%, 38.61% and (Fig. 5A-D), respectively. These research results are consistent with the findings of Abenoza, Benito, Saldaña, et al. (2013) and other researchers.

3.6. Effect of PEF on the *in vitro* antioxidant activity of VOO

Commonly used metrics such as ORAC, FRAP, ABTS, and DPPH are

used to evaluate a substance's *in vitro* antioxidant potential (Wang, Li, Li, & Luo, 2021). PEF treatment demonstrated a significant improvement in the capacity of VOO to scavenge DPPH and ABTS+ free radicals, as well as an increase in its FRAP and ORAC. The treatment resulted in an increase of 30.0%, 61.3%, 68.1%, and 47.6%, respectively, compared to the control group (Fig. 6A-D). This result shows a certain similarity with the previous result where it was shown that PEF treatment enhanced the antioxidant capacity of walnut oil and improved DPPH (Rábago-Panduro, Romero-Fabregat, Martín-Belloso, & Welti-Chanes, 2021).

A strong and statistically significant positive connection between DPPH and the total phenolic and flavonoid content was found by correlation analysis ($r = 0.67, 0.66$). (Fig. 7.) Similarly, the ABTS assay demonstrated a strong positive correlation with both total phenolics and total flavonoids ($r = 0.74, 0.72$). Furthermore, a robust and statistically significant positive association ($r = 0.83, 0.82$) was seen by the FRAP assay between the total phenolic and total flavonoid content in VOO that had undergone PEF treatment. The Oxygen Radical Absorbance Capacity (ORAC) exhibited a robust and statistically significant positive correlation ($r = 0.71, 0.70$) with the total phenolic and total flavonoid content. In addition, there is a significant positive correlation between α -tocopherol, total phenols, and total flavonoids ($r = 0.65, 0.60$). It was discovered that the improvement of *in vitro* antioxidant activity in VOO

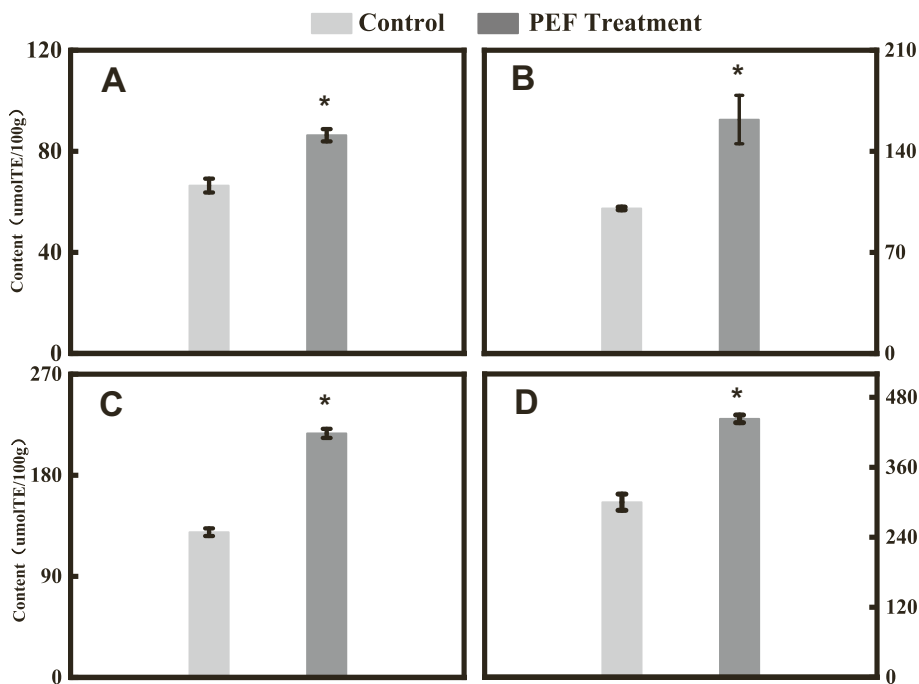


Fig. 6. Effects of PEF treatment on the free radical scavenging rate of DPPH (A) and ABTS (B), the FRAP (C), and ORAC (D) of VOO. Bars indicate standard errors. Asterisks indicate significant differences ($P < 0.05$).

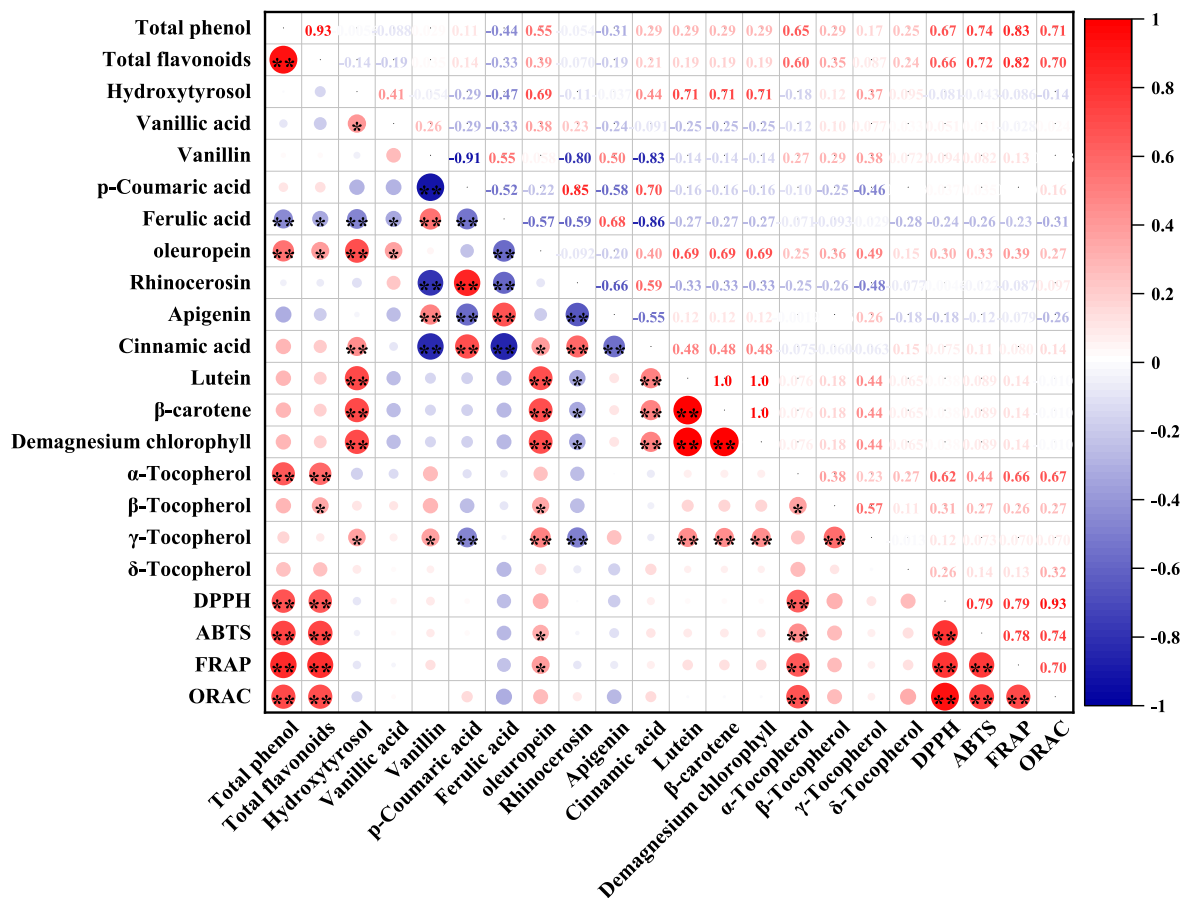


Fig. 7. Correlation analysis between active ingredients treated with PEF and antioxidant activity of VOO *in vitro* (* ≤ 0.05 , ** ≤ 0.01).

was significantly influenced by the presence of total phenolics and total flavonoids. VOO has antioxidant properties attributed to the presence of tocopherols and sterols, which are antioxidant components (Hamze et al., 2022). These elements are crucial in boosting VOO's antioxidant capabilities. Bermúdez-Oria, Rodríguez-Juan, and Rodríguez-Gutiérrez (2021) used correlation analysis to show that polyphenols are positively correlated with antioxidant activity, which is consistent with the findings of this study. However, VOO also contains antioxidant components such as sterols (Hamze et al., 2022). These elements play a role in antioxidation; however, further research is necessary to elucidate the precise mechanism through which they exert their effects.

4. Conclusion

In the current study, PEF treatment improved the oil yield in VOO. It also increased the levels of total phenolics, total flavonoids, tocopherol and oleuropein. PEF also increased the *in vitro* antioxidant activity of VOO, which was strongly associated with the increase in total phenolics, flavonoids and oleuropein. However, the application of PEF had no significant effect on the concentrations of three saturated fats, four unsaturated fats, saponification value, K232, K270, ΔK, and three pigments in VOO. In addition, PEF treatment can improve the oil yield and functional components, which can provide a basis for factory production. Considering the excellent properties of PEF treatment in improving the yield and quality of VOO, this technology should be considered for VOO processing.

CRedit authorship contribution statement

Siyuan Yang: Data curation, Writing – original draft, Writing – review & editing. **Sha Li:** Resources. **Gang Li:** Software. **Chao Li:** Investigation. **Wei Li:** Methodology. **Yang Bi:** Project administration, Funding acquisition, Conceptualization. **Juan Wei:** Validation, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to have influenced the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

Acknowledgments

This research received financial support from the following funding projects: “The Exploration, Integrated Demonstration and Application of Key Technology for High Quality Olive Oil”(21ZD4NK045) (Major Science and Technology Projects of Gansu Province); “Research on Key Technology Improvement, Equipment Development and Their Applications for Increasing Oil Extraction Rate from Fresh Olive Fruits” (2022-L · YGL-06) (Science and Technology Projects of Longnan Olive Industry Innovation Consortium in 2022).

References

- Abenoza, M., Benito, M., Saldaña, G., et al. (2013). Effects of pulsed electric field on yield extraction and quality of olive oil. *Food and Bioprocess Technology*, 6, 1367–1373. <https://doi.org/10.1007/s11947-012-0817-6>
- Andrejč, D. C., Butinar, B., Knez, Ž., Tomazič, K., & Marevc, M. K. (2022). The effect of drying methods and extraction techniques on oleuropein content in olive leaves. *Plants*, 11(7), 865–882. <https://doi.org/10.3390/plants11070865>
- Andreou, V., Kourmbeti, E., Dimopoulos, G., Psarianos, M., Katsaros, G., & Taoukis, P. (2022). Optimization of virgin olive oil yield and quality applying nonthermal

- processing. *Food and Bioprocess Technology*, 15(4), 891–903. <https://doi.org/10.1007/s11947-022-02788-2>
- Angersbach, A., Heinz, V., & Knorr, D. (2000). Effects of pulsed electric fields on cell membranes in real food systems. *Innovative Food Science and Emerging Technologies*, 1(2), 135–149. [https://doi.org/10.1016/S1466-8564\(00\)00010-2](https://doi.org/10.1016/S1466-8564(00)00010-2)
- Bermúdez-Oria, A., Rodríguez-Juan, E., & Rodríguez-Gutiérrez, G. (2021). Determination of pigments in virgin and extra-virgin olive oils: A comparison between two near UV-Vis spectroscopic techniques. *Antioxidants*, 10(12), 1858. <https://doi.org/10.3390/antiox10121858>
- Borello, E., & Domenici, V. (2019). Determination of pigments in virgin and extra-virgin olive oils: A comparison between two near UV-vis spectroscopic techniques. *Foods*, 8(1), 18–30. <https://doi.org/10.3390/foods8010018>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT- Food Science and Technology*, 28, 25–30. <https://doi.org/10.1002/elan.202060600>
- Čurko, N., Lukić, K., Tušek, A. J., Balbino, S., Tomislava, T. V., Tomašević, M., ... Ganić, K. K. (2023). Effect of cold pressing and supercritical CO₂ extraction assisted with pulsed electric fields pretreatment on grape seed oil yield, composition and antioxidant characteristics. *LWT- Food Science and Technology*, 184(000), Article 114974. <https://doi.org/10.1016/j.lwt.2023.114974>
- Demirag, O., & Konuskan, D. B. (2021). Quality properties, fatty acid and sterol compositions of east mediterranean region olive oils. *Journal of Oleo Science*, 70(1), 51–58. <https://doi.org/10.5650/jos.ess20179>
- Eduardo, P., & Martínez, D.M.I. (2015). Olive oil pilot-production assisted by pulsed electric field: Impact on extraction yield, chemical parameters and sensory properties. *Food Chemistry*, 167, 497–502. <https://doi.org/10.1016/j.foodchem.2014.07.029>
- European Union Commission. (2011). On the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of the European Communities*. No 2568/91, L248, 1-83 <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:31991R2568>
- Francesco, L., Francesco, C., Lucia, C., Luca, T., Francesco, C., & Vito, P. M. (2021). Analysis of peroxide value in olive oils with an easy and green method. *Food*, 130, 108295. <https://doi.org/10.1016/J.FOODCONT.2021.108295>
- Gentile, L., Uccella, N. A., & Sivakumar, G. (2017). Oleuropein: Molecular dynamics and computation. *Current Medicinal Chemistry*, 24(39), 4315–4328. <https://doi.org/10.2174/0929867324666170912102623>
- Guderjan, M., Elez-Martínez, P., & Knorr, D. (2006). 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(1), 55–62. <https://doi.org/10.1016/j.ifset.2006.07.001>
- Haji-Moradkhani, A., Rezaei, R., & Moghimi, M. (2019). Optimization of pulsed electric field-assisted oil extraction from cannabis seeds. *Journal of Food Process Engineering*, 42(4). <https://doi.org/10.1111/jfpe.13028>. e13028.1-13028.8.
- Hamze, L., Miserere, A., Molina, M. S., Maestri, D., Searles, P. S., & Rousseaux, M. C. (2022). Influence of environmental growth temperature on tocopherol and sterol oil concentrations in olive fruit. *Journal of the Science of Food and Agriculture*, 102(7), 2741–2749. <https://doi.org/10.1002/jsfa.11615>
- Huang, X. Y., Lu, C. H., & Di, D. L. (2022). A method for the separation and detection of pigment in olive oil based on frozen crystallization and high performance liquid chromatography. *Gansu Province. CNI15436530A*.
- Ikbil, S., Nizar, C., Monia, J., Samir, T., Ali, F., & Jean-Louis, L. (2023). Optimization and intensification of bioactive components and antioxidant activity of extracts from date fruit (*Phoenix dactylifera* L.) using pulsed electric fields (PEF) technology and thermal processing. *Processes*, 11(3), 884–892. <https://doi.org/10.3390/pr11030884>
- Jones, A., Pravadali-Cekic, S., Dennis, G. R., Bashir, R., Mahon, P. J., & Shalliker, R. A. (2017). Ferric reducing antioxidant potential (FRAP) of antioxidants using reaction flow chromatography. *Analytica Chimica Acta*, 967, 93–101. <https://doi.org/10.1016/j.aca.2017.02.032>
- Leone, A., Tamborrino, A., Esposto, S., Berardi, A., & Servili, M. (2022). Investigation on the effects of a pulsed electric field (PEF) continuous system implemented in an industrial olive oil plant. *Foods*, 11(18), 2758–2768. <https://doi.org/10.3390/foods11182758>
- Li, H. T., Zhang, X. Q., & Wang, X. M. (2022). Effect of ethyl maltol on the determination of acid value of compound seasoning. *Modern Food*, 28(11), 207–210. <https://doi.org/10.16736/j.cnki.cn41-1434/t.s.2022.11.053>
- Martínez-Navarro, M. E., Cebrián-Tarancón, C., Oliva, J., Salinas, M. S., & Alonso, G. L. (2021). Oleuropein degradation kinetics in olive leaf and its aqueous extracts. *Antioxidants*, 10(12), 1963–1973. <https://doi.org/10.3390/antiox10121963>
- Mateos, R., Espartero, L., & Trujillo, M. (2001). Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *Journal of Agricultural and Food Chemistry*, 49(5), 2185–2192. <https://doi.org/10.1021/jf0013205>
- Mazroei Seydani, L., Gharachorloo, M., & Asadi, G. (2022). Use of pulsed electric field to extract rapeseed oil and investigation of the qualitative properties of oils. *Journal of Food Process Engineering*, 45(11). <https://doi.org/10.1111/jfpe.14149>. e14149.1-14149.8.
- Mohseni, N. M., Mirzaei, H., & Moghimi, M. (2020a). Optimized extraction and quality evaluation of Niger seed oil via microwave-pulsed electric field pretreatments. *Food Science & Nutrition*, 8(3), 1383–1393. <https://doi.org/10.1002/fsn3.1396>
- Mohseni, N. M., Mirzaei, H., & Moghimi, M. (2020b). Optimization of canolol production from canola meal using microwave digestion as a pre-treatment method. *Foods*, 12(2), 318–335. <https://doi.org/10.3390/foods12020318>
- Rábago-Panduro, L. M., Romero-Fabregat, M. P., Martín-Belloso, O., & Welti-Chanes, J. (2021). Effect of pulsed electric fields (PEF) on extraction yield and stability of oil

- obtained from dry pecan nuts. *Foods*, 10(7), 1541–1552. <https://doi.org/10.3390/foods10071541>
- Rajha, H. N., Maria, A., Khattar, A., Kantar, S. E., & Vorobiev, E. (2019). Comparison of aqueous extraction efficiency and biological activities of polyphenols from pomegranate peels assisted by infrared, ultrasound, pulsed electric fields and high-voltage electrical discharges. *Innovative Food Science and Emerging Technologies*, 58, 102212–102220. <https://doi.org/10.1016/j.ifset.2019.102212>
- Re, R., Pellegrini, N., Proteggente, A., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Saari, N., Khalid, M. A., Ghanbari, R., & Anwar, F. (2012). Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.)—a review. *International Journal of Molecular Sciences*, 13(3), 3291–3340. <https://doi.org/10.3390/ijms13033291>
- Sarkis, J. R., Boussetta, N., Tessaro, I. C., Ferreira Marczak, L. D., & Vorobiev, E. (2015). Application of pulsed electric fields and high voltage electrical discharges for oil extraction from sesame seeds. *Journal of Food Engineering*, 153, 20–27. <https://doi.org/10.1016/j.jfoodeng.2014.12.003>
- Shorstkii, I., Mirshekarloo, M. S., & Koshevoi, E. (2015). Application of pulsed electric field for oil extraction from sunflower seeds: Electrical parameter effects on oil yield. *Journal of Food Process Engineering*, 40(1). <https://doi.org/10.1111/jfpe.12281>. e12281.1–12281.7.
- Stübler, A. S., Lesmes, U., Heinz, V., Rauh, C., Shpigelman, A., & Aganovic, K. (2019). Digestibility, antioxidative activity and stability of plant protein-rich products after processing and formulation with polyphenol-rich juices: Kale and kale-strawberry as a model. *European Food Research and Technology*, 245(11), 2499–2514. <https://doi.org/10.1007/s00217-019-03362-5>
- Tian, S. Q., Sun, Y., Ch, Z. C. H., Yang, Y. Q., & Wang, Y. B. (2019). Functional properties of polyphenols in grains and effects of physicochemical processing on polyphenols. *Journal of Food Quality*, 2019, 1–8. <https://doi.org/10.1155/2019/2793973>
- Veneziani, G., Esposito, S., Taticchi, A., Selvaggini, R., Sordini, B., Lorefice, A., ... Servili, M. (2019). Extra-virgin olive oil extracted using pulsed electric field technology: Cultivar impact on oil yield and quality. *Frontiers in Nutrition*, 6, 134–141. <https://doi.org/10.3389/fnut.2019.00134>
- Wang, C. H., Xue, H., Cui, Q. Z., Liu, Q. Q., Zhang, J. G., & Rao, G. D. (2022). Genome-wide identification of the cytochrome P450 superfamily in *Olea europaea* helps elucidate the synthesis pathway of oleuropein to improve the quality of olive oil. *Scientia Horticulturae*, 304(15), 111291–111301. <https://doi.org/10.1016/j.scienta.2022.111291>
- Wang, D., Li, W. X., Li, D., Li, L., & Luo, Z. S. (2021). Effect of high carbon dioxide treatment on reactive oxygen species accumulation and antioxidant capacity in fresh-cut pear fruit during storage. *Scientia Horticulturae*, 281, 109925–109934. <https://doi.org/10.1016/j.scienta.2021.109925>
- Wang, Q. J., Li, Y. F., Sun, D. W., & Zhu, Z. W. (2018). Enhancing food processing by pulsed and high voltage electric fields: Principles and applications. *Critical Reviews in Food Science and Nutrition*, 58(13), 2285–2298. <https://doi.org/10.1080/10408398.2018.1434609>
- Zeng, X. A., Han, Z., & Zi, Z. H. (2010). Effects of pulsed electric field treatments on quality of peanut oil. *Food Control*, 21(5), 0–614. <https://doi.org/10.1016/j.foodcont.2009.09.004>