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ORIGINAL ARTICLE

# Combined impact of pulsed electric field and ultrasound on bioactive compounds and FT-IR analysis of almond extract

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**Abstract** The aim of this paper is to investigate the combined impact of pulsed electric field (PEF) and ultrasound (US) to evaluate the physicochemical, bioactive compounds and chemical structure of almond extract. Almond extract was first treated with PEF and then with US. Combined treatment (PEF–US) has attained the highest value of total phenolics, total flavonoids, condense tannins, anthocyanin contents and antioxidant activity in DPPH, reducing power and metal chelating activity than all other treatments. Among all those treatments, there was slightly visible difference in the color. Moreover, FT-IR spectra indicate that the effect of PEF-US on almond extract did not produce new carbonyl compounds, but led to the higher concentration of these compounds. This study demonstrated that the PEF-US could be useful for the extraction of bioactive compounds as well as improving the stability of volatile compounds.

**Keywords** Almond · Non-thermal techniques · Bioactive compounds · FT-IR analysis

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## Introduction

Almond (*Prunus dulcis*) kernels are edible in its natural state and have a high commercial value for food industries and widely used as a main ingredient in food manufacturing (Borràs et al. 2014). On the other hand, almond nuts are good sources of micronutrients and variety of phytonutrients. Almond nut also contains significant amounts of fiber and vitamins such as vitamin E. Due to this lineup of essential and non-essential nutrients, almonds consumption have also been linked with reduction of gallstones formation, certain cancer risks, heart disease and multiple positive metabolic effects (Gama et al. 2018). To improve and preserve the nutritional profile of almond nuts it's necessary to process with novel processing technologies.

Some of the novels processing technologies are the best alternative for the thermal treatments. These novel technologies also used for extractions of bioactive compounds, such as pulsed electric field (PEF), ultrasound (US), microwave (MW) assisted extraction, high voltage electric discharges assisted extraction, supercritical fluids extraction and high-pressure assisted extraction (Gabrić et al. 2018). PEF-assisted extraction has a positive impact on obtaining valuable components from different natural sources and recently used for nonthermal pasteurization of food (Mtaoua et al. 2017). US assisted extraction increase the rates and extent of mass transfer due to its mechanical effects on the process by enhancing the penetration of solvent into the matrix due to disruption of the cell walls through acoustical cavitation (Rhazi et al. 2015). These techniques have a positive impact on yield and extraction quality with a minimum usage of organic solvent as well as energy expenditures and production time reducing. Also enhance the nutritional value of food products, due to the

minimum thermal decrepitude of heat sensitive nutrients (Aadil et al. 2013, 2018). Among these novel techniques, PEF and US have attained some attention due to their economy, simplicity, constancy, and efficiency for the detection of bioactive components (Roobab et al. 2018). The main purpose of this study was to evaluate the combined impact of these novel techniques on the phenolics, flavonoids, condense tannins and anthocyanins, antioxidant activity, volatile compounds and FT-IR analysis of almond extract isolated from the almond seeds.

## Materials and methods

### Chemicals and reagents

All these chemicals and regents were used for the determination of bioactive compounds, and antioxidant activities. Methanol; n-Hexane; Folin–Ciocalteu reagent; Sodium carbonate; DPPH (2,2-diphenyl-1-picrylhydrazyl); Gallic acid; Sodium nitrite; Aluminum chloride; Sodium hydroxide; (+)-Catechin; Vanillin; Hydrochloric acid; Sodium acetate buffer; Potassium chloride buffer; Potassium ferricyanide; Sodium phosphate buffer; Trichloroacetic acid; Ferric chloride; Ascorbic acid; Iron (II) sulfate and Ferrozine were purchased from Aladdin Industrial Corporation and Macklin Biochemical Corporation, Shanghai, China.

### Sample preparation

Almond seeds (*Prunus dulcis*) procured from local market of Guangzhou, China were ground in milling machine (YF-1000, Shanghai, China) and defatted with n-hexane. Methanolic extraction was carried out using defatted almond powder 6 g/100 ml at room temperature (40 ± 2.0 °C) for 1 h in water bath.

### Pulsed electric field (PEF) and ultrasound (US) treatments

Preliminary trails were conducted to determine the optimum conditions for PEF and US treatment. PEF treatment was done in a continuous PEF system (SCUT-PEF Team), South China University of Technology, Guangzhou, China. Almond seed extract 250 mL was pumped (Longer Pump YZ1515x, Longer Precision Pump Co., Ltd, Hebei, China) at a flow rate of 40 mL/min with electric field (EF) strength of 18 kV $cm^{-1}$ for 500 µs, pulse frequency of 1 kHz, through the treatment chamber. Digital oscilloscope (Tekway DTS1102B, USA) was used to control the input voltage and sample temperature with the help of attached coils that were submerged in a water bath to observe the temperature not exceeding 35 °C after treatment. US treatment was given to the extract 250 mL in an ultrasonic bath (SKYMEN JP-031S, Skymen Cleaning Equipment Shenzhen Co. Ltd., Shenzhen, China) at a frequency, radiation and temperature of 40 kHz, 200 W and 35 °C, respectively for 20 min. The temperature was maintained by the water circulation 0.5 L/min flow rate. Combined treatment of PEF-US was as described above. Then centrifugation (JW-3021 HR, Anhui Jiaven Equipment's Industry Co. Ltd, China) was done at 4000 rpm for 10 min at 4 °C. The extract samples (Untreated, PEF, US and PEF-US) was evaporated under vacuum at 40 °C followed by lyophilization in a freeze-dryer (Scientz-18 N, Zhejiang, China) to obtain the crude extract (powder).

### Determination of antioxidant components in almond extracts

For the determination of antioxidant components each crude extract was re-dissolved in methanol at a concentration of 10 mg/mL.

### Total phenolic contents (TPC), condense tannins, total flavonoid contents (TFC), and anthocyanins content (TAC)

TPC was measured by the method described by Ince et al. (2014) with some minor modifications. A sample solution 500 µL was mixed with 0.5 mL of Folin–Ciocalteu phenol reagent and 1 mL of dd $H_2O$. Then 2.5 mL of $Na_2CO_3$ (20%) solution were added before incubating at room temperature in the dark for 20 min. The absorbance against a blank was measured at 735 nm (TU-1810 series of UV–visible, General Analysis of General Instrument Co. Ltd., Beijing, China).

The condensed tannins were determined by the method proposed by Reátegui et al. (2014). A sample solution 100 µL was mixed with 1.5 mL of vanillin (4%) prepared with methanol and then 750 µL of concentrated HCl were added. Afterwards, incubation was done at room temperature in the dark for 20 min. The absorbance against blank was calculated at 500 nm.

TFC was determined by using the method described by The et al. (2015) with some minor changes. A sample solution 500 µL was mixed with 75 µL of 5% $NaNO_2$ solution and 1.25 mL of dd $H_2O$. After 6 min, 150 µL of $AlCl_3 \cdot H_2O$ (10%) solution were added. After 5 min, 0.5 mL of 1 M NaOH solution were added. The absorbance against blank was estimated at 510 nm.

A standard curve was prepared for TFC, TPC and condensed tannins by using Gallic acid ($y = 0.0018x - 0.0513$; $R^2 = 0.9991$), (+)-Catechin ($y = 0.0035x + 0.032$; $R^2 = 0.9988$) and (+)-Catechin

($y = 0.0055x + 0.0211$; $R^2 = 0.9995$) respectively. Results were described as mg GAE/g and mg CE/g extract (dry weight, DW). The pH-differential method was used to determine the TAC described by Lee et al. (2005).

### DPPH, reducing power and metal chelating activity

The free radical scavenging activity of the sample was calculated according to the method proposed by Lu and Yin (2018). Take different concentration (50–250 µL) of each sample mixed with 1 mM DPPH solution (50 µL) prepared with methanol was incubated in the dark at ambient temperature for 30 min. The absorbance against blank was estimated at 517 nm. The scavenging activity was calculated as the following equation:

$$\text{Scavenging effect}\% = [\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/\text{Abs}_{\text{control}} \times 100$$

$EC_{50}$ means the effective concentration of sample that can decrease DPPH concentration by 50%.

The reducing power activity was measured as described by Luengo et al. (2013). Different concentrations of almond seed extracts were mixed with 200 µL of 10 mg/mL potassium ferricyanide, and 200 µL of 0.2 M, pH 6.6 sodium phosphate buffer and then were incubated for 30 min at 50 °C, after that, 200 µL, 100 mg/mL of Trichloroacetic acid was added. The mixtures were incubated again at the same temperature for 5 min to drop the reaction process. A volume of the reaction mixture (680 µL) was mixed with distilled water (680 µL) and 68 µL of ferric chloride (10 mg/mL).

Metal chelating activity was estimated as described previously, by adding 200 µL of 0.1 mM $FeSO_4$ and 400 µL of 0.25 mM ferrozine later into 200 µL of extract (Babu et al. 2012). After that incubated at ambient temperature for 10 min, absorbance of the mixture was observed at 562 nm. Chelating activity was estimated by using the formula:

$$\%\text{chelating activity} = \left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}} \times 100$$

### Physiochemical characteristics

The pH values of samples were determined with a digital pH-meter (PHS-3S, INESA, Shanghai, China) and electric conductivity was determined by using conductivity meter (DDS-11A, Nanjing, China). The viscosity was estimated by using Brookfield viscometer (DV2-T, MA, USA) by using spindle 3 at 100 rpm and titratable acidity were measured by the method reported by Aadil et al. (2013). The hunter color values ($L^*$ $a^*$ $b^*$) of control, US, PEF and PEF-US samples were determined using a Colorimeter (CR-400 Chroma-Meter, Osaka, Japan) at room temperature (25 °C) (Mtaoua et al. 2017). The following formulas can be used to calculate the hue angle ($h^0$), saturation ($C^*$), browning Index ($BI$) and total color difference ($\Delta E$) as defined in Eqs. (1-4).

$$Hue\left(h^0\right) = tan^{-1}(b*/a*) \quad (1)$$

$$C* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (2)$$

$$BI = [100(x - 0.31)]/0.17 \quad (3)$$

$$\Delta E = \left\{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2\right\}^{1/2} \quad (4)$$

### Determination of volatile compounds

A volume of 2 µL of methanolic extract sample after solid stage extraction with sodium sulphate ($Na_2SO_4$) was used for GC/MS analysis. The analysis was performed on a GC/MS system (Mass Hunter GC/MS Acquisition, Agilent Technologies, Inc.). The GC was equipped with an HP-INNO-Wax column (dimension: 30Mts, ID: 250 µm, Film thickness: 0.25 µm). The carrier gas was helium set to flow at 1.5 mL/min. The injector was worked in split mode at the 260 °C temperature. The chromatographic employed conditions were optimized for the complete separation of the target compounds. The oven was programmed from 120 °C (3.0 min) to 250 °C with 5 °C/min and maintained for 5.0 min. The mass spectrometer (MS) was set: ion temperature at 280 °C, Electron impact ionization at 70 eV, current emission (250 µA). Contrary to the earlier reported method where SIM mode was used. The MS was functioned in the current study in taking benefit of the high compassion of the trap analyzer of ion in full scan mode. These way possible chemical interferences were eliminated.

### Fourier transforms infrared (FT-IR) spectroscopy

The IR spectra were collected using FT-IR spectrometer (Vector 33, Bruker, Ettlingen, Germany). Functional groups of the almond seed extract were measured using FT-IR. The samples were placed in potassium bromide pellet and compressed by pressure. Then, to obtain the spectrum, samples were placed in the light path to allow the infrared light to pass through them. The spectra were determined in the average IR range (400–4000 $cm^{-1}$), with 4 $cm^{-1}$ resolution.

### Statistical analyses

Statistical analyses were conducted using SPSS software, version 16.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA (analysis of variance) was used to describe significant differences between means. Duncan's Multiple Range test was performed to carry out comparisons between means. The significance level was defined as $p < 0.05$. All analyses were done in triplicates.

## Results and discussion

### Impact of PEF, US and PEF-US on TPC and TFC

As compared to the untreated extract, all treatments (PEF, US and PEF-US) have a significant ($p < 0.05$) increase in TPC and TFC (Table 1). The increase in TPC with US treatment might be due to the release of bound form of phenolics caused by rupture cell membranes through cavitation process (Aadil et al. 2013). A similar outcomes in TPC and TFC was observed for nettel by US and MW treatment (Ince et al. 2014), and for grape by-product extract by US, PEF and high hydrostatic pressure (HHP) treatment (Corrales et al. 2008). Similar results were observed for PEF treatment for ginseng root extraction (Lu and Yin 2018). A significant increase in TPC and TFC might be due to the effect of US and PEF with enzymes. The highest levels of TPC and TFC in PEF-US treatment may be due to the complementary effects of both techniques on the extract. The earlier study on combined US and MW treatment showed the better extraction of bioactive compounds from soybean germ oil (Cravotto et al. 2008). This study showed that PEF-US may be better for higher extraction of bioactive compound especially TPC and TFC.

### Impact of PEF, US and PEF-US on total anthocyanins and condense tannin contents

Treated methanolic extract (PEF, US and PEF-US) showed significant ($p < 0.05$) increase in anthocyanins and condense tannin contents than untreated extract (Table 1). The change in anthocyanin contents were observed by the cavitation process that resulted into disintegrated particles increase in the diffusion rates (Tiwari et al. 2009). Earlier, a significant increase in anthocyanins for grape skin treated by PEF and US was observed (Corrales et al. 2008) and also in red cabbage treated by US (Ravanfar et al. 2015). During PEF treatment, electric field intensity higher than the cell membrane potential punctured the membrane causing improvement in dissolution rate (Zhou et al. 2015). PEF-US treatment also showed a significant increase in total anthocyanins as compared to all other treatments and similar results for black berry bagasse treated with combined supercritical $CO_2$ and US was observed (Reátegui et al. 2014). Moreover, a significant increase in condense tannin contents were observed in MW treated black wattle barks (Rhazi et al. 2015) which supports our findings. The possible reason for the increase in PEF-US treatment might be due to some chemical effects of PEF and US that prove to be best treatment.

### Impact of PEF, US and PEF-US on DPPH activity

US, PEF and PEF-US treated extract showed significant ($p < 0.05$) increase in DPPH activity than untreated extract (Table 2). PEF-US treated extract exhibited the highest scavenging activity followed by PEF and US. PEF under certain electric field strengths brought protein structural changes, such as the quaternary structure, molecular weight, polarization of molecule, which may decrease or increase DDPH radical inhibition (Lin et al. 2012). US treatment was hydroxylation of flavanols causing positive impact on antioxidant activity (Soria and Villamiel 2010). Earlier reported that powerful scavenging DPPH activity was observed in PEF treated ginseng root extract as

**Table 1** Effect of PEF, US and PEF-US treatment on TPC, TFC, condense tannins and anthocyanin contents of almond extract

| Treatments | Total phenolics (mg GAE/g dried extract) | Total flavonoids (mg CE/g dried extract) | Condense tannin content (mg CE/g dried extract) | Total anthocyanins (mg/L) |
|---|---|---|---|---|
| Untreated | $15.90 \pm 0.10^{d}$ | $7.13 \pm 0.046^{d}$ | $0.853 \pm 0.006^{d}$ | $0.882 \pm 0.08^{c}$ |
| US | $18.38 \pm 0.09^{c}$ | $8.41 \pm 0.058^{c}$ | $1.05 \pm 0.008^{c}$ | $1.051 \pm 0.03^{b}$ |
| PEF | $19.22 \pm 0.12^{b}$ | $8.60 \pm 0.041^{b}$ | $1.18 \pm 0.007^{b}$ | $1.103 \pm 0.04^{b}$ |
| PEF-US | $21.20 \pm 0.06^{a}$ | $9.72 \pm 0.051^{a}$ | $1.48 \pm 0.013^{a}$ | $1.283 \pm 0.09^{a}$ |

Mean values in a column with different letters (a–d) are significantly different ($p < 0.05$)

*GAE* gallic acid equivalent, *CE* catechin equivalent

**Table 2** Effect of PEF, US and PEF-US treatment on $EC_{50}$values of DPPH radical scavenging and antiradical activity of almond extract

| Treatments | Mean Reduction (%) | $EC_{50}$ Value (DPPH radical scavenging activity) | Slop ± SD | $X^2$ (DF) | [a]Antiradical activity |
|---|---|---|---|---|---|
| Untreated | 36.872[d] | 27.576 (12.12–36.22)[d] | 1.877 ± 0.27 | 0.733 (3) | 0.036 ± 0.002[a] |
| US | 39.434[c] | 23.938 (11.93–36.86)[c] | 1.824 ± 0.26 | 0.367 (3) | 0.042 ± 0.003[a] |
| PEF | 40.336[b] | 21.957 (10.50–33.57)[b] | 1.778 ± 0.25 | 0.507 (3) | 0.046 ± 0.002[a] |
| PEF-US | 42.870[a] | 19.588 (09.02–30.59)[a] | 1.752 ± 0.28 | 0.647 (3) | 0.051 ± 0.004[a] |

Mean values in a column with different letters (a–d) are significantly different ($p < 0.05$); $EC_{50}$: effective concentration of sample that can decrease DPPH concentration by 50%; [a]Antiradical activity: $1/EC_{50}$. $EC_{50}$ value of DPPH

compared to hydrolytic enzyme processing (Lu and Yin 2018), US treated whole mung bean, hull and cotyledon (Singh et al. 2017) as well as for US, PEF and HHP treated grape skin extracts (Corrales et al. 2008). The significant increase of DPPH was recorded for PEF-US may be due to the synergistic effect of both treatments. The results were in agreements with the previous findings obtained for combined treatment MW and PEF for extract from canola seed cake (Teh et al. 2015).

## Impact of PEF, US and PEF-US on reducing power and metal chelating activity

The potential of plant extracts to reduce $Fe^{+3}$ to $Fe^{+2}$ may serve as a notable indicator of its antioxidant potential. The reducing power of the compounds has been used to assess their ability to donate an electron (Maksimović et al. 2005). US, PEF, and PEF-US treatment significantly increased the reducing power and metal chelating activity of almond extract (Fig. 1a, b). Similar results of reducing power were observed for orange peel extract treated with PEF (Luengo et al. 2013) and adinandra nitida leave extract treated with US (Liu et al. 2013). The highest reducing power was observed for PEF-US may be due to higher TPC in the almond extract. The findings are in agreement with the previous results reported for combined treatment of MW and PEF for extract from canola seed cake (Teh et al. 2015). Significant increase in metal chelating ability after US assisted extraction of bioactive compounds for banana was also reported (Babu et al. 2012). This may be change in polarization and structure by PEF. As a result, it may be easier to provide electrons, so that the antioxidant capacity was increased (Lin et al. 2012).

## Impact of PEF, US and PEF-US on optical properties

Hunter color values ($L^*$, $a^*$, and $b^*$) of PEF, US and PEF-US treated extracts are shown in Table 3. The $a^*$ value was the lowest for PEF-US and highest for the untreated extract. Results indicated that decrease in a* value reflecting increase in greenness. However, $b^*$ value for PEF-US was significantly higher than PEF, US and untreated extract. $L^*$ value was decreased after PEF, US and PEF-US methods. An increase in $b^*$ value was observed in PEF, US and PEF-US than untreated, reflecting increase in yellowness. Moreover, the total color difference was lower for PEF–US while the highest for US. Similarly, C* and BI values were highest for PEF-US treatment than other methods. The change in color caused by PEF may be linked with the electroporation and release of intracellular content (Grimi et al. 2010). While variation in color by US treatment may have occurred by carotenoids isomerization reactions or producing of free radicals (Santhirasegaram et al. 2013). Mtaoua et al. (2017) did not find any significant changes by applying high intensity PEF to date palm extract. Bioactive compounds can also react with each other and other compounds, such as artificial polymeric compounds generated during processing through non-enzymatic reactions (Shoji 2007).

## Impacts of PEF, US and PEF-US on physicochemical properties

In this study, viscosity, pH and TA was not significantly affected by PEF, US and PEF-US than untreated extract (Table 3). Earlier reported, US treatment was not significantly changed the pH and acidity of vegetable protein and PEF treatment not changed viscosity of olive oil extract (Abenoza et al. 2013; O'sullivan et al. 2016). Overall EC of liquid food products is due to the presence of nutrients like vitamins, minerals, proteins and lipids (Martín et al. 1994). A significant increase in EC was found by PEF, US and PEF-US than untreated extract. Similar findings regarding EC were recorded for combined treatment US and ohmic hydro-distillation for extract from zenyan oil (CVETKOVIĆ et al. 2018). Protein mixture results were 1.861, 1.927 and 1.944 mg/mL for US, PEF, and PEF-US, and a significant difference ($p < 0.05$) was observed in PEF-US sample than control (1.80).

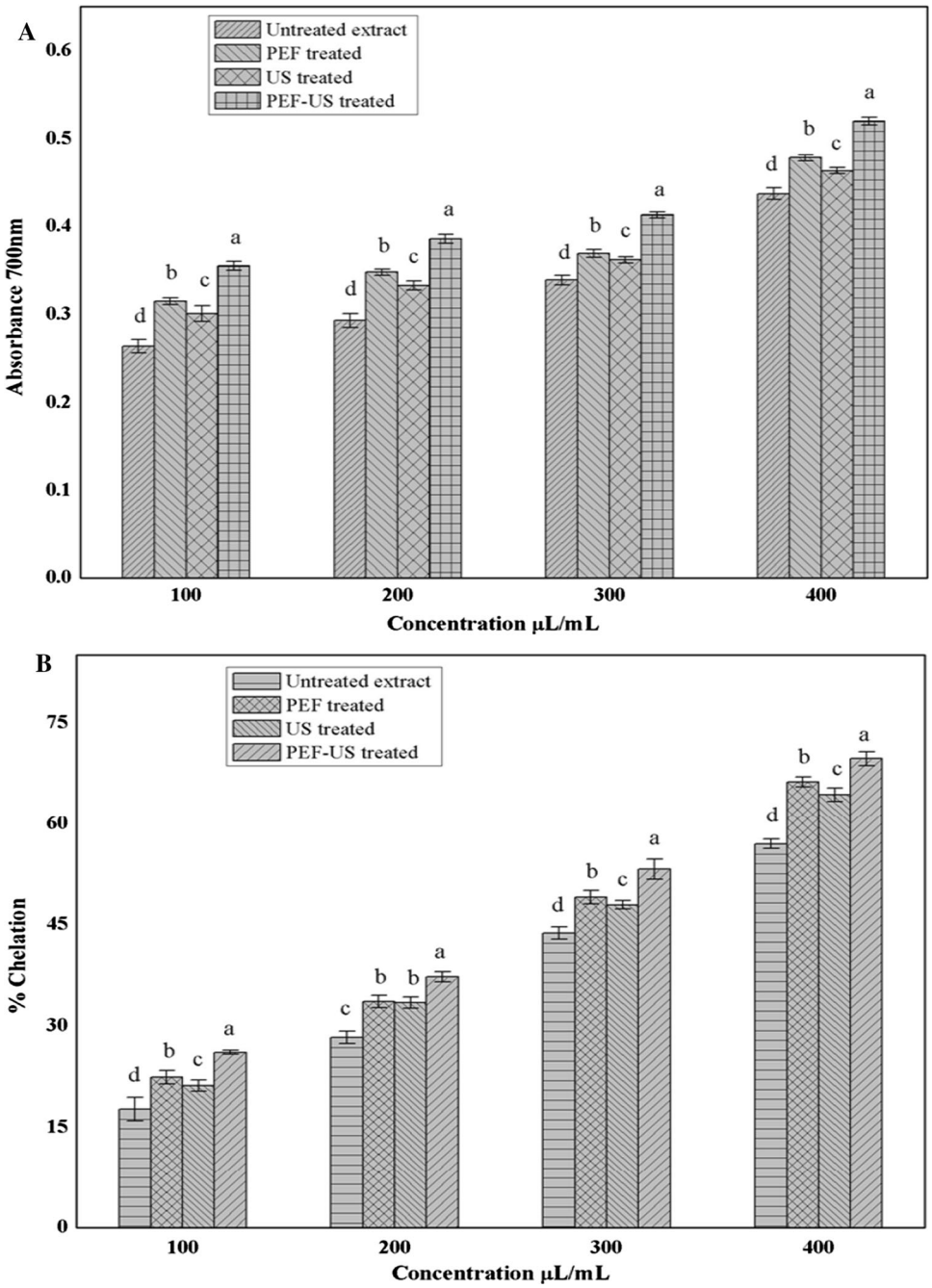


**Fig. 1** Effect of PEF, US and PEF-US treatment on reducing power assay (**a**), metal chelating activity (**b**) of almond extract

## Impact of PEF, US and PEF-US on volatile compounds

The highest number of compounds and highest peak percent area were found in PEF-US treated extract followed by PEF and US (Table 4). According to their functional groups, methyl, ester, ether and acid compounds were found in higher proportion during all treatment than untreated extract. Almond extract can be treated with nonthermal technologies to increase the extraction of volatile compounds, the same trend in other kinds of wine produced from different raw materials was reported (Saldaña et al. 2017). Methyl esters are a kind of fatty acid ester that is obtained by transesterification of fats with methanol, these compounds were identified may be reaction of some fat residues and methanol. PEF might because considerable change in volatile compounds could possibly be due to an enhanced release of matrix-bound compounds (Mtaoua et al. 2017). PEF treatment supported the intracellular contents extraction, through permeabilization resulting into more yield, enhance extraction efficiency and intracellular metabolites extraction (Mtaoua et al. 2017).

**Table 3** Effect of PEF, US and PEF-US treatment on color, viscosity, electrical conductivity (EC), pH, titratable acidity (TA) and protein mixture of almond extract

| Treatments | Color parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | *L** | *a** | *b** | [a]*Hue* (h°) | [b]*C** | [c]*BI* | [d]ΔE |
| Untreated | 50.69 ± 0.66[a] | − 1.01 ± 0.03[a] | 5.10 ± 0.39[c] | − 78.91 ± 0.28[c] | 5.19 ± 0.38[c] | 9.070 ± 0.95[c] | – |
| US | 50.24 ± 0.05[a] | − 1.14 ± 0.02[a] | 5.43 ± 0.04[b] | − 78.14 ± 0.23[b] | 5.54 ± 0.04[b] | 9.420 ± 0.04[b] | 1.27 ± 0.87[a] |
| PEF | 49.48 ± 0.68[b] | − 1.11 ± 0.07[a] | 5.23 ± 0.05[b] | − 78.01 ± 0.20[b] | 5.35 ± 0.07[b] | 9.657 ± 0.05[b] | 0.81 ± 0.37[b] |
| PEF-US | 50.35 ± 0.10[a] | − 1.26 ± 0.01[b] | 5.74 ± 0.06[a] | − 77.61 ± 0.25[a] | 5.88 ± 0.06[a] | 10.222 ± 0.29[a] | 0.90 ± 0.38[b] |

| | Viscosity (mPa/s) | EC (µs/cm) | pH | TA (%) | Total Protein (mg/mL) |
|---|---|---|---|---|---|
| Untreated | 1.66 ± 0.06[b] | 229.6 ± 4.5[d] | 7.06 ± 0.01[b] | 0.13 ± 0.07[a] | 1.80 ± 0.005[d] |
| US | 1.67 ± 0.08[b] | 242.1 ± 3.6[c] | 7.04 ± 0.02[a] | 0.14 ± 0.06[a] | 1.861 ± 0.01[c] |
| PEF | 1.68 ± 0.09[b] | 251.4 ± 3.0[b] | 7.03 ± 0.03[a] | 0.14 ± 0.08[a] | 1.927 ± 0.01[b] |
| PEF-US | 1.70 ± 0.07[a] | 260.3 ± 3.05[a] | 7.02 ± 0.05[a] | 0.15 ± 0.09[a] | 1.944 ± 0.02[a] |

Mean values in a column with different letters (a–d) are significantly different ($p < 0.05$); [a]Hue ($h^0$): $\tan^{-1}$ (b*/a*); [b]C*: Chroma values; [c]BI: Browning Index; [d]ΔE: Color difference

The increase in components with US treatment may be due to the release of bound form of phenolics caused by rupture cell membranes through cavitation process (Aadil et al. 2013). Similar comparative results were observed for US, MW, hydro-distillation, soxhlet extraction and cold maceration treatment for *Lonicera macranthoides* volatile frictions (Wu et al. 2015) as well as MW treated essential oil from aromatic herbs (Lucchesi et al. 2004). A significantly higher amount of volatile compounds were identified after MW treatment from *Marchantia convoluta* as compared to phytosol extraction (Yan et al. 2008) as well as MW assisted hydro-distillation treated essential oil from cinnamomum cassia as compare to hydro-distillation (Jeyaratnam et al. 2016).

## Impact of PEF, US and PEF-US on FT-IR profile

The infrared spectroscopy characteristic absorption band of the almond extract mostly appeared in wave number and also presented that the treated and untreated extract spectra have the same band positions which indicate that identical compounds were observed in each extract (Fig. 2). Thus, the differences between untreated, US, PEF, and PEF-US extract were the percentages of transmittance of the identical carbonyl. The carboxylic acid existing in the almond extract is presented by the broad absorbance peak of O–H stretching vibration between 2400 and 3400 $cm^{-1}$. This observation clearly confirms that the presence of carboxylic acid because the O–H stretch appeared in very broad band spectrum which centers on 3000 $cm^{-1}$ and somewhat hides the C–H stretching bands (Pavia et al. 2008). The C–H deformation vibrations between 1350 and 1475 $cm^{-1}$, strong absorbance peak of C–H vibrations between 2800 and 3000 $cm^{-1}$ indicates the presence of alkanes. Absorption bands at 2924–2928 $cm^{-1}$ and 2852–2859 $cm^{-1}$ correspond to symmetric and asymmetric stretching vibrations of methyl ($CH_3$) groups, respectively. The narrow and sharp band recorded at 1750 $cm^{-1}$ is indicated C=O stretching vibration of ester groups (Szalontai et al. 2003). While some ester carbonyl groups appear as ketones in same general area, in this study ketones can be terminated by recording the broad and strong C–O stretching vibrations that appeared in region 1000–1300 $cm^{-1}$ where absorption of ketones appeared as narrower and weaker bands. The aliphatic secondary amines existing near 1500 $cm^{-1}$, the N–H bending vibration was very weak and normally not recorded. The major differences in transmittance percentage of US, PEF and PEF-US can be found from three compounds: amine, ester and carboxylic acids. The increase in transmittance percentage was recorded in ester (at 1000–1300 $cm^{-1}$), carboxylic acid (around 3412 $cm^{-1}$) and primary and secondary amines (around 1535 and 1637 $cm^{-1}$; while decreases were observed in alkanes (around 2856 and 2924 $cm^{-1}$) ester (around 1246 and 1750 $cm^{-1}$ respectively). The increase in transmittance percentage in ester and decrease in primary and secondary amines might be due to the release of volatiles and formation of color respectively, during PEF, US and PEF-US treatments. Outcomes of the PEF, US and PEF-US described major changes in the transmittance of the carbonyl compounds. Finally, there was significant decrease in transmittance percentage of untreated, PEF, US and PEF-US treated extract in term of esters formation and significant increase in transmittance percentage in term of primary, secondary amines and carboxylic acid.

**Table 4** Effect of PEF, US and PEF-US treatment on volatile compounds of almond extract

| %Area | | | | Component |
|---|---|---|---|---|
| Untreated | US | PEF | PEF-US | |
| 1.18 | 1.67 | 1.44 | – | Paromomycin |
| 9.53 | 54.2 | 14.55 | 9.09 | Dimethyl ether |
| 0.29 | 0.36 | – | – | L-Glucose, 6-deoxy-3-O-methyl- |
| 1.99 | 2.85 | 3.16 | – | Acetic acid, methoxy- |
| 0.2 | 2.95 | 2.34 | 2.74 | D-Mannosamine |
| 0.33 | – | 0.58 | 0.37 | Bicyclo [6.2.0] decan-9-one, 10,10-dichloro- |
| 0.53 | 1.09 | 1.11 | 1.29 | Benzenepropanoic acid, alpha.-(hydroxyimino)- |
| 0.28 | – | – | – | Melezitose |
| 0.41 | – | – | – | D-Streptamine, O-6-amino-6-deoxy-.alpha. -D-glucopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-(methylamino)-.beta.-L-arabinopyranosyl-( |
| 1.19 | – | – | – | 2-Azido-2,4,4,6,6-pentamethylheptane |
| 1.19 | 1.68 | 1.38 | – | 11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester |
| 0.25 | – | – | 0.48 | [1-(3,3-Dimethyloxiran-2-ylmethyl)-3,7-dimethylocta-2,6-dienyl] trimethylsilane |
| 0.27 | – | – | – | Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl- |
| 2.67 | 5.42 | 100 | 28.17 | 1-Heptatriacotanol |
| 2.57 | 4.83 | 3.46 | 4.56 | 9-Octadecenoic acid, methyl ester, (E)- |
| 13.71 | 13.61 | 11.8 | 13.43 | Estra-1,3,5(10)-trien-17. beta. -ol |
| 1.46 | 0.77 | 0.86 | 0.82 | 2-Myristynoyl pantetheine |
| 100.0 | 100.0 | 83.25 | 100 | 6-Octadecenoic acid |
| – | 9.97 | 2.63 | 1.67 | Octaethylene glycol monododecyl ether |
| – | 0.44 | – | – | Alpha-l-rhamnopyranosyl |
| – | 0.44 | – | – | 4-Amino-1,5-pentandioic acid |
| – | 0.76 | – | – | Tetrahydro-22-desoxy-tomatillidine |
| – | 0.34 | – | – | 1-Pyridinepropanoic acid, hexahydro-3-(hydroxymethyl)- |
| – | 2.1 | 1.68 | 2.07 | 1,3-Propanediol, 2-methyl-, dipropanoate |
| – | 0.29 | 0.24 | – | 5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dimethyl-.alpha.-methylene-2-oxo-, methyl ester |
| – | 0.46 | 0.28 | 0.56 | 1,25-Dihydroxyvitamin D3, TMS derivative |
| – | – | 0.25 | – | [1,1′-Bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester |
| – | – | 0.20 | – | 13,16-Octadecadiynoic acid, methyl ester |
| – | – | 0.23 | – | 2-Trimethylsiloxy-6-hexadecenoic acid, methyl ester |
| – | – | 0.27 | 0.38 | 4-(cis-2,3,4,trans-6-Tetramethyl-3-cyclohexenyl)-2-butanone semicarbazone |
| – | – | 0.28 | 0.30 | 3,6,9,12-Tetraoxatetradecan-1-ol, 14-[4-(1,1,3,3-tetramethylbutyl) phenoxy]- |
| – | – | 0.26 | 0.32 | 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2′,3′]pyrrolo[4′,5′-d]pyridazine |
| – | – | 0.21 | – | 9,10-Secochoesta-5,7,10(19)-triene-3,24,25-triol, (3.beta.,5Z,7E)- |
| – | – | 10.84 | 48.64 | Bis (2-ethylhexyl) phthalate |
| – | – | 0.20 | 20.45 | 1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis |
| – | – | – | 0.44 | Beta-d-Allopyranoside, methyl 6-deoxy-2-O-methyl- |
| – | – | – | 3.05 | Thietane, 2,4-dimethyl- |
| – | – | – | 0.43 | 2,5-Octadecadiynoic acid, methyl ester |
| – | – | – | 0.20 | 1-Tributylsilyloxy-3-phenylpropane |
| – | – | – | 0.73 | Tetrahydro-22-desoxy-tomatillidine |
| – | – | – | 0.23 | Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2′-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)- |
| – | – | – | 1.58 | 1-Pentanone, 1-(p-anisyl)-3-methyl- |
| – | – | – | 2.57 | Gamma Sitosterol |
| 138.05 | 204.23 | 241.5 | 244.57 | Total |

* (−): not detected

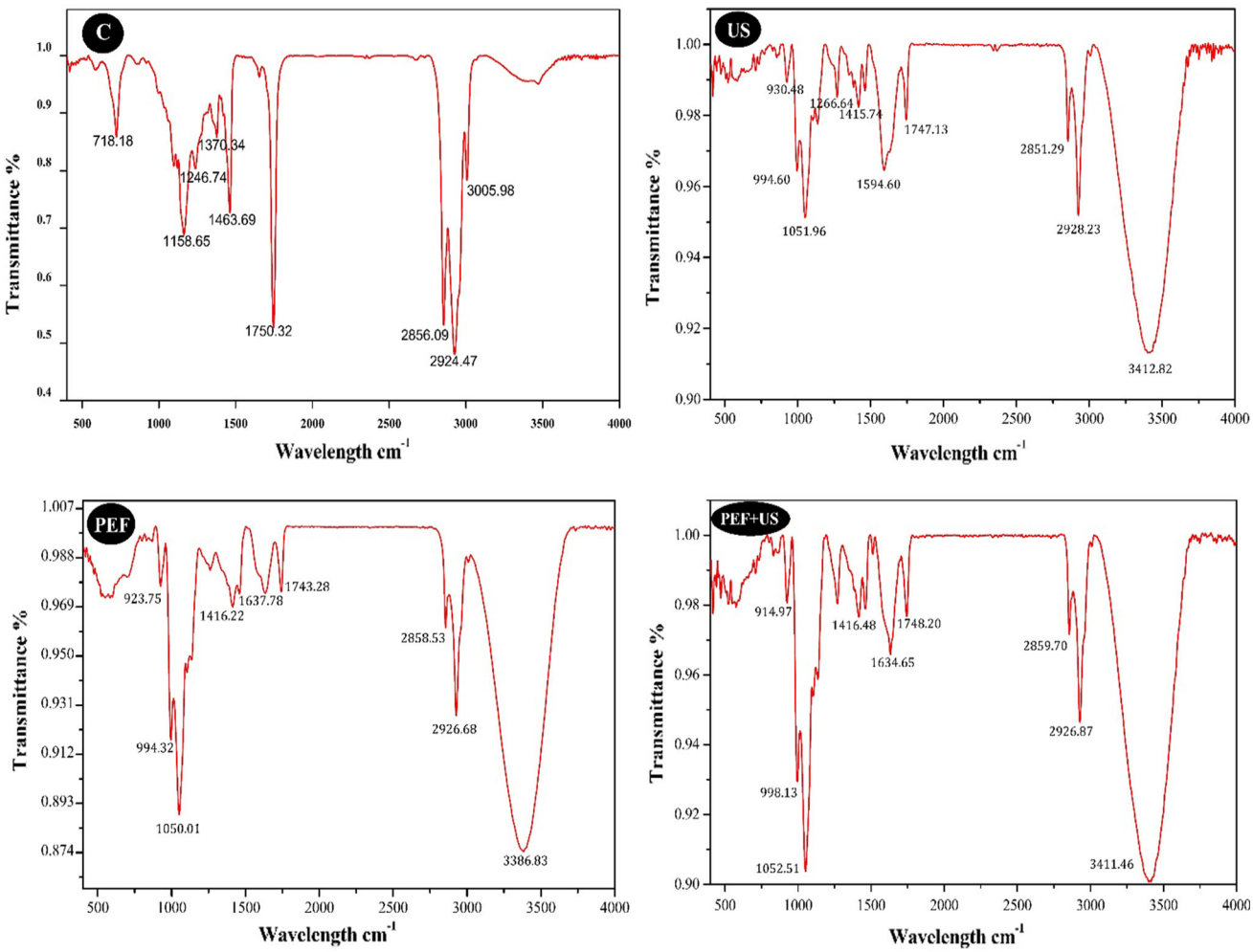


**Fig. 2** Effect of PEF, US and PEF-US treatment on Fourier transforms infrared spectra (FT-IR) of almond extract

## Conclusion

Our findings confirmed that combination of nonthermal technologies is a useful alternative technique in the food processing industries but economically little expensive as compare to individuals. This study depicts combined treatment (PEF-US) resulted in improvement of TPC, TFC, DPPH activity, reducing power and metal chelating activity. Moreover, also improved condenses tannins, anthocyanins and number of volatiles compounds. The FT-IR indicates processing through PEF, US and PEF-US did not produce new carbonyl compounds, but led to increases in concentration as indicated by the percentage of transmittance of compounds. In general, combined treatment can be used at commercial level to produce the safe, healthy and high-quality foods to increase the market value.

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## References

Aadil RM, Zeng X-A, Han Z, Sun D-W (2013) Effects of ultrasound treatments on quality of grapefruit juice. Food Chem 141:3201–3206

Aadil RM et al (2018) Combined effects of pulsed electric field and ultrasound on bioactive compounds and microbial quality of grapefruit juice. J Food Process Preserv 42:e13507

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

Babu MA, Suriyakala M, Gothandam K (2012) Varietal impact on phytochemical contents and antioxidant properties of *Musa acuminata* (banana). J Pharm Sci Res 4:1950

Borràs E, Amigo JM, van den Berg F, Boqué R, Busto O (2014) Fast and robust discrimination of almonds (*Prunus amygdalus*) with respect to their bitterness by using near infrared and partial least squares-discriminant analysis. Food Chem 153:15–19

Corrales M, Toepfl S, Butz P, Knorr D, Tauscher B (2008) Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. Innov Food Sci Emerg Technol 9:85–91

Cravotto G, Boffa L, Mantegna S, Perego P, Avogadro M, Cintas P (2008) Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. Ultrasonics sonochem 15:898–902

Cvetković A, Jurina T, Valinger D, JURINJAK TUŠEK A, Benković M, GAJDOŠ KLJUSURIĆ J (2018) The estimation of kinetic parameters of the solid-liquid extraction process of the lavender flower (Lavandula × hybrida L.). Croat J Food Sci Technol 10:64–72

Gabrić D, Barba F, Roohinejad S, Gharibzahedi SMT, Radojčin M, Putnik P, Bursać Kovačević D (2018) Pulsed electric fields as an alternative to thermal processing for preservation of nutritive and physicochemical properties of beverages: a review. J Food Proc Eng 41:e12638

Gama T, Wallace HM, Trueman SJ, Hosseini-Bai S (2018) Quality and shelf life of tree nuts: a review. Sci Hortic 242:116–126

Grimi N, Mamouni F, Lebovka N, Vorobiev E, Vaxelaire J (2010) Acoustic impulse response in apple tissues treated by pulsed electric field. Biosys Eng 105:266–272

Ince AE, Sahin S, Sumnu G (2014) Comparison of microwave and ultrasound-assisted extraction techniques for leaching of phenolic compounds from nettle. J Food Sci Technol 51:2776–2782

Jeyaratnam N, Nour AH, Akindoyo JO (2016) Comparative study between hydrodistillation and microwave-assisted hydrodistillation for extraction of Cinnamomum Cassia oil. J Eng Appl Sci 11:2647–2652

Lee J, Durst RW, Wrolstad RE (2005) Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Inter 88:1269–1278

Lin S, Guo Y, You Q, Yin Y, Liu J (2012) Preparation of antioxidant peptide from egg white protein and improvement of its activities assisted by high-intensity pulsed electric field. J Sci Food Agri 92:1554–1561

Liu B, Ma Y, Liu Y, Yang Z, Zhang L (2013) Ultrasonic-assisted extraction and antioxidant activity of flavonoids from Adinandra nitida leaves. Trop J Pharm Res 12:1045–1051

Lu CW, Yin YG (2018) Comparison of antioxidant activity of ginseng root extracts obtained by pulsed electric field and hydrolytic enzyme processing. IOP Conf Ser: Earth Environ Sci 185(1):157

Lucchesi ME, Chemat F, Smadja J (2004) Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. J Chromatogr A 1043:323–327

Luengo E, Álvarez I, Raso J (2013) Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields. Innov Food Sci Emerg Technol 17:79–84

Maksimović Z, Malenčić Đ, Kovačević N (2005) Polyphenol contents and antioxidant activity of Maydis stigma extracts. Biores Technol 96:873–877

Martín O, Zhang Q, Castro A, Barbosa-Cánovas G, Swanson B (1994) Revisión: empleo de pulsos eléctricos de alto voltaje para la conservación de alimentos. Microbiología e ingeniería del proceso. Rev Esp Cienc Tecnol Aliment 1:1–34

Mtaoua H, Sánchez-Vega R, Ferchichi A, Martín-Belloso O (2017) Impact of high-intensity pulsed electric fields or thermal treatment on the quality attributes of date juice through storage. J Food Process Preserv 41:e13052

O'sullivan J, Murray B, Flynn C, Norton I (2016) The effect of ultrasound treatment on the structural, physical and emulsifying properties of animal and vegetable proteins. Food hydrocol 53:141–154

Pavia DL, Lampman GM, Kriz GS, Vyvyan JA (2008) Introduction to spectroscopy, 4th edn. Brookescole Publishers, California

Ravanfar R, Tamadon AM, Niakousari M (2015) Optimization of ultrasound assisted extraction of anthocyanins from red cabbage using Taguchi design method. J Food Sci Technol 52:8140–8147

Reátegui JLP, da Fonseca Machado AP, Barbero GF, Rezende CA, Martínez J (2014) Extraction of antioxidant compounds from blackberry (*Rubus* sp.) bagasse using supercritical $CO_2$ assisted by ultrasound. J Supercritl Fluids 94:223–233

Rhazi N, Hannache H, Oumam M, Sesbou A, Charrier B, Pizzi A, Charrier-El Bouhtoury F (2015) Green extraction process of tannins obtained from Moroccan *Acacia mollissima* barks by microwave: modeling and optimization of the process using the response surface methodology RSM. Arab J Chem. https://doi.org/10.1016/j.arabjc.2015.04.032

Roobab U, Aadil RM, Madni GM, Bekhit AED (2018) The impact of nonthermal technologies on the microbiological quality of juices: a review. Compr Rev Food Sci Food Saf 17:437–457

Saldaña G, Cebrián G, Abenoza M, Sánchez-Gimeno C, Alvarez I, Raso J (2017) Assessing the efficacy of PEF treatments for improving polyphenol extraction during red wine vinifications. Innov Food Sci Emerg Technol 39:179–187

Santhirasegaram V, Razali Z, Somasundram C (2013) Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. Ultrason Sonochem 20:1276–1282

Shoji T (2007) Polyphenols as natural food pigments changes during food processing. Am J Food Technol 2:570–581

Singh B, Singh N, Thakur S, Kaur A (2017) Ultrasound assisted extraction of polyphenols and their distribution in whole mung bean, hull and cotyledon. J Food Sci Technol 54:921–932

Soria AC, Villamiel M (2010) Effect of ultrasound on the technological properties and bioactivity of food: a review. Trends Food Sci Technol 21:323–331

Szalontai B, Kóta Z, Nonaka H, Murata N (2003) Structural consequences of genetically engineered saturation of the fatty acids of phosphatidylglycerol in tobacco thylakoid membranes. An FTIR study. Biochemistry 42:4292–4299

Teh SS, Niven BE, Bekhit AEDA, Carne A, Birch EJ (2015) Microwave and pulsed electric field assisted extractions of polyphenols from defatted canola seed cake. Inter J Food Sci Technol 50:1109–1115

Tiwari B, O'donnell C, Patras A, Brunton N, Cullen P (2009) Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage. Eur Food Res Technol 228:717–724

Wu C, Wang F, Liu J, Zou Y, Chen X (2015) A comparison of volatile fractions obtained from *Lonicera macranthoides* via different extraction processes: ultrasound, microwave, Soxhlet extraction, hydrodistillation, and cold maceration. Integ Med Res 4:171–177

Yan C, XING YU J, Xing T, XIAO QING C (2008) Comparison of volatile components from *Marchantia convoluta* obtained by microwave extraction and phytosol extraction. J Chilean Chem Soc 53:1518–1522

Zhou Y, Zhao X, Huang H (2015) Effects of pulsed electric fields on anthocyanin extraction yield of blueberry processing by-products. J Food Process Preserv 39:1898–1904

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