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The ultrasound application does not affect to the thermal properties and chemical composition of virgin olive oils

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ABSTRACT

In this work, the effects of high power ultrasound treatment (40 kHz) on virgin olive oil (VOO) for different times (0, 15, 30 min) were studied, in order to verify if extent modifications in their chemical composition and thermal properties. The effects of the different ultrasound treatments on VOOs were determined considering the following parameters: quality index (free acidity, K_{232} and K_{270}), lipid profile (fatty acids and triglycerides composition) minor components (phenols, tocopherols, pigments and volatiles) and thermal properties (crystallization and melting) by Differential Scanning Calorimetry (DSC).

During the ultrasound treatments, bubbles growth was present in the VOO due to the phenomenon of cavitation and a slight increase of the temperature was observed. In general, the ultrasound treatments did not cause alterations on VOO parameters evaluated (oxidation state, lipid profile, minor components and thermal profiles). However, a slight decrease was observed in some volatile compounds.

1. Introduction

In the last decades, the virgin olive oils (VOO) sector has carried out a conversion of the traditional extraction process to a new and highly automated one, through of technological innovations mainly focused on the extraction process yield and quality improvement of the product [1–6]. The VOOs extraction process could be defined as the orderly set of unit operations that are used to achieve the separation of the oil from the rest of the components of the olive. According to the definition of virgin olive oils of the International Olive Council [7], these operations are exclusively mechanical or physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration.

Recently, emerging technologies are being applied into the olive paste malaxation process [8–9], such as ultrasound (US) waves [10–23], microwave [24–26], pulsed electric field [27–31] among others [2,22,32,33]. These technologies are being used with the aim to establish an alternative system to the traditional malaxation that allows reducing time and improving the process efficiency [6,8,9,11]. Among them, the US technology seems to be a promising application in the field of VOOs industry, due to its mechanical and thermal effects when applied on olive paste [12–15,19,20,34]. Besides, according to several studies, the olive oils obtained from treated olive paste with US in the malaxation step did not present changes in the quality indices, fatty acid composition and volatile aromatic compounds of the VOO, however controversial results on phenols content have been reported by different authors [12,14,20,21,23,34,35].

The US are sound waves with frequencies from 20 kHz to some GHz, higher than the audible limit of the human ear [9]. When the US is applied on a continuum fluid, it determines the alteration of positive and negative pressures inside it. When the negative pressure falls below the vapor pressure of the fluid itself, it produces cavities and tiny bubble growth. If the bubble growth reaches a critical size, it implodes causing the phenomenon of cavitation, the most important effect in high-power ultrasound and responsible of mechanical effect [10,17,36]. In addition, a thermal effect occurs when kinetic energy of the US waves is converted into the thermal energy due to turbulence increment in the matter [37].

So far, all the studies carried out that included the use of the US in the olive oil extraction process were conducted by applying the US on olive fruits or olive pastes, in order to improve the extractability of the oil and its quality characteristics [8,10,11,17,34]. However, the effect of the direct application of this emerging technology on the chemical composition and thermal properties of VOOs has not been thoroughly described up to now. Femenia et al. [38] described a method for

Abbreviations: VOO, Virgin Olive Oil; US, Ultrasound; DSC, Differential Scanning Calorimetry; TAG, triacylglycerol; FA, free acidity; FAME, fatty acid methyl ester

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preventing the total or partial crystallization of olive oil during storage at low temperature by application of US energy, allowing to retain the physical–chemical and sensory properties of the product. The VOO storage at low temperatures (lower than 6–8 °C) implies the formation of white deposits in the oils, mainly due to due to triglyceride crystallization. Nowadays, it is more than demonstrated that thermal properties, as crystallization point, is directly correlated with the FAMEs and TAG composition of the vegetable oils [39–41], including olive oil [42–44]. According to this, it is to be expected that VOO thermal profile could be altered by US treatment and thus prevent the crystallization of the oils as described by Femenia et al. [38].

On the other hand, the effect of US application on other vegetable oils has been studied. Chemat et al. [45,46] determined the effects of US on refined sunflower oil samples, obtaining differences between untreated and US treated samples. The peroxide value increased after US treatment, while off-flavour compounds for example hexanal and limonene resulting from the ultrasonic degradation of sunflower oil were observed. However, no significant changes were observed regarding to the fatty acid composition before and immediately after the treatment. Chen et al. [47] studied the effects of US parameters on the crystallization behavior of palm oil. They observed that US treatment significantly reduced the induction time, accelerated the crystallization rate and changed the crystallization mechanism of palm oil by producing smaller and uniformly crystals. Maruyama et al. [48] observed that the sonication induced the crystallization of coconut and palm oils. Patrick et al. [49] developed a system which can investigate the effect of ultrasound on the crystallization of fats under controlled conditions covering a range of intensities and cooling rates. Rincon-Cardona et al. [50] researched the effect of US on the crystallization behavior, melting profile and elasticity of a soft stearin fraction of high-stearic high-oleic sunflower oil. Results showed that US can be used to induce and increase the rate of crystallization of the soft stearin.

The aim of this study was to investigate the changes in the VOOs thermal and physico-chemical characteristics that occur as a consequence of the ultrasound treatment. For this purpose, thermal parameters (cooling and melting) by Differential Scanning Calorimetry (DSC), quality indexes (free acidity and absorption at 232 and 270 nm), lipid profiles (FAMEs and TAG) and minor components (tocopherols, phenols, pigments and volatile compounds) of the oils before and after the ultrasonic treatments were determined.

2. Materials and methods

2.1. Materials

Two filtered virgin olive oils (VOOs) from ‘Arbequina’ and ‘Picual’ cultivars were used in this experiment. Around 3 L of each oil was divided in aliquots that were packaged in clear glass bottles of 250 mL.

2.2. Ultrasound treatment

In order to sonicate the oils by the aid of ultrasonic waves, a SELECTA (mod.3000513) ultrasonic bath was used with a tank dimensions (W × D × H mm) were 300 × 140 × 150, a tank capacity of 6 L, power generator of 150w and frequency of 40 KHz. For each oil, three bottles were placed into the bath and then the tank was filled with distilled water until cover them. The oils samples were sonicated for different times (0, 15, 30 and 60 min) and the temperature was monitoring. After sonication of the samples were directly analyzed.

2.3. Analytical determinations

2.3.1. Oil quality indices

Free acidity (FA) and UV-specific extinction coefficients (K_{232} and K_{270}) were determined according to the analytical methods of the European Official Method of Analysis (EU Regulations 2568/91) [51]. FA was expressed as percentage of oleic acid, while K_{232} and K_{270} extinction coefficients were calculated from absorption values measured at 232 and 270 nm, respectively.

2.3.2. Fatty acids and triglycerides determination

Fatty acid methyl esters (FAMEs) and triacylglycerols (TAGs) profiles were obtained according to the European Regulation 2568/91 [51].

2.3.3. Tocopherol content

Tocopherols composition was determined by HPLC according to the IUPAC method 2432 [52]. Detection and quantification was carried out in an Agilent 1200 HPLC equipped with a quaternary pump and UV-Vis detector set a 295 nm. The results were expressed as mg kg^{-1} of oil.

2.3.4. Pigments content

Carotenoid and chlorophyllic pigments were determined spectrophotometrically at 470 and 670 nm according to Mínguez-Mosquera et al. [53] in a Cary 50 Bio spectrometer (Varian Inc., USA). The carotenoid and chlorophylls content were expressed as mg kg^{-1}.

2.3.5. Total polar phenol content

Phenolic content was carried out according to the method described by Vázquez-Roncero et al. [54]. The phenolic components were extracted from an oil-in-hexane solution with methanol:water (60:40) and their concentration was obtained by colorimetric measurement at 725 nm using Folin-Ciocaltea reagent [54]. The absorbance measurements were performed in an UV-vis spectrophotometer Varian Cary Bio50 (Varian, Spain). The results are expressed as mg kg^{-1} of caffeic acid.

2.3.6. HPLC analysis of phenolic compounds

The individual phenolic compounds were determined according to Beltrán et al. [55]. First, he olive oil sample (1.5 g) with 100mLof a standard solution (0.002 g of syringic acid/100 mL of methanol) was dissolved in n-hexane (1 mL). Then, the phenolic compounds were extracted with 1.25 mL of methanol/water (60:40 v/v) twice and raised to a final volume of 2.5 mL with the same methanol/water solution. Finally, phenolic compounds were quantified at 280 nm using a HP Agilent 1100 HPLC system equipped with an autosampler, quaternary pump, and diode array detector. A mixture of water/acetic acid (98:2v/v) (solvent A) and methanol/acetic acid (98:2 v/v) (solvent B) was used as mobile phase. While, the coloumn was a reversed-phase C18 Pecosphere (83 4.6 mm i.d., 3 mm particle size, Brown Lee Columns) with an injection volume of 20 mL and a flow rate of 0.45 mL/min. The solvent gradient changed according to the following conditions with a total run time of 70 min: 90% A–10% B for 10 min, 80% A–20% Bin 8 min then remained for 2 min, 60% A–40% B in 10 min, 50%A–50% B in 10 min, and 100% B in 10 min until the end of the run. Syringic acid was used as internal standard and the response factors determined by Mateos et al. [56]. The results were expressed as mg/kg.

2.3.7. Analysis of volatile compounds

Solid-phase microextraction (SPME) followed by GC-FID were used to analyze the volatile fraction in the VOO samples studied according to the method described by Sánchez-Ortiz et al. [57]. Briefly, Olive oil samples were tempered at room temperature and then placed (1.000 ± 0.001 g) in a 10 mL vial heater at 40° C for a 10 min equilibration time. Then, volatile compounds from headspace were adsorbed on by exposing the solidphase microextraction (SPME) fiber DVB/Carboxen/PDMS 50/30 μm 1 cm (Supelco Co. Bellefonte, PA) for 50 min at 40 °C in the headspace of the sample, and then retracted into the needle and immediately transferred and desorbed for 5 min into the injection port of a gas chromatograph equipped with an FID. Volatiles were analyzed by triplicate using a Varian CP 3800 GC equipped with a Supelcowax 10 capillary column (30 m × 0.25 mm, 0,25 μm,

Sigma-Aldrich Co. LLC). Operating conditions were as follows: Helium was the carrier gas; injector and detector at 250 °C; and column held for 5 min at 40 °C and then programmed at 4 °C min^{-1} to 200 °C. Compound identification was carried out on a HRGC-MS Varian under identical conditions for GC, matching against the Wiley/NBS Library, and by GC retention time against standards. For the quantification of the different volatile compounds, individual calibration curves obtained by adding known amounts of the different compounds to deodorized olive oil were used. Reference compounds used for identification and quantification of volatile compounds were supplied by Sigma-Aldrich (Bellefonte, PA). The volatile compounds were clustered in derivatives of the LOX pathway (Aldehydes LOX, Alcohols LOX, Esters LOX and Ketones LOX) and non-derivative from LOX pathway (Aldehydes non-LOX, Alcohols non-LOX and Esters non-LOX) according to their chemical nature. The results were expressed as mg of volatile compounds per kilogram of oil.

2.3.8. Differential Scanning Calorimetry (DSC)

Samples of olive oil (5–7 mg) were weighted in aluminum pans of 40 μL and analyzed with a DSC822 (Mettler Toledo, Switzerland). Firstly, oil samples were equilibrated at 25 °C for 5 min and then cooled to −80 °C at a rate of 5 °C/min. After equilibrated at −80 °C for 5 min, the oil samples were heated to 25 °C at a rate of 5 °C/min and finally equilibrated at 25 °C for 1 min. An air flow was purged in the DSC cell at 100 mL/min. The curves of the DSC thermograms were analyzed with a with STAR Software (Version 8.10, METTLER TOLEDO) to obtain enthalpy (DH, J/g), temperature of the major peak of crystallization phase (P_c, °C), temperature of the major peak of melting phase (P_{m1},°C), temperature of the minor peak of melting phase (P_{m2},°C), initial temperature of transition (t_o,°C), end temperature of transition (t_e, °C) and range of the transition (R, °C, difference between t_o and t_e). Triplicate analyses were performed per oil.

2.4. Statistical Analysis

Analysis of variance was applied, significant differences between the untreated and US treated oils were determined applying Tukey's test $p < 0.05$ (Statistix 9.0 software, Tallahassee, FL USA). The results were expressed as mean ± standard deviation ($n = 3$).

3. Results and discussion

In this work, VOOs of two different olive varieties ('Arbequina' and 'Picual') were sonicated in an ultrasonic bath (40 kHz; 150 W). First, should be highlighted the bubbles growth present in the oil during the US treatments, as can be observed in the Fig. 1. These typical bubbles, as commented in introduction section, are due to presence of positive and negative pressures produced by the sonication waves known as the phenomenon of cavitation [36,58]. Besides, the US process was accompanied of a slight increase of the oil temperature, from 22.1 ± 0.2 °C at the beginning of the treatments to 23.9 ± 0.3, 25.5 ± 0.5 and 30.1 ± 0.6 °C after 15, 30 and 60 min of sonication, respectively. This thermal effect occurs when kinetic energy of the waves is converted into the thermal energy due to turbulence increment in the matter [37].

3.1. Ultrasound effects on quality parameters

Table 1 shows a comparison of the quality parameters (FA, K_{232} and K_{270}) of untreated and US treated oils for 15, 30 and 60 min. In general, for both varieties, the quality parameters studied did not present significant differences between the untreated and US treated oils.

The FA in oils is the result of the degree of breakdown of the TAGs, due to hydrolysis reaction [4]. The oxidation parameter K_{232} is related to the formation of hydroperoxides, conjugated dienes, carboxylic compounds, and conjugated trienes, while K_{270} depends on secondary oxidation products formed from the initial compounds detected at 232 nm [59]. As observed, the values of these quality parameters show that sonication did not degrade the oils. According to these quality parameters, all oils were classified in the 'extra virgin' category as established by EU regulation [7]. Similar results were described by Feminia at al. [38], when applied ultrasound to EVOO of the 'Empeltre' variety.

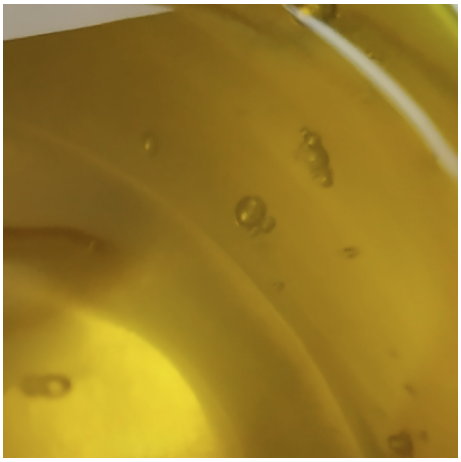

Fig. 1. Detail of bubbles growth in the oils during US treatment due to cavitation phenomenon.

3.2. Ultrasound effects on FAMEs and TAGs composition.

The FAMEs and TAGs composition of the untreated and US treated virgin olive oils are shown in Table 2. It is well established by many authors that the fatty acid composition of olive oil is strongly influenced by cultivar [60,61] among others agronomical factors [62–64]. As expected, there was a high degree of variability in FAMEs composition for the oils from these two different olive varieties although their values are similar to those published previously [44,65,66]. For both oils, the predominant FAME were oleic acid (C18: 1), higher in the 'Picual' variety with 78%, whereas the major TAG was Triolein (OOO + PoPP) with a 47 and 32%, corresponding to 'Picual' and 'Arbequina' varieties, respectively.

Regarding to the effect of the sonication on FAME and TAG composition, for both olive oil varieties (Table 2), no significant differences ($P > 0.05$) were found between untreated and US treated samples after 60 min. Results indicate no changes on lipid profile, such as breakdown of fatty acid carbon chains [67]. Similar results were obtained by Feminia et al. [38] in VOO and by other authors for other vegetable oils, such as sunflower [45,46] and interesterified soybean oil [68].

3.3. Ultrasound effects on VOOs thermal properties.

Table 3 shows cooling and melting thermal parameters obtained from the oil DSC thermograms for both oil varieties. Although the thermograms are not reported, the oils (untreated and US treated) showed a typical DSC cooling and melting thermograms for this type of vegetable oil, observing differences between olive oil varieties for these thermal parameters studied, as reported [42,44,65,66]. In general, the thermal parameters studied did not show differences between untreated and US treated oils.

DSC cooling thermograms from 25 to −80 °C showed two exothermic events, a well-defined main peak (P_c) and other secondary peak, not so well defined. The crystallization peaks in the DSC thermograms showed an initial temperature of crystallization (t_o) with values from −15,4 to −16.6 °C and from −12.8 to −13.1 °C, peaks for temperature of crystallization (P_c) with values from −41.4 to −41.6 °C

Table 1
Ultrasound effects on quality index and minor components of VOOs.

VOO variety	Picual				Arbequina			
US time (min)	0	15	30	60	0	15	30	60
Quality indices								
F.A (%)	0.18 ± 0.01^{A}	0.19 ± 0.02^{A}	0.18 ± 0.01^{A}	0.18 ± 0.01^{A}	0.22 ± 0.01^{A}	0.22 ± 0.01^{A}	0.23 ± 0.01^{A}	0.22 ± 0.01^{A}
K_{232}	1.87 ± 0.01^{A}	1.88 ± 0.04^{A}	1.87 ± 0.01^{A}	1.91 ± 0.02^{A}	2.00 ± 0.08^{A}	2.04 ± 0.04^{A}	2.01 ± 0.02^{A}	2.03 ± 0.03^{A}
K_{270}	0.14 ± 0.00^{A}	0.14 ± 0.01^{A}	0.14 ± 0.01^{A}	0.15 ± 0.01^{A}	0.15 ± 0.01^{A}	0.14 ± 0.01^{A}	0.15 ± 0.01^{A}	0.15 ± 0.02^{A}
Minor components								
Polyphenols [mg kg^{-1}]	473 ± 2.3^{A}	478.2 ± 5.0^{A}	478.6 ± 7.6^{A}	475.5 ± 6.0^{A}	358.1 ± 5.1^{A}	345.6 ± 3.6^{AB}	336.2 ± 6.4^{B}	343.8 ± 7.7^{AB}
Tocopherols T. [mg kg^{-1}]	308.9 ± 1.1^{A}	308.4 ± 1.7^{A}	312.5 ± 3.2^{A}	315.4 ± 2.3^{A}	309.7 ± 2.4^{A}	308.4 ± 1.6^{A}	310.3 ± 3.1^{A}	303.9 ± 1.1^{A}
α-Tocopherol [mg kg^{-1}]	283.3 ± 0.8^{A}	282.4 ± 1.9^{A}	285.1 ± 2.1^{A}	286.8 ± 2.0^{A}	290.4 ± 1.7^{A}	289.2 ± 1.1^{A}	292 ± 1.1^{A}	287.8 ± 1.5^{A}
β-Tocopherol [mg kg^{-1}]	6.9 ± 0.4^{B}	7.2 ± 0.5^{AB}	7.8 ± 0.5^{AB}	8.6 ± 0.6^{A}	10.6 ± 0.6^{A}	10.9 ± 0.5^{A}	10.3 ± 1.8^{A}	8.9 ± 0.2^{A}
γ-Tocopherol [mg kg^{-1}]	18.6 ± 0.8^{A}	18.9 ± 0.5^{A}	19.6 ± 1.3^{A}	20 ± 0.9^{A}	8.7 ± 0.4^{A}	8.3 ± 0.0^{A}	8 ± 0.7^{A}	7.2 ± 0.5^{A}
Carotenoids p. [mg kg^{-1}]	7.3 ± 0.0^{A}	7.4 ± 0.1^{A}	7.4 ± 0.1^{A}	7.4 ± 0.1^{A}	8.5 ± 0.2^{A}	8.3 ± 0.2^{A}	8.6 ± 0.1^{A}	8.7 ± 0.2^{A}
Chlorophyll *p*. [mg kg^{-1}]	19.5 ± 0.1^{A}	19.6 ± 0.2^{A}	19.6 ± 0.0^{A}	19.7 ± 0.3^{A}	21.6 ± 0.3^{A}	20.9 ± 0.2^{B}	21.1 ± 0.1^{AB}	21.3 ± 0.2^{AB}

*Mean ± sd (n = 3). Different letters in the same row and VOO variety indicate significant differences (P < 0.05) between the different times of US treatment.

and from −47.2 to −47.9 °C and temperatures of end crystallization (t_e) with values ranged from −47.0 to −47.9 °C and from −54.9 to −55.6 °C, for 'Picual' and 'Arbequina' oils, respectively. These differences between olive oil varieties for peak maximum and shapes were previously attributed to the difference in FAMEs and TAGs composition/or initial oxidative status of the oils [43,44,65,66,69]. However, comparing the oils before and after US treatments, the thermal parameters of crystallization peaks did not present variations, as can observe in Table 2.

In the reverse thermal process (melting), DSC heating profiles from −80 to 25 °C showed an endothermic phase transition. It showed two well defined peaks, with similar melting shapes that have also reported by other authors [39,43,44,65,66,69–71]. The melting peaks in the DSC thermograms exhibited initial melting temperatures (t_0) with values ranged from −18.9 to −19.0 °C and from −21.7 to –23.0 °C, temperatures of melting for the minor peak (P_{m1}) with values from −2.5 to −2.7 °C and from −4.5 to −4.9 °C, temperatures of melting for the major peak (P_{m2}) with values comprised from −6.8 to −6.9 and from −7.2 to −7.3 °C, and temperatures of end melting (t_e) with values ranged between 10.1 and 10.3 °C and 10.4 to 10.5 °C, for the 'Picual'

Table 2
Ultrasound effects on fatty acid composition and triacylglycerol profile of VOOs.

VOO variety	Picual		Arbequina	
US time (min)	0	60	0	60
Fatty Acids (%)				
C12:0	< 0.01	< 0.01	< 0.01	< 0.01
C14:0	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}
C16:0	12.18 ± 0.02^{B}	12.37 ± 0.06^{A}	15.17 ± 0.18^{A}	15.28 ± 0.11^{A}
C16:1	1.10 ± 0.01^{A}	1.16 ± 0.02^{A}	1.55 ± 0.03^{A}	1.57 ± 0.02^{A}
C17:0	0.05 ± 0.00^{A}	0.05 ± 0.00^{A}	0.12 ± 0.00^{A}	0.12 ± 0.00^{A}
C17:1	0.1 ± 0.00^{A}	0.1 ± 0.00^{A}	0.25 ± 0.00^{A}	0.25 ± 0.00^{A}
C18:0	2.67 ± 0.00^{A}	2.65. ± 0.01^{A}	1.94 ± 0.02^{A}	1.94 ± 0.01^{A}
C18:1	78.40 ± 0.02^{A}	78.22 ± 0.06^{B}	68.25 ± 0.14^{A}	68.12 ± 0.12^{A}
C18:2	4.00 ± 0.03^{A}	4.02 ± 0.00^{A}	11.19 ± 0.04^{A}	11.19 ± 0.00^{A}
C18:3	0.64 ± 0.01^{A}	0.64 ± 0.00^{A}	0.62 ± 0.01^{A}	0.62 ± 0.00^{A}
C20:0	0.39 ± 0.00^{A}	0.38 ± 0.00^{B}	0.40 ± 0.02^{A}	0.40 ± 0.00^{A}
C20:1	0.26 ± 0.00^{A}	0.26 ± 0.00^{A}	0.31 ± 0.01^{A}	0.31 ± 0.00^{A}
C22:0	0.12 ± 0.00^{A}	0.10 ± 0.00^{B}	0.12 ± 0.01^{A}	0.13 ± 0.00^{A}
C22:1	< 0.01	< 0.01	< 0.01	< 0.01
C24:0	0.07 ± 0.00^{A}	0.06 ± 0.00^{A}	0.06 ± 0.01^{A}	0.06 ± 0.00^{A}
TAGs (%)				
LLL	0.06 ± 0.01^{A}	0.06 ± 0.01^{A}	0.16 ± 0.02^{A}	0.17 ± 0.00^{A}
OLLn + PoLL	0.20 ± 0.0^{A}	0.18 ± 0.01^{B}	0.32 ± 0.03^{A}	0.33 ± 0.01^{A}
PLLn	0.06 ± 0.01^{A}	0.05 ± 0.01^{A}	0.09 ± 0.00^{A}	0.10 ± 0.00^{A}
OLL	0.64 ± 0.01^{A}	0.65 ± 0.01^{A}	2.52 ± 0.03^{A}	2.55 ± 0.03^{A}
OOLn + PoOL	1.31 ± 0.01^{A}	1.33 ± 0.01^{A}	1.51 ± 0.03^{A}	1.47 ± 0.02^{A}
PLL + PoPoO	0.28 ± 0.00^{A}	0.27 ± 0.02^{A}	1.09 ± 0.04^{A}	1.14 ± 0.02^{A}
POLn + PPoPo + PPoL	0.67 ± 0.00^{A}	0.63 ± 0.02^{B}	0.89 ± 0.05^{A}	0.92 ± 0.02^{A}
OOLn + LnPP	6.26 ± 0.02^{A}	6.24 ± 0.05^{A}	13.70 ± 0.06^{A}	13.74 ± 0.01^{A}
PoOO	2.11 ± 0.02^{A}	2.11 ± 0.02^{A}	2.10 ± 0.03^{A}	2.12 ± 0.03^{A}
SLL + PLO	3.04 ± 0.04^{A}	3.01 ± 0.03^{A}	8.78 ± 0.05^{A}	8.80 ± 0.03^{A}
PoOP + SPoL + SOLn + SPoPo	0.98 ± 0.04^{A}	0.94 ± 0.01^{A}	1.42 ± 0.09^{A}	1.37 ± 0.00^{A}
PLP	0.25 ± 0.02^{A}	0.18 ± 0.06^{A}	1.33 ± 0.04^{A}	1.26 ± 0.07^{A}
OOO + PoPP	46.63 ± 0.03^{B}	47.05 ± 0.18^{A}	31.87 ± 0.29^{A}	31.80 ± 0.19^{A}
SOL	0.34 ± 0.02^{A}	0.37 ± 0.05^{A}	0.78 ± 0.09^{A}	0.78 ± 0.08^{A}
POO	26.08 ± 0.08^{A}	26.16 ± 0.08^{A}	24.29 ± 0.27^{A}	24.20 ± 0.08^{A}
POP	4.29 ± 0.05^{A}	4.19 ± 0.07^{A}	5.19 ± 0.11^{A}	5.26 ± 0.06^{A}
SOO	5.26 ± 0.02^{A}	5.21 ± 0.13^{A}	2.96 ± 0.04^{A}	2.88 ± 0.14^{A}
POS + SLS	1.33 ± 0.00^{A}	1.39 ± 0.04^{A}	1.12 ± 0.0^{A}	1.10 ± 0.06^{A}

*Mean ± sd (n = 3). Different letters in the same row and VOO variety indicate significant differences (P < 0.05) between the different times of US treatment.

Table 3
Ultrasound effects on DSC parameters of the VOOs.

VOO variety	Picual				Arbequina			
US time (min)	0	15	30	60	0	15	30	60
Cooling treatment								
t_o (°C)	−15.4 ± 0.1^{A}	−16.3 ± 0.2^{B}	−16.6 ± 0.1^{B}	−15.3 ± 0.4^{A}	−13.1 ± 0.0^{B}	−12.8 ± 0.4^{AB}	−13.0 ± 0.0^{AB}	−12.8 ± 0.1^{A}
P_c (°C)	−41.4 ± 0.0^{A}	−41.6 ± 0.1^{A}	−41.6 ± 0.1^{A}	−41.4 ± 0.1^{A}	−47.2 ± 0.2^{A}	−47.8 ± 0.3^{B}	−47.2 ± 0.1^{A}	−47.9 ± 0.1^{B}
t_e (°C)	−47.0 ± 0.0^{A}	−47.0 ± 0.2^{A}	−47.2 ± 0.1^{A}	−47.2 ± 0.1^{A}	−54.9 ± 0.4^{A}	−55.5 ± 1.0^{A}	−55.2 ± 0.2^{A}	−55.6 ± 0.3^{A}
R	31.7 ± 0.1^{AB}	30.7 ± 0.1^{BC}	30.6 ± 0.1^{C}	31.9 ± 0.5^{A}	41.7 ± 0.3^{A}	42.8 ± 0.5^{A}	42.2 ± 0.2^{A}	42.8 ± 0.4^{A}
Heating treatment								
t_o (°C)	−19.0 ± 0.1^{A}	−18.9 ± 0.0^{A}	−18.9 ± 0.1^{A}	−19.0 ± 0.1^{A}	–22.7 ± 0.7^{BC}	−21.7 ± 1.3^{A}	–23.0 ± 0.1^{C}	−21.6 ± 0.2^{AB}
Pm_1 (°C)	−2.7 ± 0.1^{A}	−2.6 ± 0.0^{A}	−2.5 ± 0.1^{A}	−2.6 ± 0.3^{A}	−4.5 ± 0.0^{A}	−4.8 ± 0.2^{AB}	−4.8 ± 0.1^{B}	−4.9 ± 0.1^{B}
Pm_2 (°C)	6.9 ± 0.0^{A}	6.8 ± 0.1^{A}	6.9 ± 0.0^{A}	6.9 ± 0.1^{A}	7.3 ± 0.0^{A}	7.2 ± 0.1 ^{A}	7.2 ± 0.0^{A}	7.2 ± 0.1^{A}
t_e (°C)	10.2 ± 0.1^{A}	10.3 ± 0.2^{A}	10.1 ± 0.1^{A}	10.1 ± 0.0^{A}	10.5 ± 0.1^{A}	10.4 ± 0.1^{A}	10.5 ± 0.0^{A}	10.5 ± 0.1^{A}
R	29.2 ± 0.1^{A}	29.2 ± 0.2 ^{A}	29.0 ± 0.1^{A}	29.1 ± 0.1^{A}	33.2 ± 0.7^{A}	32.2 ± 1.3^{A}	32.5 ± 0.1^{A}	32.1 ± 0.3^{A}

*Mean ± sd (n = 3). Different letters in the same row and VOO variety indicate significant differences ($P < 0.05$) between the different times of US treatment. P_c, temperature of the major peak of crystallization phase; P_{m1}, temperature of the major peak of melting phase; P_{m2}, temperature of the minor peak of melting phase; t_o and T_e, initial and end temperature of the transition phase, respectively; R, range of the transition phase (temperature difference between t_o and t_e).

and 'Arbequina' oils, respectively. As can be observed, the US application did not change the thermal properties of the VOOs.

These results obtained from DSC analysis of the oils did not agree with the behavior described by Femenia et al. [38] in their invention for a method for preventing the total or partial crystallization of olive oil during storage at low temperatures through the application of ultrasonic energy. However, unfortunately in this patent, no studio on their thermal properties supports this claim. Thus, according to the results obtained in the present work, prevention the total or partial crystallization cannot be attributed to changes in the cooling point or thermal profile due to the US treatments.

3.4. Ultrasound effects on VOOs minor components.

Table 1 also shows a comparison of the minor components (phenols, tocopherols and pigments) of the untreated and US treated VOOs. In general, the content of these minor components were not influenced by US treatments, independently of the time applied.

The total phenols content of the VOOs studied (untreated and US treated) were ranged between 473 and 478 mg/kg for 'Picual' oils and 336–358 mg/kg for 'Arbequina' oils, a higher content for the 'Picual' oils that can be explained by the variety [60]. Phenols degradation mainly depends on the availability of oxygen that is promoted by light, heat, metals, and enzymes [72]. Phenolic content was not affected by the ultrasound treatments, although a slight tend to decrease could be observed in US treated 'Arbequina' oils that could be explained by the slight increase of the temperature during the US treatment.

Simple phenols (Hydroxytyrosol and Tyrosol) and secoiridoids (3,4-DHPEA-EDA, p-HPEA-EDA, 3,4-DHPEA-EA and p-HPEA-EA) content of oils were also analyzed (Fig. 2). These simple phenols and its derivatives are strongly related to the VOO shelf life because of their antioxidant ability and bioactive activities. Besides, they are responsible of the inclusion of the VOO on the nutrition and health claims made on foods by European Union [73]. As expected, due to the olive variety [61], both oils showed different phenolic profile. The 'Picual' variety oils was characterized for a higher Hydroxytyrosol, Tyrosol, 3,4-DHPEA-EA and p-HPEA-EA content, while 'Arbequina' had a higher p-HPEA-EDA content. Phenolic components did not vary their concentration by US treatment.

The total tocopherol content (sum of α, β, and γ forms) is also shown in Table 1. These compounds are natural antioxidants present in the VOO and are also included in the health claims by European Union 432/2012 [73]. Although 'Picual' oils have higher tocopherol content, in this work similar concentrations were found in both oils. It can be explained for the influence of other agronomic factors, such as the fruit ripeness [74]. As expected, the α-tocopherol was the major tocopherol detected in both oil varieties. Concerning to the effect of the ultrasound treatments, the total tocopherol content (and their individual forms α, β, and γ) was not influenced for the sonication process and neither for its thermal increase associated. Therefore none degradation process mentioned above are present during the ultrasonic process.

The pigments (carotenoids and chlorophylls) in addition to play an important role in the VOO oxidative stability [75], they are mainly responsible for the color of VOOs [53,76]. The pigment content of VOOs varies according to olive variety, fruit ripeness, and extraction and storage conditions, among others factors [77]. In the present work, both carotenoids and chlorophylls content were very similar for both oils. The main factors that degrade the pigments are the light, temperature and oxygen exposure [77]. As can be observed in the Table 3, the ultrasound treatment did not affect the pigment content in the oils studied, thus no degradation process were caused due to the application of this emerging technology.

3.5. Ultrasound effects on VOOs volatile compounds.

Volatile fraction of the VOOs (without treatment and US treatment) is shown in Fig. 3. Volatile compounds were grouped according to non-LOX derivative and LOX derivatives and their chemical nature: aldehydes, alcohols, esters and ketones as described and listed in Section 2.3.7.

The oils used in this work showed different volatile profiles that can be attributed to the influence of the olive variety on the content of these compounds [78], agronomical factors, such as fruit ripeness and climatic conditions [79], or technological factors [80].

Respect to the effect of the ultrasound treatments on volatile fraction, in general, large changes in the VOOs volatile content were not observed for the first 30 min of sonication. However, after 60 min, a slight decrease of the volatiles fraction was observed, more notable for 'Picual' variety. Alcohols non-LOX and esters non-LOX slightly decreased in both varieties. The LOX fraction, aldehydes, alcohols and esters, also decreased only for 'Picual' oil, showing the esters non-LOX a greater decrease, until 24 and 17% for 'Picual' and 'Arbequina' variety, respectively. Aldehydes non-LOX and Ketones remained stable for both varieties. The slight reduction of some of these volatile compounds can be explained for the increase of temperature during US treatment. In other vegetable oils (sunflower), other authors have described some off-flavour compounds, such as hexanal and limonene resulting from the ultrasonic degradation Chemat el al. [46], although this effect was not observed in this work.

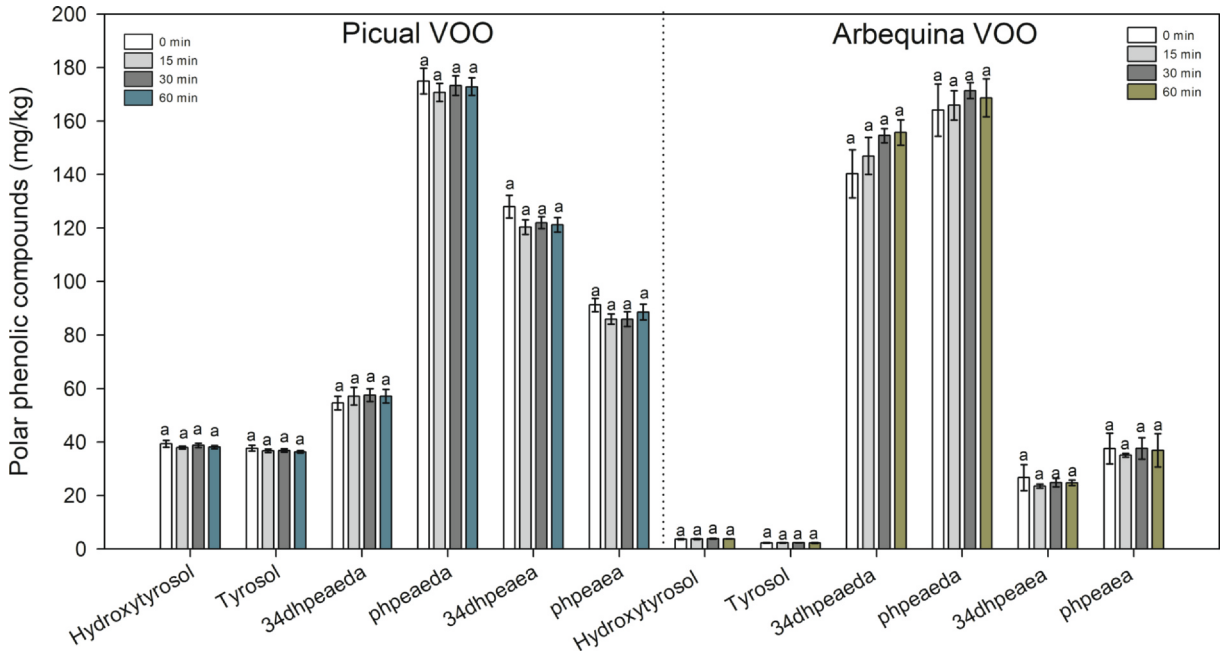

Fig. 2. Ultrasound effects on VOOs phenolic compounds. Different letters for each VOO variety and the same phenol compound indicate significant differences (p = 0.05) between the different times of US treatment.

4. Conclusion

The results obtained in this study have revealed that ultrasound waves are a clean technology that in general does not affect the chemical composition and thermal properties of virgin olive oils. During US treatment, could be observed bubbles in the oil due to phenomenon of cavitation and a slight increase of the oil temperature was registered. The VOO quality parameters (free acidity and oxidation parameters) and minor component (tocopherols, pigments and phenols) were not affected by the US treatment. However a slight decrease was observed in some volatile compounds after 60 min of sonication, until a 24% less of esters non-LOX for 'Picual' oil. The lipid profile, which is related with the oils thermal properties, was not affected by sonication treatments. This was also confirmed by DSC study, because no differences in the cooling and melting parameters studied were observed either between untreated and US treated oils.

Therefore, according to results obtained in this work and those indicated in the introduction, all indicates that this emerging technology seems to be a promising application in the field of VOOs industry. Since, the effects of the US aplication on olive paste are merely thermal–mechanical (facilitating the separation of oil from solids in the extraction process) and they do not alter the quality, composition and thermal properties of the VOOs.

CRediT authorship contribution statement

Abraham Gila: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft. **Araceli Sánchez-Ortiz:** Formal analysis, Resources. **Antonio Jiménez:** Conceptualization, Methodology, Formal analysis, Supervision. **Gabriel Beltrán:** Conceptualization, Methodology, Formal analysis, Supervision, Funding acquisition.

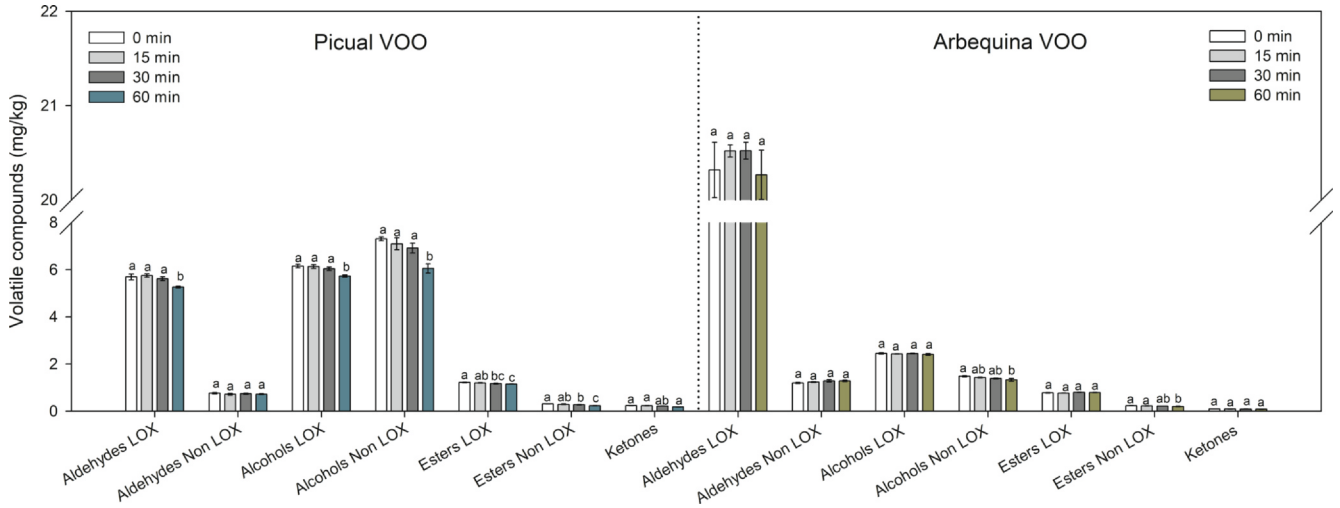

Fig. 3. Ultrasound effects on VOOs volatile compounds. Different letters for each VOO variety and the same volatile compound indicate significant differences (p = 0.05) between the different times of US treatment.

Declaration of Competing Interest

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

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