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Physicochemical characterization of virgin olive oil obtained using an ultrasound-assisted extraction at an industrial scale: Influence of olive maturity index and malaxation time

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ABSTRACT

Ultrasound-assisted extraction is an innovative technique applied to the extraction process for virgin olive oil (VOO), which is generally employed to increase plant efficiency and improve product quality. A high-power ultrasound (US) device was introduced at an industrial plant that can process at 2 tons/h to evaluate the technique's physicochemical impact on quality parameters of VOO that was caused by an intensive mass transfer induced by acoustic cavitation process and shockwaves. The impact on oil yield was also evaluated with respect to the ripening stage and malaxation time. No significant effects on the legal and commercial parameters of VOO (including quality indices, sterols, triterpene dialcohols, waxes and diacylglycerols) were found for olives at medium-early ripening stage. Significant physical changes, increased extraction yield (22.7%), enhanced phenol content (10.1%) were observed in US-VOO compared to control (C) oil extracted with a traditional process at an early maturity index.

1. Introduction

The quality of virgin olive oil originates in the orchard and its management, continues to be impacted through the regulation of operative parameters and technical considerations in the extraction process and evolves during the entire storage period until the oil is consumed. The first step involves the genetic origin of the olive, the geographical growing area, the climate and the agronomic practices, with attention often being particularly focused on fertilization and water availability (Caruso et al., 2017; Rallo et al., 2018). The second step concerns the regulation of the main variables (temperature, oxygen, enzymatic activities and the disruption level of olive cell tissues) involved in mechanical extraction processes of olive oil that are connected to increases in parameters associated with quality (Bejaoui, Sánchez-Ortiz, Sánchez, Jiménez, & Beltrán, 2017; Esposto et al., 2013; Selvaggini et al., 2014; Servili et al., 2015; Veneziani et al., 2015). The last step has a central role in preserving a high level quality after all other efforts have been undertaken, where factors that could drastically reduce the shelf-life of the product are controlled.

Many of the latest innovative technologies that have been applied in olive oil extraction plants have had the primary aim of increasing extractability of oil, while preserving or improving the quality characteristics in VOO. A common role of microwaves, pulsed electric field, ultrasound and similar technologies is to cause a major cellular breakdown and release intracellular contents to enhance the olive oil yield (Clodoveo et al., 2017; Puertolas & Martinez de Maranon, 2015; Leone et al., 2018). Each different technology differently impacts olive cellular structure due to thermal and non-thermal treatments that are strictly related to sudden and rapid alterations in temperature, pressure, energy, electrical potential, or any combination of these. Extraction technologies produce structural modifications in the olive tissues, causing different effects like development of pores, increased cellular permeability and collapse of cellular membranes (Chemat et al., 2017). Novel treatments are often pursued for an additional increase in the crushing and malaxation phases; these treatments seek to improve the release of oil drops that are primarily located inside of the vacuoles of the mesocarp cells present in olive drupes. This process also causes a simultaneous release of other intracellular matter that allows different antioxidant compounds to diffuse into the aqueous and oily phases (Chemat et al., 2017). In addition to increased oil yields, several recent technological studies, concerning heat exchangers, pulsed electric field and ultrasound, showed there is a concomitant effect of improved VOO

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quality due to enrichment of the relative content of α-tocopherols and/or hydrophilic phenols that are strictly related to the health and sensory properties of the product (Iqdiam et al., 2018; Puertolas & Martinez de Maranon, 2015; Almeida, Valli, Bendini, & Toschi, 2017; Esposto et al., 2013; Veneziani et al., 2018a). The disruption effects on olive paste cells can also cause a release of other compounds (volatile compounds, waxes, sterols, metals, triterpene dialcohols, pro-oxidants, etc…) with properties that negatively impact olive oil characteristics (OJEC, 2015) and its antioxidant stability (Chemat et al., 2017). These possibly negative impacts on VOO quality through using novel technologies is scarcely addressed in the literature.

Ultrasound-assisted extraction, which is based on effects that cause cavitation that enables accelerated heat and mass transfers, is one of the recent technologies applied to the mechanical extraction process of VOO before and after the malaxation phase. The first studies evaluating high-power ultrasound were carried out at lab-scale processing plants, and these studies showed a limited effect on the efficiency of oil yield, a reduction in phenolic compounds and an increase in the content of α-tocopherols, chlorophylls and carotenoids (Jiménez, Beltrán, & Uceda, 2007; Clodoveo, Durante, La Notte, Punzi, & Gambacorta, 2013). More recent experimental tests led to an improved working efficiency of ultrasound systems that also caused a further increased oil yield and an increase in their phenolic fraction, which was probably due to better control of both the parameters involved in the oxidation processes and of the enzymatic activities responsible for the reduction of the main phenolic compounds (Almeida et al., 2017; Clodoveo et al., 2017; Juliano et al., 2017, Leone et al., 2018). Some latest studies that examined the effects of ultrasound on olive oil extractability and its effects on concerns relating to quality were performed by evaluating data obtained in lab-scale or semi-industrial processing plants (Almeida et al., 2017; Bejaoui et al., 2017; 2018; Iqdiam et al., 2018; Juliano et al., 2017). This paper examines the impact of a high-power ultrasound technology on the physicochemical characteristics of VOO extracted by an industrial processing plant with a working capacity of 2 tons/h. Large-scale extractions were carried out using olives at three different ripening stages and applying three different times of malaxation to better evaluate the variables that can affect the process of disrupting olive cell membranes and cell walls, the consequent effect on oil yield, and the effects on the release of extractable compounds capable of modifying VOO quality.

2. Materials and methods

2.1. Chemicals

Phenolic alcohols such as (*p*-hydroxyphenyl)ethanol (*p*-HPEA) and (3,4-dihydroxyphenyl)ethanol (3,4-DHPEA) were purchased from Cabru s.a.s. (Arcore, Milan, Italy) and Fluka (Milan, Italy), respectively. Vanillic acid, α-tocopherol, 2,2-diphenyl-1-picrylhydrazil (DPPḢ), the analytical standards of aldehydes, alcohols, esters and ketones, sterols, triterpene dialcohols and waxes were supplied by Sigma-Aldrich (Milan, Italy). The isomer of oleuropein aglycon (3,4-DHPEA-EA), the dialdehydic forms of elenolic acid linked to tyrosol and hydroxytyrosol (*p*-HPEA-EDA and 3,4-DHPEA-EDA), (+)-1-acetoxypinoresinol and (+)-pinoresinol were obtained as reported by Veneziani et al. (2017).

2.2. VOO mechanical extraction process

Trials were conducted with Ogliarola garganica olives that were harvested in the Apulia region from mid-October to mid-November at three different ripening stages: 0.88, 2.82 and 3.31 were representative maturity indices (MI) in the control (C1, C2 and C3) and ultrasound VOOs (US1, US2 and US3), respectively. The maturity index was calculated as reported by Beltran, Uceda, Jimenez, and Aguilera (2003). Olives were processed within 48 h from harvesting and each VOO, obtained from batch of olives at different ripening stage and/or at a different malaxation time (control and experimental trial), was extracted three times using a Pieralisi industrial system (Pieralisi S.p.A., Jesi, Ancona, Italy) with a working capacity of 2 tons/h, installed at Frantoio “Il Nocciolino” (Ponte Valleceppi, Perugia, Italy). The VOO extraction plant was equipped with a hammer-type FP HP 30 INOX crusher (2800 rpm), four MOLINOVA TG MOD 600 malaxers, a Leopard 5 decanter and a BRAVO vertical separator.

The Leopard decanter, based on multi phase decanter (DMF) technology, is a two-phase centrifuge able to produce a little hydrated pomace, similar to one obtained from a three-phase decanter, and recovering a certain amount of pulp from the pomace to obtain an olive “paté” characterized by a high moisture content without the presence of olive pits. The bowl discharging device of DMF technology, automatically controlled, allows to extract the olive oil without the addition of water with the advantages of three-phase decanter extraction technology.

The experimental tests were carried out using a high-power UIP4000 hdT ultrasoundsystem (Hielscher Ultrasonics GmbH, Teltow, Germany). The ultrasound industrial equipment produced by Hielscher GmbH, and installed by Seneco Science (Seneco S.r.l., Milano, Italy), is for continuous operation, and is composed by a generator with a touch screen control panel, with the possibility of a remote control by ethernet connection and a transducer with 4 kW power and 20 kHz; to be in position to work in continuous operation the ultrasound equipment have a flow cell special designed with inlet at the bottom and outlet at the upper part, connection DN90. At the outlet part there are connection for temperature and pressure control. A pinch valve is used to regulate the pressure inside the flow cell. A special designed radial cascatrode in titanium is located inside of the flow-cell to provide an adequate cavitation; the amplitude of cavitation is possible to regulate by the touch-screen control panel. The unit was positioned between the crusher and the malaxer, connection is by food-grade pipes only. The olive paste, coming out from the crusher was processed in continuous mode with ultrasound at a frequency of 20 kHz, a power of 2.8 kW, 3 bars of pressure and an 80% of amplitude. The ultrasound treatment was followed by a malaxation phase regulated at a kneading time of 30 min at 25 °C. The batch of olives at an early ripening stage (0.88 MI) was also processed at two additional malaxation times of 10 min and 20 min (C4 and US4 control and ultrasound tests, respectively).

2.3. VOO physicochemical analysis

2.3.1. Turbidity grade

A Ratio Turbidimeter Hach Model 18,900 (Hach Company Loveland, Colorado, US) was used to detect the turbidity level of the VOO. The values of the turbidity grade were expressed in nephelometric turbidity units (NTU).

2.3.2. Moisture content

The moisture contents of VOO samples were evaluated as described in the ISO 662 method (ISO 662, 1998). Five grams of each oil sample were weighted in an aluminum capsule and placed in a oven (Binder GmbH, Tuttlingen, Germany) at 105 °C for approximately 5 h until a constant weight was obtained.

2.3.3. Oil content

The Soxhlet extractor was utilized to analyze paté and pomace oil content where 10 g of dried sample with 5 g of pumice stone was loaded into a thimble made from thick filter paper and placed inside the main compartment of the Soxhlet extractor. The process was undertaken for six hours using hexane as an extraction solvent. The solvent was removed by using a Rotavapor R-210 rotary evaporator (Buchi Italia s.r.l, Cornaredo, Italy); afterwards, the residual oil content was detected (AOAC, 1995).

2.3.4. Oil color

VOO color measurements were carried out over the whole visible spectrum (380–770 nm) using a Cary 100 Scan UV–Visible Spectrophotometer (Varian, Walnut Creek, CA, U.S.A.) with illuminant D65 and a 10° observer. CIELAB color space was used to evaluate the scalar coordinates ($L^*a^*b^*$) and the angular coordinates of chroma (C^*) and hue (h) related to psychophysical characteristics of color (Moyano, Heredia, & Meléndez-Martínez, 2010). L^*, a^* and b^* coordinates described the color parameters of lightness, red (positive)/green (negative) color components and yellow (positive)/blue (negative) color components, respectively. All of the recorded data were elaborated on using Cary WinUV Color software. The Euclidean distance between two points were assessed in three-dimensional space in control and ultrasound VOOs, and the values of color differences were calculated using the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

2.3.5. Antioxidant activity

The antioxidant activities of VOOs were obtained in VOOs that had been extracted with and without an ultrasound-assisted extraction process and were assessed for their Radical Scavenging Capacity (RSC) using the 2,2-difenil-1-picrilidrazil (DPPḢ) radical. Total antioxidant capacity was evaluated directly dissolving the VOO sample in ethyl acetate as was described in Mancebo-Campos, Salvador, and Fregapane (2014). The assays were spectrophotometrically performed with a Cary 100 Scan UV–Visible spectrophotometer (Varian, Walnut Creek, CA, U.S.A.), where 0.5 ml of ethyl acetate solution, at different concentrations of sample (0.1–0.4 ml), was added to 3 ml of DPPH ethyl acetate solution. Measurements were carried out at 515 nm in cuvettes that were kept at room temperature in the dark for 20 min. For each VOO samples the concentration of antioxidant, able to reduce to 50% the initial concentration of DPPḢ solution (EC_{50}), was detected. The RSC value was expressed as mg of oil/ml of mixture reaction (MR).

2.3.6. Quality indices

The values of free acidity, peroxide value, K_{232}, K_{270} and ΔK were detected according to the analysis methods described by Regulation (EU) 2015/1830 (OJEC, 2015).

2.3.7. Sterols, triterpene dialcohols and waxes

The sterol composition, total sterols, amount of erythrodiol and uvaol, and waxes were detected using the official methods of analysis (Regulation (EU) 2015/1830, OJEC, 2015).

2.3.8. Diacylglycerols (DAGs)

1,2- and 1,3 diacylglycerols contents in control and ultrasound-treated VOOs were evaluated using the methods of analysis described by the IOC (2013).

2.3.9. Chlorophylls and carotenoids

The analysis of VOO pigments were carried out following the spectrophotometric method described by Mínguez-Mosquera, Rejano-Navarro, Gandul-Rojas, SanchezGomez, and Garrido-Fernandez (1991). The absorbance at 670 nm and 470 nm was detected to determine the chlorophylls and carotenoids content, respectively. Chlorophyll and carotenoid total fractions were expressed as mg/kg of VOO using the respective coefficient of extinction (613 and 2000).

2.3.10. α-Tocopherol

HPLC–DAD–FLD was used to detect the content of α-tocopherol in VOOs. Samples were prepared by dissolving one gram of oil into 10 ml of *n*-hexane that was then filtered with a 0.25-nm polyvinylidene difluoride (PVDF) syringe filter (Agilent Technologies, Santa Clara, CA, USA) and injected into an HPLC system. The α-tocopherol content was evaluated at an excitation wavelength of 294 nm and at an emission wavelength of 300 nm as described by Esposto et al. (2015).

2.3.11. Hydrophilic phenols

An Agilent Technologies system Model 1100 (Agilent Technologies, Santa Clara, CA, USA), that consists of a vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment, a diode array detector (DAD), and a fluorescence detector (FLD) was used to perform HPLC analysis of the main phenolic compounds in VOO. The extraction methods and the chromatographic tests were carried out as described by Selvaggini et al. (2014). The quantitative and qualitative compositions of hydrophilic phenols were separated and determined using a Spherisorb ODS1 column (5 µm, 4,6 mm × 250 mm, Waters, Milford, MA, USA) and was elaborated using ChemStation software (Agilent Technologies, Palo Alto, CA, USA).

2.3.12. Volatile compounds

Headspace solid-phase microextraction followed by gas chromatography–mass spectrometry (HS-SPME/GC-MS) was used to identify the quantitative and qualitative volatile composition of VOO treated or untreated with the ultrasound system. Gas chromatographic analysis of aldehydes, alcohols, esters and ketones that were primarily concerned with the development of VOO sensory notes was carried out using a Varian 4000 GC-MS controlled by Varian MS Workstation Software, Version 6.6. The instrument's working parameters for the identification, detection and quantification of VOO volatile compounds were adjusted using the analysis method reported by Veneziani et al. (2015).

2.4. Statistical analysis

All the physicochemical data of oil treated and untreated with ultrasound-assisted extraction were elaborated on by using SigmaPlot Software 12.3 (Systat Software Inc., San Jose, CA, USA) for the one-way analysis of variance (ANOVA), where differences were considered to be significant at $p < 0.05$.

3. Results and discussion

3.1. Physicochemical characterization of VOO

The impact of ultrasound-assisted extraction on the physiochemical composition of VOO was evaluated in an industrial plant that was capable of processing Ogliarola garganica olives at a working capacity of 2 tons/h at an early maturity index of 0.88 for a conventional malaxation time of 30 min.

The ultrasound system did not modify the legal quality indices of free acidity, peroxide value and spectrophotometric constants, as reported by other studies (Clodoveo et al., 2013; Juliano et al., 2017).

Epicuticular and intracuticular waxes, mainly found in the epicarp of olive drupe as three dimensional complexes or embedded in the cutin matrix (Lanza & Di Serio, 2015), can modify their own availability and solubility relating to levels of physical shock and increased temperature. The cavitation process due to ultrasound waves causes sudden temperature increases and disruptive effects on the fruit skin that did not change the waxes content in either type of VOO, as is shown in Table 1.

The oils extracted from the three distinct layers of olive pericarp are characterized by different sterolic compositions (Guillaume, Ravetti, Lala Ray, & Johnson, 2012). The different fruit tissues of skin, pulp and seed show different concentrations of sterols that are mainly represented as β-sitosterol, campesterol, delta-5-avenasterol and stigmasterol, with the highest total content of sterols detected in the endocarp oil (which is approximately two-fold higher than what is observed in the mesocarp and epicarp). The possible more abundant extraction, as a result of ultrasound treatment, of oil contained in the cells of skin and seed tissues and characterized by different concentrations of sterols did not determine any significant alteration of the

Table 1
Physicochemical parameters and anioxidant activity of VOO control (C1, C2 and C3) and extracted with ultrasound system (US1, US2 and US3) using Ogliarola garganica olives at different maturity index and malaxed for 30 min.[a]

Maturity index	C1	US1	C2	US2	C3	US3
	0.88		*2.82*		*3.31*	
Turbidity (NTU)	131 ± 28a	64 ± 24b	240 ± 28a	218 ± 16a	300 ± 26a	235 ± 25b
Oil moisture content (%)	0.160 ± 0.01a	0.131 ± 0.01b	0.178 ± 0.01a	0.172 ± 0.03a	0.195 ± 0.01a	0.176 ± 0.005b
Free fatty acid (% oleic acid)	0.25 ± 0.0000a	0.25 ± 0.0000a	0.29 ± 0.007a	0.31 ± 0.007a	0.27 ± 0.007a	0.27 ± 0.007a
Peroxide values ($meqO_2$/kg)	4.7 ± 0.6a	4.9 ± 0.7a	7.3 ± 1.0a	7.3 ± 0.4a	5.8 ± 0.1a	5.7 ± 0.1a
K_{232}	1.974 ± 0.01a	1.968 ± 0.02a	2.156 ± 0.09a	2.075 ± 0.02a	2.087 ± 0.02a	2.06 ± 0.04a
K_{270}	0.195 ± 0.003a	0.197 ± 0.003a	0.187 ± 0.02a	0.184 ± 0.002a	0.195 ± 0.008a	0.186 ± 0.01a
ΔK	−0.006 ± 0.0003a	−0.006 ± 0.0003a	−0.004 ± 0.0004a	−0.004 ± 0.0007a	−0.004 ± .0004a	−0.004 ± 0.0004a
1.2/1.3 DAGs (%)	99.3 ± 0.3a	98.7 ± 0.4a	82.25 ± 3.3a	83.1 ± 3.1a	86.6 ± 0.1a	86.0 ± 2.0a
Waxes (mg/kg)	19.9 ± 0.8a	18.2 ± 0.2a	13.8 ± 1.3a	14.5 ± 0.6a	12.2 ± 1.4a	15.3 ± 1.1b
Cholesterol (%)	0.2 ± 0.07a	0.2 ± 0.07a	0.2 ± 0.03a	0.2 ± 0.01a	0.3 ± 0.007a	0.2 ± 0.03a
Brassicasterol (%)	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 00.0a
24-Methylenecholesterol (%)	0.1 ± 0.01a	0.1 ± 0.02a	0.1 ± 0.01a	0.1 ± 0.0a	0.2 ± 0.007a	0.2 ± 0.0a
Campesterol (%)	3.3 ± 0.2a	3.2 ± 0.02a	3.2 ± 0.01a	3.2 ± 0.01a	3.1 ± 0.0	3.1 ± 0.01
Campestanol (%)	0.2 ± 0.01a	0.2 ± 0.02a	0.2 ± 0.06a	0.2 ± 0.0a	0.2 ± 0.04a	0.2 ± 0.07a
Stigmasterol (%)	0.5 ± 0.01a	0.5 ± 0.02a	0.6 ± 0.007a	0.5 ± 0.02a	0.5 ± 0.06a	0.5 ± 0.05a
Δ7-Campesterol (%)	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Δ5,23-Stigmastadienol (%)	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Chlerosterol (%)	1.0 ± 0.02a	1.1 ± 0.1a	1.1 ± 0.01a	1.0 ± 0.04a	1.1 ± 0.007a	1.0 ± 0.01a
β-Sitosterol (%)	85.0 ± 0.2a	85.5 ± 0.3a	85.7 ± 0.1a	86.0 ± 0.3a	84.4 ± 0.08a	84.5 ± 0.3a
Sitostanol (%)	1.3 ± 0.09a	1.3 ± 0.01a	1.2 ± 0.07a	1.2 ± 0.1a	1.5 ± 0.007a	1.4 ± 0.1a
Δ5-Avenasterol (%)	6.4 ± 0.05a	6.2 ± 0.1a	6.1 ± 0.07a	5.8 ± 0.01a	6.9 ± 0.1a	7.0 ± 0.08a
Δ5,24-Stigmastadienol (%)	1.1 ± 0.02a	1.0 ± 0.1a	0.8 ± 0.07a	0.9 ± 0.0a	1.0 ± 0.04	1.0 ± 0.03a
Delta-7-Stigmastenol (%)	0.3 ± 0.01a	0.2 ± 0.1a	0.3 ± 0.04a	0.4 ± 0.2a	0.3 ± 0.007a	0.4 ± 0.1a
Delta-7-Avenasterol (%)	0.7 ± 0.02a	0.5 ± 0.2a	0.5 ± 0.007a	0.5 ± 0.01a	0.6 ± 0.007a	0.6 ± 0.03a
App *β*-sitosterol (%)[b]	94.8 ± 0.2a	95.1 ± 0.4a	94.9 ± 0.0a	94.9 ± 0.2a	94.8 ± 0.04a	94.9 ± 0.1a
Total sterols (mg/kg)	1273.4 ± 33a	1301.5 ± 31a	995.1 ± 5.0a	981.4 ± 8.5a	904.4 ± 13.8a	952.8 ± 3.1b
Erythrodiol and uvaol (%)	1.4 ± 0.04a	1.1 ± 0.5a	2.2 ± 0.1a	2.4 ± 0.2a	2.4 ± 0.1a	2.6 ± 0.2a
Chlorophyll (mg/kg)	4.0 ± 0.09a	4.9 ± 0.4b	4.4 ± 0.4a	4.5 ± 0.01a	3.1 ± 0.2a	2.9 ± 0.3a
Carotenoid (mg/kg)	3.1 ± 0.02a	3.7 ± 0.3b	3.3 ± 0.2a	3.5 ± 0.04a	2.8 ± 0.06a	2.8 ± 0.2a
Colour: L*	90.151 ± 0.1a	93.171 ± 1.6b	80.419 ± 1.6a	81.765 ± 0.8a	82.081 ± 2.1a	82.215 ± 0.6a
a*	−5.277 ± 0.2a	−5.644 ± 0.1b	−4.907 ± 0.1a	−5.061 ± 0.02a	−4.132 ± 0.1a	−4.299 ± 0.01a
b*	48.315 ± 0.5a	53.204 ± 2.0b	44.016 ± 2.8a	46.110 ± 0.01a	40.541 ± 1.1a	38.644 ± 2.2a
C*	48.744 ± 0.4a	53.498 ± 2.1b	44.290 ± 2.8a	46.367 ± 0.04a	40.751 ± 1.1a	38.595 ± 1.9a
h	96.22 ± 0.2a	96.06 ± 0.4a	96.38 ± 0.6a	96.03 ± 0.3a	95.82 ± 0.04a	95.74 ± 0.5a
EC_{50}-$DPPH^{\cdot}$ (mg/ml MR)	7.05 ± 0.3a	6.57 ± 0.2b	9.33 ± 0.3a	8.62 ± 0.3b	9.2 ± 0.2a	8.94 ± 0.5a

[a] The data are the mean values of three indipendent extractions, ± standard deviation. For each different maturity index, the values in each row having different letters (a-b) are significantly different from one another ($p < 0.05$).

[b] App β-sitosterol: Δ5,23-stigmastadienol + chlerosterol + *β*-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol. DAGs = diacylglycerols; MR = reaction mixture; L*a*b* = CIE coordinates; C* = chroma; h = hue.

sterols composition compared to the control test, as shown by sterols content of C1 and US1 VOOs (Table 1).

As previously reported relating to VOO sterol concentration, the amount of triterpene dialcohols is largely influenced by cultivar, ripening stage and environmental conditions (Lukić, Lukić, Krapac, Sladonja, & Piližota, 2013) and technological factors (Guillaume et al., 2012). The concentration of erythrodiol and uvaol also varies in relation to a single part of the olive fruit. All of these aspects could suggest that ultrasound treatment may impact the content of triterpene dialcohols, but chemical analysis determined no differences in these compounds relating to ultrasound extraction (Table 1).

Ultrasound-assisted extraction can modify the chemical structure of some compounds during the treatment of the food matrix that can induce isomerization, degradation effects, or both. The analysis of diacylglycerols did not show any alteration of the content of 1,2- and 1,3-diacylglycerols, confirming the other qualitative parameters of VOO samples analyzed.

The turbidity grade and moisture content of VOOs were also assessed after the separation process by using a vertical centrifuge. The data reported in Table 1 show how this innovative technological treatment determines an improved separation of the water phase from the oil phase, highlighted by a significant reduction in the turbidity and moisture level of US1 oil (Table 1); these values confirm the results from the study by Veneziani et al. (2018b) that related water content to veiled oil.

The data from the CIELAB coordinates showed that there were slight increases of lightness (L*), green value (a*) and yellow value (b*) in VOO obtained by ultrasound treatment that were probably due to the reduced turbidity grade and were likely connected to oil transparency (Gordillo, Ciaccheri, Mignani, Gonzalez-Miret, & Heredia, 2011) and increased pigment contents (chlorophylls and carotenoids) as described by other authors (Clodoveo et al., 2013; Iqdiam et al., 2018). The chroma value was also enhanced from the ultrasound-assisted extraction, while the hue data showed no difference when compared with the control (Table 1). The Euclidean distance (ΔE) between two points in the three-dimensional CIELAB color space, which respectively belong to experimental (US1) and control oil (C1), reached a value of 5.76 CIELAB units, which is a difference in color that is easily distinguished by the human eye, as shown by the range of values used to correlate visual and numerical analyses of CIELAB units (Gordillo et al., 2011).

The analysis of chlorophylls and carotenoids content showed an increase of 21.5% and 15.4% of the US1 VOO, confirming as mentioned above about oil color changes due to the impact of ultrasound technology.

The physical and chemical effects of the cavitation phenomenon caused by ultrasound treatment resulted in a significant enhancement of the extraction yield that increased from 11.9 kg of oil per 100 kg of olives in the C1 sample to 14.6 kg in the US1 sample, with a 22.7% increase when compared to oil obtained by the control test in olives that were at an early ripening stage. The data was confirmed by the

Table 2
VOO extraction yield, moisture and oil content of paté and pomace obtained from olives at different maturity index and for a malaxation time of 30 min.

Maturity index	C1	US1	C2	US2	C3	US3
	0.88		*2.82*		*3.31*	
Extraction yield (%)	11.9 ± 0.3a	14.6 ± 0.3b	17.5 ± 0.2a	18.1 ± 0.2b	17.8 ± .3a	17.3 ± 0.2a
Paté moisture content (%)	74.8 ± 3.5a	77.6 ± 2.1a	76.7 ± 1.5a	76.7 ± 1.2a	80.6 ± 1.3a	78.1 ± 2.1a
Paté oil content (% d.w.)	26.2 ± 2.3a	20.4 ± 2.2b	24.9 ± 0.3a	26.8 ± 2.5a	21.4 ± 2.1a	23.9 ± 3.1a
Pomace moisture content (%)	50.8 ± 3.5a	46.8 ± 0.5a	47.3 ± .1a	47.6 ± 0.3a	49.4 ± 0.7a	48.9 ± 0.2a
Pomace oil content (% d.w.)	3.9 ± 0.4a	4.0 ± 0.1a	3.6 ± 0.3a	3.3 ± 0.02a	4.2 ± 0.6a	3.6 ± 0.2a

[a]The data are the mean values of three independent extractions, ± standard deviation. For each different maturity index the values in each row having different letters (a-b) are significantly different from one another ($p < 0.05$). C1, C2 and C3 = control test; US1, US2 and US3 = ultrasound test; dw = dry weight.

Table 3
Phenolic composition (mg/kg) of VOOs control (C1, C2 and C3) and extracted with ultrasound system (US1, US2 and US3) processing Ogliarola garganica olives at different maturity index for 30 min of malaxation time.[a]

Maturity index	C1	US1	C2	US2	C3	US3
	0.88		*2.82*		*3.31*	
3,4-DHPEA	1.1 ± 0.1a	1.4 ± 0.1b	5.4 ± 1.8a	5.1 ± 0.4a	5.1 ± 0.2a	5.1 ± 0.6a
p-HPEA	3.0 ± 0.7a	3.6 ± 0.1a	6.5 ± 1.1a	6.2 ± 0.1a	5.8 ± 0.1a	5.7 ± 0.1a
Vanillic acid	0.4 ± 0.04a	0.3 ± 0.1a	0.4 ± 0.1a	0.4 ± 0.01a	0.3 ± 0.003a	0.3 ± 0.04a
3,4-DHPEA-EDA	588.5 ± 30.0a	666.6 ± 34.7b	475.5 ± 6.5a	485.2 ± 6.4a	442.1 ± 31.3a	430.9 ± 40.7a
p-HPEA-EDA	202.4 ± 3.2a	204.7 ± 3.9a	169.8 ± 4.2a	189.4 ± 4.0b	181.4 ± 9.5a	175.7 ± 8.8a
3,4-DHPEA-EA	187.1 ± 3.9a	207.8 ± 2.7b	166.1 ± 6.3a	170.9 ± 7.6a	153.4 ± 17.8a	150.5 ± 9.9a
Ligstroside aglycone	18.5 ± 0.1a	20.4 ± 0.5b	18.5 ± 0.5a	17.0 ± 0.1b	18.0 ± 0.9a	17.8 ± 0.6a
(+)-1-Acetoxypinoresinol	40.3 ± 1.3a	39.3 ± 2.1a	38.2 ± 1.2a	38.7 ± 1.1a	38.8 ± 1.1a	39.1 ± 1.0a
(+)-Pinoresinol	22.0 ± 0.4a	23.3 ± 0.3b	23.6 ± 1.0a	22.8 ± 0.4a	21.6 ± 1.1a	20.2 ± 0.2a
Total phenols	1063.3 ± 30.5a	1167.4 ± 35.1b	904.1 ± 10.8a	935.9 ± 10.4b	866.4 ± 41.3a	845.3 ± 43.9a
α-Tocopherol	245.3 ± 2.7a	272.9 ± 1.9b	272.1 ± 5.4a	268.7 ± 4.1a	267.7 ± 0.8a	261.4 ± 5.1a

[a] The data are the mean values of three independent VOO extractions, ± standard deviation. For each different maturity index the values in each row having different letters (a-b) are significantly different from one another ($p < 0.05$).

Table 4
Volatile composition (μg/kg) of VOOs control (C1, *C*2 and C3) and extracted with ultrasound system (US1, US2 and US3) processing Ogliarola garganica olives at different maturity index.[a]

Maturity index	C1	US1	C2	US2	C3	US3
	0.88		*2.82*		*3.31*	
Aldehydes						
(*E*)-2-Pentenal	40 ± 1a	42 ± 2a	30 ± 1a	30 ± 0a	32 ± 3a	35 ± 3a
Hexanal	430 ± 14a	357 ± 7b	333 ± 9a	351 ± 2b	250 ± 1a	277 ± 44a
(*E*)-2-Hexenal	20,352 ± 383a	19,357 ± 117b	14,250 ± 35a	14,245 ± 390a	9944 ± 1081a	11,238 ± 628a
(*E,E*)-2,4-Hexadienal	118 ± 4a	102 ± 2b	69 ± 1a	67 ± 1a	55 ± 5a	62 ± 1b
2,4-Hexadienal (i)	203 ± 11a	173 ± 5b	117 ± 1a	114 ± 3a	93 ± 8a	108 ± 1b
Sum of C_5 and C_6 aldehydes	21,142 ± 384a	20,031 ± 117b	14,799 ± 36a	14,807 ± 390a	10,373 ± 1081a	11,719 ± 629a
Alcohols						
Ethanol	2037 ± 111a	2249 ± 130a	2873 ± 39a	2844 ± 73a	1284 ± 78a	1366 ± 131a
Benzyl alcohol	79 ± 4a	77 ± 1a	93 ± 1a	87 ± 4a	117 ± 10a	98 ± 4b
Phenylethyl alcohol	157 ± 10a	150 ± 4a	236 ± 3a	212 ± 12b	332 ± 25a	272 ± 16b
1-Pentanol	15 ± 1a	15 ± 0.2a	12 ± 1a	12 ± 1a	35 ± 20a	41 ± 4a
1-Penten-3-ol	589 ± 12a	587 ± 27a	410 ± 10a	415 ± 10a	423 ± 12a	399 ± 11a
(*E*)-2-Penten-1-ol	60 ± 3a	61 ± 2a	33 ± 1a	33 ± 1a	51 ± 0.2a	53 ± 2a
(Z)-2-Penten-1-ol	455 ± 10a	449 ± 18a	356 ± 11a	360 ± 6a	336 ± 21a	324 ± 14a
1-Hexanol	464 ± 30a	432 ± 23a	442 ± 13a	423 ± 0.3b	417 ± 6a	397 ± 8b
(*E*)-2-Hexen-1-ol	285 ± 9a	274 ± 24a	248 ± 11a	230 ± 0a	219 ± 2a	206 ± 5b
(*Z*)-3-Hexen-1-ol	464 ± 8a	383 ± 17b	219 ± 12a	221 ± 11a	212 ± 20a	221 ± 1a
Sum of C_5 and C_6 alcohols	2331 ± 36a	2200 ± 49b	1719 ± 26a	1693 ± 16a	1691 ± 37a	1641 ± 20a
Esters						
Hexyl acetate	34 ± 2a	34 ± 2a	41 ± 3a	25 ± 1b	11 ± 1a	12 ± 1a
(*Z*)-3-Hexenyl acetate	80 ± 2a	63 ± 3b	11 ± 0.2a	13 ± 2b	20 ± 2a	20 ± 1a
Sum of esters	113 ± 3a	97 ± 4b	51 ± 3a	37 ± 2b	30 ± 2a	32 ± 1a
Ketones						
3-Pentanone	96 ± 2a	102 ± 2a	304 ± 23a	267 ± 17b	71 ± 1a	72 ± 0.3a
1-Penten-3-one	268 ± 11a	286 ± 18a	122 ± 34a	149 ± 2a	165 ± 2a	165 ± 2b
6-Methyl-5-hepten-2-one	3 ± 0.2a	2 ± 0.5b	4 ± 0a	4 ± 0.3a	3 ± 0a	3 ± 0a
Sum of ketones	366 ± 11a	391 ± 18b	430 ± 41a	420 ± 17a	240 ± 2a	240 ± 2a

[a] The data are the mean values of three independent VOO extractions, ± standard deviation. For each different maturity index the values in each row having different letters (a-b) are significantly different from one another ($p < 0.05$).

Table 5
Phenolic composition (mg/kg) of VOOs control (C4) and extracted with ultrasound system (US4) at different malaxation times processing Ogliarola garganica olives at 0.88 maturity index.[a]

Malaxation time	C4			US4		
	10 min	20 min	30 min	10 min	20 min	30 min
3,4-DHPEA[a]	1.7 ± 0.3aAB	2.5 ± 0.5aA	1.1 ± 0.1aB	1.6 ± 0.1aA	2.1 ± 0.5aA	1.4 ± 0.1bA
p-HPEA	4.6 ± 0.5aA	3.8 ± 1.2aA	3.4 ± 0.5aA	3.7 ± 0.4aA	5.0 ± 1.4aA	3.4 ± 0.3aA
Vanillic acid	0.4 ± 0.1aA	0.5 ± 0.1aA	0.4 ± 0.06aA	0.5 ± 0.1aA	0.5 ± 0.1aA	0.3 ± 0.1aA
3,4-DHPEA-EDA	506.6 ± 19.0aA	597.0 ± 15.5aB	601.9 ± 41.0aB	555.2 ± 26.2aA	651.5 ± 24.9bB	715.6 ± 37.3bB
p-HPEA-EDA	188.7 ± 11.2aA	203.0 ± 4.2aA	205.5 ± 3.7aA	187.9 ± 7.3aA	204.9 ± 6.2aB	204.4 ± 4.9aB
3,4-DHPEA-EA	176.0 ± 11.2aA	184.5 ± 10.1aA	187.3 ± 2.4aA	169.0 ± 7.2aA	200.9 ± 8.4aB	205.5 ± 1.7bB
Ligstroside aglycone	16.9 ± 0.6aA	20.1 ± 0.3aB	18.5 ± 0.2aC	16.6 ± 0.5aA	20.0 ± 0.9aB	19.8 ± 0.4bB
(+)-1-Acetoxypinoresinol	38.4 ± 1.9aA	42.1 ± 1.1aA	42.2 ± 2.5aA	39.9 ± 2.2aAB	43.6 ± 0.5aA	37.7 ± 2.1aB
(+)-Pinoresinol	21.9 ± 1.2aA	23.3 ± 0.6aA	22.5 ± 0.6aA	20.7 ± 1.0aA	22.4 ± 0.3aAB	23.7 ± 0.8aB
Total phenols	955.3 ± 24.9aA	1076.8 ± 19.0aB	1082.7 ± 41.3aB	995.0 ± 28.2aA	1150.7 ± 27.1bB	1211.8 ± 37.7bB
α-Tocopherol	267.7 ± 10.2aA	270.7 ± 11.0aA	242.8 ± 1.9aB	301.4 ± 10.2bA	306.8 ± 7.7bA	270.8 ± 3.1bB

[a] The data are the mean values of three independent VOO extractions, ± standard deviation. For each different malaxation times the values in each row having different letters (a-b) are significantly different from one another ($p < 0.05$). For control (C4) and ultrasound (US4) test the values in each row having different letters (A-C) are significantly different from one another ($p < 0.05$).

residual oil content value in the olive pomace and paté that were lower in the US1 sample compared to the C1 sample (Table 2).

The introduction of ultrasound treatment of crushed olive paste during the VOO mechanical extraction process caused a release of phenolic compounds into the oily phase that caused an increased amount of hydrophilic phenols in US1 oil compared to the corresponding control oil (Table 3). The percentage increase was 9.8% and was mainly due to the aglycon derivatives of oleuropein (3,4-DHPEA-EDA, 3,4-DHPEA-ED and 3,4-DHPEA) that are among the most important bioactive molecules characterized by their activities that convey health and sensory benefits (Di Maio et al., 2011, Parkinson & Cicerale, 2016). The lipophilic fraction, mainly represented by α-tocopherol, was also positively enhanced, with a higher concentration of approximately 30 mg/kg that represented an increase of 11.3% compared to the content of the C1 sample. Both increases in hydrophilic and lipophilic concentrations of VOO phenols that are due to the improvement of cellular degradation of the olive drupe are in accordance with other studies that showed different enhancements of the phenolic fraction when using different ultrasound systems and different operating parameters (Almeida et al., 2017; Clodoveo et al., 2017; Juliano et al., 2017, Leone et al., 2018). However, other studies did not confirm an increase in the hydrophilic fraction of phenolic compounds, but ultrasound seems to have a universal positive effect on the α-tocopherol content in all experimental investigations (Bejaoui, Beltran, Aguilera, & Jimenez, 2016; Clodoveo et al., 2013; Iqdiam et al., 2018; Jimenez et al., 2007). The higher amount of bioactive compounds in US1 virgin olive oil, characterized by significant biological properties such as antioxidant activities (Di Maio et al., 2011, Servili et al., 2014), was also confirmed by RSC analysis that used the DPPḢ radical. The antioxidant capacity of the US1 sample showed a 6.8% reduction in the EC_{50} value compared to the control, pointing out a major increase in total antioxidant activity of VOO that was extracted with ultrasound (Table 1).

C1 and US1 oil samples were also subjected to an assessment of volatile compounds that showed a global decrease in such compounds (Table 4) after ultrasound treatment (Kalua, Bedgood Jr., Bishop, & Prenzler, 2013). The sum of C_5 and C_6 saturated and unsaturated aldehydes was 21142 μg/kg and 20031 μg/kg for C1 and US1 VOOs, respectively, with a 5.3% decrease. The reduction of aldehydes was mainly observed in the concentration of hexenal, (*E*)-2-hexenal and 2,4-hexadienal (i), which are responsible for the "green" sensory note in VOO (Kalua, Bedgood, Bishop, & Prenzler, 2013). The reduction of the sum of C_5 and C_6 saturated and unsaturated alcohols and of the sum of esters in US1 oil (5.6% and 14.2%, respectively) was essentially due to the decreased concentration of 1-hexanol, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate. The decrease in the volatile fraction of US1 VOO could be due to a partial inactivation of the enzymes belonging to the lipoxygenase pathway that resulted from disruption or denaturation of enzymatic molecular structures due to the physical and chemical effects of the cavitation phenomena, which resulted in a significant reduction in levels of enzymatic activity after the crushing phase and during the 30 min of the malaxation step (Terefe, Buckow, & Versteeg, 2015).

3.2. Impact of the ultrasound system on VOO extracted at different malaxation times

The high increase of extraction yield obtained in the US1 VOO suggested that a supplementary investigation should be pursued on the impact on oil extractability using the same technology with the same cultivar at the same maturity index (0.88) but with a reduction in the time of malaxation to determine how a hypothetical conversion of the traditional mechanical extraction process to a continuous extraction system might be achieved. A new test was repeated using three different malaxation times (10 min, 20 min and 30 min) for VOO extraction using control conditions (C4) and the ultrasound system (US4). The oil extraction yield showed a progressively decrease reducing the time of malaxation (data not shown). The US4 VOO extracted at 20 min of malaxation time showed a reduced oil yield of 0.4 kg per 100 kg of olives compared to the US4 VOO extracted at 30 min of malaxation time, but which still had a higher value than that of the control at 20 min of malaxation time (and represented a 9.2% increase). After 10 min of malaxation VOO that was extracted using ultrasound registered an extraction yield that was no longer improved than when it was compared to the control, underlining that a malaxation phase longer than 10 min is necessary to improve oil extractability with a high-power ultrasound system at an early ripening stage of the processed olives.

The decreased malaxation time also caused a progressive reduction in the hydrophilic phenols content in both the control and ultrasound VOOs, with a reduction from 30 min to 10 min representing 11.8% and 17.9% decreases, respectively. The amount of phenolic compounds in US4 were always higher than what was observed in C4 at each respective malaxation time even if the data obtained with 10 min of malaxation was not statistically significant (Table 5).

Alpha-tocopherol was increasingly concentrated at 30 min, 20 min and 10 min of malaxation where US4 VOOs exhibited 11.5%, 13.3% and 12.6% increases when compared to controls, respectively, demonstrating low variability as a function of time.

The highest values of volatile composition were observed in the VOOs extracted at 20 min of malaxation in both the C4 and US4 trials (Table 6).

No significant differences were detected in the volatile composition

Table 6
Volatile composition (μg/kg) of VOOs control (C4) and extracted with ultrasound system (US4) at different malaxation times processing Ogliarola garganica olives at 0.88 maturity index.[a]

Malaxation time	C4			US4		
	10 min	20 min	30 min	10 min	20 min	30 min
Aldehydes						
(*E*)-2-Pentenal	42 ± 2aA	46 ± 4aA	40 ± 1aA	43 ± 2aA	42 ± 2aA	44 ± 1bA
Hexanal	439 ± 11aA	434 ± 34aA	414 ± 6aA	395 ± 9bA	478 ± 19aB	362 ± 18bA
(*E*)-2-Hexenal	21968 ± 438aA	21970 ± 2395aA	20710342aA	22197 ± 753aA	22603 ± 282aA	19390 ± 141bB
(*E,E*)-2,4-Hexadienal	219 ± 5aA	223 ± 32aA	112 ± 1aB	216 ± 4aA	230 ± 9aA	99 ± 1bB
2,4-Hexadienal (i)	1293aA	130 ± 20aA	189 ± 5aB	135 ± 5aA	135 ± 6aA	167 ±
Sum of C_5 and C_6 aldehydes	22796 ± 438aA	22802 ± 2395aA	21464 ± 343aA	22986 ± 753aA	23489 ± 282aA	20061 ± 143bB
Alcohols						
Ethanol	2373 ± 41aA	2334 ± 114aA	2073 ± 88aB	1998 ± 155bA	2306 ± 34aB	2066 ± 126aAB
Benzyl alcohol	130 ± 14aA	143 ± 7aA	81 ± 0aB	157 ± 17aA	129 ± 9aB	77 ± 1bC
Phenylethyl alcohol	267 ± 25aA	264 ± 23aA	152 ± 9aB	336 ± 25bA	271 ± 13aB	155 ± 25aC
1-Pentanol	15 ± 1aA	16 ± 1aA	16 ± 1aA	17 ± 3aA	13 ± 1bB	15 ± 0aC
1-Penten-3-ol	590 ± 40aA	570 ± 42aA	604 ± 14aA	580 ± 36aAB	551 ± 34aA	619 ± 12aB
(*E*)-2-Penten-1-ol	86 ± 5aA	75 ± 6aA	59 ± 26aA	87 ± 2aA	74 ± 2aA	61 ± 20aA
(*Z*)-2-Penten-1-ol	469 ± 34aA	485 ± 47aA	469 ± 13aA	412 ± 25aA	455 ± 17aAB	466 ± 11aB
1-Hexanol	342 ± 11aA	488 ± 101aA	427 ± 15aA	358 ± 19aA	391 ± 25aAB	414 ± 12aB
(*E*)-2-Hexen-1-ol	354 ± 11aA	332 ± 67aA	274 ± 14aA	305 ± 32aAB	306 ± 24aA	241 ± 1bB
(*Z*)-3-Hexen-1-ol	302 ± 5aA	447 ± 10aB	453 ± 13aB	354 ± 32bA	404 ± 30aA	404 ± 8bA
Sum of C_5 and C_6 alcohols	2157 ± 55aA	2413 ± 137aB	2301 ± 40aAB	2114 ± 66aA	2193 ± 60aA	2219 ± 30bA
Esters						
Hexyl acetate	44 ± 7aAB	53 ± 3aA	35 ± 1aB	37 ± 3aA	34 ± 6bA	36 ± 3aA
(*Z*)-3-Hexenyl acetate	53 ± 1aA	100 ± 10aB	83 ± 1aC	55 ± 2aA	87 ± 8aB	67 ± 2bA
Sum of esters	97 ± 7aA	153 ± 11aB	118 ± 2aC	92 ± 3aA	121 ± 10bB	102 ± 3bA
Ketones						
3-Pentanone	103 ± 13aA	90 ± 8aA	99 ± 1aA	110 ± 19aA	68 ± 16aB	99 ± 2aAB
1-Penten-3-one	275 ± 20aA	287 ± 18aA	284 ± 8aA	272 ± 13aA	260 ± 9aA	309 ± 13bB
6-Methyl-5-hepten-2-one	3 ± 0aA	3 ± 0.5aA	3 ± 1aA	3 ± 0aA	3 ± 0.4aA	2 ± 0aB
Sum of ketones	381 ± 24aA	380 ± 20aA	385 ± 8aA	385 ± 23aA	331 ± 19bB	410 ± 13bA

[a] The data are the mean values of three independent VOO extractions, ± standard deviation. For each different malaxation time the values in each row having different letters (a-b) are significantly different from one another ($p < 0.05$). For control (C4) and ultrasound (US4) test the values in each row having different letters (A-C) are significantly different from one another ($p < 0.05$).

between C4 VOOs and US4 VOOs at 10 and 20 min of malaxation. The data did not confirm the decrease of volatile compounds showed at 30 min of malaxation (Tables 4 and 6) for the olives harvested at an early maturity index. For that reason, the variability of volatile fraction seem to be mainly influenced by malaxation parameters rather than ultrasound treatment as described by other authors (Iqdiam et al., 2018; Kalua, Bedgood Jr., Bishop, & Prenzler, 2006; Reboredo-Rodriguez, Gonzalez-Barreiro, Cancho-Grande, & Simal-Gandara, 2014; Selvaggini et al., 2014).

3.3. Impact of ultrasound system processing olives at different maturity indices

This experimental study also evaluated VOO extraction yield as measured using Ogliarola garganica olives at other two different maturity indices: 2.82 and 3.31 for control (C2 and C3) and ultrasound tests (US2 and US3), respectively, performed at 30 min of malaxation time. The ultrasound system showed a reduced capacity for improving the oil extraction yield in increasingly ripe olives, as higher maturity indices coincided with a decreased oil yield compared to oil extracted from olives at a 0.88 MI (Table 2). However, US2 sample showed a significant enhancement of oil yield from 17.5 kg per 100 kg of olives of C2 sample to 18.1 kg with a 3.4% increase as compared to control VOO, whereas US3 did not show a difference in oil yield when compared to the control (Table 2). This decreasing trend of oil extraction yield could be related to a physiological increase of the activity of endogenous depolymerizing enzymes (pectinase, cellulase and hemicellulase) during the ripening of the olive fruit that have the same roles of cell wall and membrane degradation that occur in the cavitation phenomenon caused by ultrasound waves that may promote the release of oil and other organic material from the olive cells before and during the VOO mechanical extraction process (Dag et al., 2011; Vierhuis et al., 2001). Increasingly intensive enzymatic activity may reduce the impact of ultrasound treatment on extraction yield and thus limit its efficacy when olives are processed at a medium-late ripening stage.

No significant differences were detected from the analysis of free acidity, peroxide value, K_{232}, K_{270} and ΔK between experimental and control VOOs extracted from fruits at the other two ripening stages (Table 1).

The turbidity grade and moisture content of VOOs showed the same decreasing trend pointed out for the oils obtained from olives at an early maturity index (0.88 MI) but with a more limited impact. The reduction of moisture in the VOOs treated with ultrasound was 9.0% and 9.7%, respectively for US2 and US3 compared to control test (Table 1). On the whole, the turbidity levels were higher than the values showed for the first ripening stage of olives and the same reduction effect of US treatment was detected for both the other VOOs, even if the data was not statistically significant for the VOO obtained from fruits at 2.82 MI (Table 1).

The analysis of CIE Lab color space did not show any significant effects due to the use of US system compared to the test conducted processing olives at 0.88 MI, the data of VOOs form olives at 2.82 and 3.31 MI were supported by the analysis of chlorophylls and carotenoid that were also not significant confirming absence of color alterations.

As shown for the first stage of fruit ripening, the other two batches of olives did not show differences of triterpene dialcohols and 1,2- and 1,3-diacylglycerols, whereas the content of waxes and total sterols increased in the last ripening stage, with values of 25.4% and 5.6% compared to the control trial, respectively (Table 1). The results suggest a more intensive effect of ultrasound treatment on the release of waxes

and sterols into the water/oily phase from olive cells at a late ripening stage, when the different tissues of fruit are subjected to high levels of depolymerizing enzymatic activities. However, the sterol content, that decreased during the olives ripening in both US ad C tests, did not modified its percentage composition after the ultrasound treatment for all the VOOs extracted from fruits at different maturity indices (Table 1). The high percentage increase of waxes compared to control at the highest maturity index did not compromise the quality of the product with a content (15.3 mg/kg) abundantly below the limit (≤150 mg/kg) fixed by the regulation of European Union (OJEC, 2015).

The minor effect of high-power ultrasound also concerned the phenolic fraction (Table 3) showing there was no significant impact on both the concentration of hydrophilic phenols and the concentration of α-tocopherol that is further confirmed by results from Bejaoui et al. (2017) in olives at an advanced stage of development and ripening. Others studies even showed reduced phenolic content accompanied by reduced extraction yields in olive fruits that were processed at a maturity index over 1.9 units (Clodoveo et al., 2013; Jimenez et al., 2007).

Concentrations of volatile compounds in VOOs extracted at 2.82 and 3.31 maturity indices did not follow the same trend observed in the test using olives at the early ripening stage, showing no significant differences between ultrasound-treated and untreated oil samples (Table 4).

4. Conclusions

The impact of high-power ultrasound technology on oil yield and quality parameters were evaluated, along with a first time of evaluating how it impacted the concentration of sterols, triterpene dialcohols, waxes, diacylglycerols and other physicochemical characteristics of VOOs extracted at different maturity indices of olives. No effects were observed on VOO legal and quality characteristics, maintaining an unchanged commercial category in the product, with the only exception of a slight increase of waxes and total sterols at the highest maturity index. Hydrophilic phenols and α-tocopherols were increased in their concentrations in many VOOs that were extracted using high-power ultrasound. The ultrasound system showed a positive impact on VOOs obtained from olive fruits at an early ripening stage, as it was able to cause a highly disruptive effect on the cells that are still characterized with intense physiological activities and thus guaranteed an abundant release of intracellular contents into the liquid medium. This phenomenon allowed an increased oil yield and a significant improvement in the phenolic fraction that are, however, negatively influenced by the enhancement of fruit ripening. The cavitation phenomena and shock-waves caused by this new technology were applied to the VOO mechanical extraction process, and their effects on cell walls and membranes seemed to have been minimized when there is increased activity of endogenous enzymes. The impact of ultrasound technology was observed to be progressively reduced when used with olives at higher maturity indices, canceling its positive effect on both oil extractability and enhancement of quality parameters in drupes at a medium-high level of ripening. The effects of the new technology should be evaluated on different cultivar at different maturity indices and further studies should be also developed on the correct management of the different operative parameters (frequency, power, pressure and amplitude) of ultrasound system to investigate the possible performances on quality and extraction yield of fruits at a medium-late ripening stages.

Declaration of interest

None.

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