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High vacuum-assisted extraction affects virgin olive oil quality: Impact on phenolic and volatile compounds

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ABSTRACT

High vacuum technology has been incorporated into a new assisted extraction system applied to virgin olive oil (VOO) processing, which was tested at a lab-scale pilot plant to evaluate its impact on the physicochemical properties of the olive paste and oil. The vacuum system induced changes in the mechanical and structural properties of the olive cells, improving the coalescence of the oil droplets due to substantial cellular and intracellular mass transfer during the process, as shown by cryo-scanning electron microscopy (Cryo-SEM) analysis. The effects on the quality characteristics of VOOs extracted from three cultivars at different malaxation temperatures were evaluated. A significant increase in the phenolic content, from 25.2% to 48.6%, was observed. The content of volatile compounds responsible for the VOO flavor decreased as a function of malaxation temperature. The reduction of some volatile molecules related to the VOO off-flavor (ethanol, ethyl acetate and acetic acid) was also shown.

1. Introduction

The technological innovations of recent decades have strongly affected the olive oil sector and most VOO mechanical extraction processes (Kalogianni, Georgiou, & Hasanov, 2019). Many emerging technologies have been studied and applied to VOO processing (Cecchi, Bellumori, Corbo, Milani, Clodoveo, & Mulinacci, 2019; Esposto et al., 2013; Iqdiam et al., 2018; Polari, Garcí-Aguirre, Olmo-García, Carrasco-Pancorbo, & Wang, 2019; Puértolas & Martinez de Maranon, 2015; Taticchi et al., 2019; Veneziani et al., 2017; 2019). These new production systems have the common aim of improving the extraction process with a particular focus on the working efficiency of the industrial plant, oil yield and quality of the VOO. Increasing the working efficiency is mainly associated with shortening the extraction time and the possibility of converting traditional discontinuous extraction plants into continuous ones (Clodoveo et al., 2017; Leone, Zagaria, Sabella, Tamborrino, & Romaniello, 2015; Veneziani et al., 2015). All these approaches consider increases in oil extractability for improving oil yield and enhancing the quality parameters related to the compounds responsible for the health and the nutritional and sensory properties of VOOs. The main results achieved through technological innovations involve increases in the phenolic contents in VOO and changes in the volatile fractions, although the impacts are often influenced by the different cultivars and their ripening stage (Almeida, Valli, Bendini & Gallina Toschi, 2017; Bejaoui et al., 2018; Veneziani et al., 2015; 2018). The enhancement of the VOO phenolic concentration was mainly due to increases in secoiridoids (aglycon derivatives of oleuropein and ligstroside), the most abundant compounds in oil, which are characterized by high antioxidant activity and health benefits (Gaforio et al., 2019; Servili et al., 2015), as confirmed by EFSA (EFSA, 2012). Instead, the modification of the volatile fraction was due to changes in the concentration of compounds belonging to the main classes of molecules (C_5 and C_6 saturated and unsaturated aldehydes, alcohols and esters), which are involved in the flavor of VOO and responsible for the most appreciated sensory notes of the product. Both the groups of chemical compounds, phenolic and volatile molecules, were influenced by technological innovations that can modify or alter their production and diffusion in the oily phase. These physicochemical processes are largely regulated by enzymatic activities (Caponio, Leone, Squeo, Tamborrino, & Summo, 2019; Hbaieb Kotti, Cortes-Francisco, Caixach, Gargouri, & Vichi, 2016; García-Rodríguez, Romero-Segura, Sanz, & Perez, 2015; Taticchi et al., 2019) that mainly involve polyphenoloxidase (PPO), peroxidase (POD) and lipoxygenase (LOX) and the associated oxidation phenomena (Esposto et al., 2015; Di Maio et al., 2013).

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In this study, a technological method based on the use of high vacuum was tested in the mechanical extraction of olive oil to evaluate its potential positive effects on whole oil extraction process. Vacuum treatment is a technological process that is commonly used in different agri-food sectors. Vacuum treatments are used in cooking, cooling, drying and freeze-drying, impregnation, evaporation and packaging systems (Saurel, 2000). A vacuum can be applied in different processes concerning the pretreatment, production, stabilization and conservation of many foodstuffs derived from fruits, vegetables, meat, fish and dairy products. Vacuum systems, when combined with different processing techniques, can preserve and/or increase the health, sensory and nutritional properties of food products, reducing the degradation effects and improving their quality parameters and shelf life (Ravichandran and Upadhyay, 2004). The removal of oxygen during food processing and the control of temperature reduce oxidation processes and allow the regulation and suppression of enzymatic activity, reducing browning and physicochemical alterations of plant materials. The effects of vacuum treatment modify the characteristics of foods due to changes in the mechanical and structural properties of tissue, including texture and porosity (Betoret, Betoret, Rocculi, & Dalla Rosa, 2015). The alterations of the cellular structure of fruit and vegetable tissues guarantee a high flow of liquids into the intracellular spaces and capillaries of the plant material that can be used to modify the physicochemical composition of the product, significantly impacting quality attributes (Radziejewska-Kubzdela, Biegańska-Marecik, & Kidoń, 2014; Zhao and Xie, 2004). Some of the characteristics of vacuum and most high vacuum techniques, such as modifying the distribution of the liquid phase into plant materials or regulating the removal of volatile compounds, have recently been the main subject of studies in the food science sector (Castro and Ross, 2012: Lagacé et al., 2019; Quarta and Monica Anese, 2011; Tylewicza et al., 2019). The physicochemical modification of fruit and vegetable tissues, due to a rapid mass transfer induced by new technologies such as vacuum impregnation and vacuum infusion, has also improved our understanding of the potential applications of vacuum in food processing (Pasławska, Stępień, Nawirska-Olszańska, & Sala, 2019; Radziejewska-Kubzdela et al., 2014). On the basis of this information, the impact of high vacuum on the virgin olive oil mechanical extraction process was evaluated. The effects of mass transfer, induced by high vacuum through the high volume of intracellular spaces of the crushed olive paste, on the main quality parameters of VOO, such as the contents of phenolic and volatile compounds strongly linked to the health benefits and the sensory notes of the product, were assessed. In this study, the Zuccardi-Bonino method, which utilizes high vacuum technology to extract oil from oily fruits such as avocado, palm fruit and olive drupes, was used (WIPO, 2019) to evaluate its physicochemical impact on olive paste and VOO. The disruption effect of high vacuum on olive tissues could improve the breakdown of cellular walls and membranes increasing the intra-and inter-cellular liquid mass transfer combined with a stripping phenomenon of volatile compounds.

2. Materials and methods

2.1. Chemicals

Methanol, water, acetic acid, butanal, isobutyl acetate, 1-nonanol and all the analytical standards of the volatile compounds evaluated in the headspace of the VOO [pentanal, (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, (*E*,*E*)-2,4-hexadienal, 2,4-hexadienal (i), 1-pentanol, 1-penten-3-ol, (*E*)-2-penten-1-ol, (*Z*)-2-penten-1-ol, 1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-3-hexen-1-ol, hexyl acetate] were supplied by Merck (Merck KGaA, Darmstadt, Germany). Tyrosol (*p*-HPEA) and hydroxytyrosol (3,4-DHPEA) were purchased from Cabru s.a.s. (Arcore, Milan, Italy) and Fluka (Milan, Italy), respectively. Lignans, such as (+)-pinoresinol and (+)-1-acetoxypinoresinol and the aglyconic derivatives of oleuropein and ligstroside [the dialdehydic forms of decarboxymethyl elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA or oleacein) and to tyrosol (*p*-HPEA-EDA or oleocanthal), 3,4-(dihydroxyphenyl)ethanol elenolic acid (3,4-DHPEA-EA or an isomer of the oleuropein aglycon), and *p*-(hydroxyphenyl)ethanol elenolic acid (*p*-HPEA-EA or ligstroside aglycon)] were extracted from VOO following the method described by Selvaggini et al. (2014).

2.2. VOO extraction process

Three Italian cultivars, Peranzana, Moraiolo and Coratina, were processed to obtain VOO controls (C) and VOOs extracted with high vacuum (V). Two batches of Moraiolo olives were harvested at the end of October 2018 and during the first week of November in the Umbria region with a maturity index values (MIs) of 1.67 and 3.58, respectively (Beltran, Uceda, Jimenez, & Aguilera, 2003). The Peranzana and Coratina olives were purchased from the Apulia region during the first weeks of November 2018 (1.3 and 1.18 MI, respectively).

The VOO experimental samples, obtained from olives (5 kg) extracted within 48 h of harvesting, were collected at the end of a lab-scale mechanical extraction process. The process was composed of a crushing phase using a hammer crusher, a malaxation phase under high vacuum conditions following the Zuccardi-Bonino method (WIPO, 2019), and an extraction phase with a swinging-bucket CRU-5000 centrifuge (Damon/IEC Division, Needham, USA). The malaxer prototype was equipped with a vacuum extractor system based on the use of a high vacuum pump with a maximum pressure up to −680 mm Hg. The vapors and gases obtained from the olive paste, through the action of the high vacuum, circulate towards an Alfa Laval Brazed Heat Exchanger (Alfa Laval Lund AB, Ronneby, Sweden), wherein the water vapor is condensed and collected in the condensate collection vessel. The condenser was kept at low temperature with a cooling liquid that recirculated through a refrigerator to maintain a temperature of 4 °C.

In a preliminary test, Moraiolo olives were processed at high temperatures (40 °C, 50 °C and 60 °C) for a brief malaxation time (10 min). All the other tests were conducted at lower temperatures (20 °C, 25 °C and 30 °C) for 30 min of malaxation with olives from the Peranzana, Moraiolo and Coratina cultivars. The control tests were carried out with the same lab-scale mechanical extraction plant without the use of high vacuum during the kneading step (25 °C for 30 min of malaxation).

The data of VOOs obtained from different cultivar are the mean values of two independent extractions.

2.3. VOO chemical analysis

2.3.1. Legal quality parameters

Some of the legally regulated quality parameters of the VOO samples from the control and experimental tests (free fatty acids, peroxide value, K_{232}, K_{270} and ΔK) were evaluated as required by the Regulation (EU) 2015/1830 (OJEC, 2015).

2.3.2. Phenolic compounds

The hydrophilic fraction of the VOO phenols was extracted following the methods described by Selvaggini et al. (2014) with some modifications. An Ultra-Turrax T 25 homogenizer (IKA Labortechnik, Staufen, Germany) was used to mix 20 g of VOO sample with 10 mL of methanol/water solution (80:20 v/v) at 17,000 rpm for 2 min. The extraction was repeated twice. The methanolic extract was derived from the two supernatants obtained by centrifugation at 2850 rpm for 10 min after each extraction process. The two supernatants were collected and concentrated by Rotavapor at 37 °C. The extract was taken with 2 mL of methanol, evaporated to dryness under a nitrogen stream, and stored at −25 °C until analysis. High-performance liquid chromatography (HPLC) analysis of the phenolic extract was carried out using an Agilent Technologies system Mod. 1100, composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment and detectors (DAD and FLD) and equipped with a C18

Table 1
Phenolic composition (mg/kg) of VOOs control (C) and VOOs extracted under vacuum conditions (V) processing Peranzana, Coratina and Moraiolo olives at different malaxation temperatures.

	Cv Coratina			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
3,4-DHPEA*	1.8 ± 0.3aA	3.9 ± 0.6bA	2.7 ± 1abA	2.9 ± 0.1abA
p-HPEA	4 ± 0.4aA	4.5 ± 0.6aA	3.8 ± 0.5aA	4.4 ± 0.1aA
Vanilic acid	0.6 ± 0.1aA	0.6 ± 0.1aA	0.7 ± 0.02aA	0.7 ± 0.1aA
3,4-DHPEA-EDA	467.9 ± 36.6aA	692.7 ± 5.1bA	732.2 ± 30.6bcA	785.6 ± 30.1cA
p-HPEA-EDA	128.6 ± 5.4aA	129.4 ± 4.2aA	131.7 ± 3.5aA	142.7 ± 12aA
(+)-1-Acetoxypinoresinol	51.1 ± 0.5aA	54.3 ± 3abA	58.3 ± 0.7bA	58.7 ± 1.7bA
(+)-Pinoresinol	29.4 ± 0.5aA	14 ± 1bA	17 ± 0.2cA	20.9 ± 1.2dA
3,4-DHPEA-EA	128.9 ± 9.2aA	177 ± 31.8bA	198.5 ± 13bA	205.9 ± 7.2bA
Ligstroside aglycone	25.7 ± 0.2aA	26.4 ± 2.7aA	28 ± 2.4aA	23.1 ± 0.5aA
Σ of phenols	**838 ± 39.3aA**	**1102.7 ± 45.6bA**	**1172.9 ± 36bcA**	**1245 ± 34.1cA**
	Cv Moraiolo			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
3,4-DHPEA	1.7 ± 0.5aA	2.2 ± 0.3aB	1.8 ± 0.2aA	4.1 ± 2aA
p-HPEA	2.1 ± 0.4aAB	2 ± 0.1aB	1.8 ± 0.1aB	2.2 ± 0.4aB
Vanilic acid	0.3 ± 0.04aB	0.3 ± 0.01aB	0.3 ± 0.05aB	0.2 ± 0.008aB
3,4-DHPEA-EDA	737.9 ± 61.8aB	906 ± 36.1bB	988.6 ± 28.4bB	982.1 ± 19.9bB
p-HPEA-EDA	75.2 ± 8.5aB	85.8 ± 1.7abB	91.5 ± 4.7bB	81 ± 2.4abB
(+)-1-Acetoxypinoresinol	23.9 ± 1aB	23.1 ± 1.1aB	21.8 ± 2.8aB	26.2 ± 0.1aB
(+)-Pinoresinol	24.4 ± 3.5aA	20.9 ± 0.2aB	20.4 ± 2.2aB	18.8 ± 1.2aA
3,4-DHPEA-EA	100 ± 18.3aA	170.2 ± 3.2bA	167.6 ± 21.3bA	171.6 ± 3.6bB
Ligstroside aglycone	18.2 ± 1.7aB	21.4 ± 0.1aB	20.8 ± 2.5aB	18.8 ± 0.3aB
Σ of phenols	**983.7 ± 67.7aB**	**1231.8 ± 36.5bB**	**1314.5 ± 41.8bB**	**1305 ± 20.8bA**
	Cv Peranzana			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
3,4-DHPEA	2.7 ± 1aA	6 ± 0.4bC	9.8 ± 0.5cB	12.9 ± 2.2cB
p-HPEA	4.2 ± 0.2aB	6 ± 0.6bC	5.1 ± 0.1abC	7.4 ± 0.6cC
Vanilic acid	0.8 ± 0.004aC	1.1 ± 0.002bC	0.5 ± 0.02cC	0.9 ± 0.05dC
3,4-DHPEA-EDA	430.9 ± 5.9aA	584.5 ± 16.3bC	589.9 ± 11.4bC	598.4 ± 38.3bC
p-HPEA-EDA	74.6 ± 1.9aB	74.9 ± 0.7aC	73.9 ± 3.2aC	73.8 ± 4.8aB
(+)-1-Acetoxypinoresinol	12.3 ± 3.7aC	13.4 ± 0.6aC	13.3 ± 2.1aC	13.2 ± 3.2aC
(+)-Pinoresinol	11.8 ± 3.9aB	13.4 ± 0.04aA	10.8 ± 0.02aC	11.3 ± 0.3aB
3,4-DHPEA-EA	33.4 ± 2.4aB	78.7 ± 3.5bB	77.2 ± 15.3bB	74.5 ± 1bC
Ligstroside aglycone	11.5 ± 1.1aC	5.7 ± 0.3bC	11.6 ± 0.4aC	13.4 ± 0.2cC
Σ of phenols	**582.2 ± 9aC**	**783.7 ± 17.1bC**	**792.1 ± 24.7bC**	**805.8 ± 38.9bB**

*The data are the mean values of two independent extractions, ± standard deviation. For each different cultivar, the values in each row having different letters (a-d) are significantly different from one another ($p < 0.05$). For each different compounds, the values in each column having different letters (A-C) are significantly different from one another ($p < 0.05$).

column (Spherisorb ODS-1 (250 mm × 4.6 mm) 5 μm particle size, supplied by Phase Separation Ltd. (Deeside, UK)). HPLC analysis was performed after sample solubilization with 1 mL methanol/water solution (50:50 v/v) and filtration through a 25 mm PVDF filter with a 0.2 μm pore size. The mobile phase was composed of 0.2% acetic acid (pH 3.1) in water (solvent A) with methanol (solvent B), and the gradient was modified as described by Selvaggini et al. (2014) with a running time of 73 min. Tyrosol, hydroxytyrosol and the other aglycon derivatives of oleuropein and ligstroside were detected by using the DAD with a wavelength of 278 nm, whereas the lignans were detected by using the FLD operated at 280 nm ex. and 339 nm.

2.3.3. Volatile compounds

The evaluation and quantification of volatile compounds in VOOs were performed by headspace-solid phase microextraction (HS-SPME) followed by gas chromatography-mass spectrometry (HS-SPME-GC/MS).

For sampling the headspace volatile compounds, SPME was applied as follows: three grams of VOO was placed in a 20 mL vial and tightly capped with polytetrafluoroethylene (PTFE) septum. 2-Methylpropyl acetate was added as an internal standard at a concentration of 9.8 mg/kg. The vial was left for 10 min at 35 °C to allow the volatiles in the headspace to equilibrate. After equilibration, the SPME fiber (a 50/30 μm divinylbenzene/Carboxen/poly(dimethylsiloxane) (DVB/CAR/PDMS) with a length of 2 cm; StableFlex, Supelco, Inc., Bellefonte, PA, USA) was exposed to the vapor phase for 30 min to sample the volatile compounds. Then, gas chromatography analysis was performed.

GC/MS analysis. The analyses of the volatile compounds were conducted with an Agilent Technologies GC 7890B equipped with a "Multimode Injector" (MMI) 7693A (Agilent Technologies, Santa Clara, CA, USA) and a thermostated PAL3 RSI 120 autosampler equipped with a fiber conditioning module and an agitator (CTC Analytics AG, Zwingen, Switzerland). The detection system was an Agilent 5977B single quadrupole GC/MSD with an EI Extractor (XTR) source (Agilent Technologies, Santa Clara, CA. USA). The volatiles adsorbed by the fiber were thermally desorbed in the hot GC injector port, which was set in splitless mode, for 5 min at 250 °C. The volatile compounds were separated on a DB-WAXetr column (50 m, 0.32 mm i.d., 1 μm film thickness) (Agilent Technologies, Santa Clara, CA, USA) using helium as the carrier gas at a constant flow rate of 1.7 mL/min. The GC oven heating program started at 35 °C; this temperature was held for 4 min, then increased to 150 °C at a rate of 4 °C/min, increased to 180 °C at a rate of 8 °C/min, held for 2 min, increased to 210 °C at a rate of 11 °C/min, and this temperature was held for 13.77 min. The total analysis time was 55 min.

The temperature of the transfer line was fixed at 215 °C. The MSD was operated in electron ionization (EI) mode, at an ionization energy of 70 eV, in scan mode, with scanning in the mass range of m/z 25 – 350 a.m.u. at a scan rate of 4.3 scan/s and in SIM for improving the detection limits. The MS source and the MS quad temperatures were

Table 2
Quality indices of VOOs control (C) and VOOs extracted under high vacuum conditions (V) processing Peranzana, Coratina and Moaiolo olives at different malaxation temperatures.

	Cv. Coratina			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
Free fatty acid (% oleic acid)*	0.44 ± 0a	0.45 ± 0a	0.43 ± 0.01a	0.44 ± 0a
Peroxide value (meqO_2/kg)	6.7 ± 0.2a	6.4 ± 0.3a	6.4 ± 0a	6.6 ± 0.2a
K_{232}	1.843 ± 0.02a	1.833 ± 0.03a	1.862 ± 0.02a	1.895 ± 0.02a
K_{270}	0.214 ± 0.004a	0.205 ± 0.006a	0.219 ± 0.005a	0.219 ± 0.002a
Δ K	−0.003 ± 0.0002a	−0.003 ± 0.0002a	−0.004 ± 0.0004a	−0.003 ± 0b
	Cv. Moraiolo			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
Free fatty acid (% oleic acid)	0.36 ± 0.03a	0.33 ± 0.01a	0.37 ± 0.04a	0.37 ± 0.03a
Peroxide value (meqO_2/kg)	4.5 ± 0.4a	4.1 ± 0.1a	4 ± 0.1a	4.6 ± 0.4a
K_{232}	1.676 ± 0.01a	1.683 ± 0.02a	1.716 ± 0.03a	1.730 ± 0.02a
K_{270}	0.159 ± 0.006a	0.165 ± 0.005a	0.162 ± 0.002a	0.173 ± 0.006a
Δ K	−0.003 ± 0.0004a	−0.004 ± 0.0002b	−0.004 ± 0.0002b	−0.004 ± 0b
	Cv. Peranzana			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
Free fatty acid (% oleic acid)	0.47 ± 0a	0.46 ± 0.01a	0.46 ± 0.01a	0.47 ± 0a
Peroxide value (meqO_2/kg)	6 ± 0.5a	6.5 ± 0.1a	6.6 ± 0.2a	6 ± 0.5a
K_{232}	1.818 ± 0.02a	1.881 ± 0.01a	1.854 ± 0.01a	1.818 ± 0.02a
K_{270}	0.164 ± 0.002a	0.177 ± 0.001a	0.169 ± 0.001a	0.164 ± 0.002a
Δ K	−0.003 ± 0.0004a	−0.003 ± 0.0003a	−0.003 ± 0.0001a	−0.003 ± 0.0004a

*The data are the mean values of two independent extractions, ± standard deviation. For each different cultivar, the values in each row having different letters (a-d) are significantly different from one another ($p < 0.05$).

Table 3
Volatile composition (μg/kg) of VOOs control (C) and VOOs extracted under high vacuum conditions (V) processing Coratina olives at different malaxation temperatures.

	Cv Coratina			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
*Aldehydes**				
Pentanal	72 ± 8a	44 ± 5b	65 ± 6ab	13 ± 4c
(*E*)-2-Pentenal	24 ± 2a	15 ± 0b	14 ± 1b	19 ± 2ab
Hexanal	459 ± 45a	428 ± 27a	341 ± 26a	157 ± 15b
(*E*)-2-Hexenal	30285 ± 1054a	23780 ± 2430b	18380 ± 776bc	16645 ± 1450c
(*E*,*E*)-2,4-Hexadienal	179 ± 25a	90 ± 6b	90 ± 10b	92 ± 7b
Sum of C_5 and C_6 aldehydes	**31019 ± 1055a**	**24358 ± 2430b**	**18889 ± 776c**	**16926 ± 1450c**
Alcohols				
Ethanol	18965 ± 1168a	11156 ± 454b	11135 ± 365b	8116 ± 781b
1-Pentanol	59 ± 5a	23 ± 0b	23 ± 4b	8 ± 0c
1-Penten-3-ol	318 ± 35a	178 ± 5b	192 ± 17b	126 ± 5b
(*E*)-2-Penten-1-ol	25 ± 3a	15 ± 0a	14 ± 1a	100 ± 43a
(*Z*)-2-Penten-1-ol	336 ± 29a	224 ± 4b	169 ± 39b	226 ± 1b
1-Hexanol	369 ± 41a	391 ± 48a	300 ± 12a	314 ± 18a
(*E*)-2-Hexen-1-ol	268 ± 6a	233 ± 26ab	153 ± 33b	172 ± 7b
(*Z*)-3-Hexen-1-ol	281 ± 28ab	340 ± 1a	320 ± 5ab	267 ± 15b
Sum of C_5 and C_6 alcohols	**1656 ± 68a**	**1405 ± 55b**	**1170 ± 56b**	**1213 ± 50b**
Esters				
Ethyl acetate	1431 ± 44a	280 ± 24b	295 ± 29b	273 ± 15b
Hexyl acetate	77 ± 8a	51 ± 2b	54 ± 5b	44 ± 5b
(*Z*)-3-Hexenyl acetate	67 ± 6ab	70 ± 6a	47 ± 5bc	41 ± 4c
Sum of esters	**143 ± 10a**	**121 ± 7ab**	**101 ± 8bc**	**85 ± 7c**
Ketones				
3-Pentanone + 2-Pentanone	346 ± 64a	236 ± 16a	251 ± 15a	228 ± 27a
1-Penten-3-one	109 ± 12a	95 ± 3a	72 ± 6a	79 ± 2a
6-Methyl-5-hepten-2-one	2 ± 1a	4 ± 0ab	5 ± 0b	3 ± 0ab
Sum of ketones	**457 ± 65a**	**336 ± 16a**	**328 ± 16a**	**310 ± 27a**
Acetic acid	12029 ± 538a	4463 ± 293b	4373 ± 202b	4290 ± 374b

*The data are the mean values of two independent extractions, ± standard deviation. For each different cultivar, the values in each row having different letters (a-d) are significantly different from one another ($p < 0.05$).

190 °C and 150 °C, respectively. The volatile compounds were identified by comparison of their mass spectra and retention times with those of authentic reference compounds and with the spectra in the NIST 2014 mass spectral library.

The volatile compounds were quantified using calibration curves for each compound by internal standard calculation, and the results are expressed in μg/kg of oil.

Table 4
Volatile composition (μg/kg) of VOOs control (C) and VOOs extracted under high vacuum conditions (V) processing Moraiolo olives at different malaxation temperatures.

	Cv Moraiolo			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
*Aldehydes**				
Pentanal	121 ± 1a	25 ± 4b	116 ± 9ac	54 ± 30bc
(*E*)-2-Pentenal	57 ± 27a	32 ± 1a	22 ± 2a	22 ± 1a
Hexanal	415 ± 163a	222 ± 17a	176 ± 14a	142 ± 24a
(*E*)-2-Hexenal	17138 ± 654a	13940 ± 1192ab	10407 ± 711bc	9214 ± 916c
(*E*,*E*)-2,4-Hexadienal	111 ± 22a	91 ± 6ab	54 ± 4bc	40 ± 1c
Sum of C_5 and C_6 aldehydes	**17842 ± 675a**	**14310 ± 1192ab**	**10775 ± 711bc**	**9472 ± 917c**
Alcohols				
Ethanol	8413 ± 674a	4646 ± 458b	5146 ± 231b	2451 ± 216c
1-Pentanol	51 ± 46a	23 ± 4qa	40 ± 4a	22 ± 3a
1-Penten-3-ol	333 ± 4a	235 ± 4b	122 ± 18c	223 ± 22b
(*E*)-2-Penten-1-ol	163 ± 15a	108 ± 4b	112 ± 6b	44 ± 11c
(*Z*)-2-Penten-1-ol	459 ± 11a	386 ± 7b	271 ± 28c	182 ± 1d
1-Hexanol	346 ± 30a	355 ± 12a	297 ± 6a	307 ± 8a
(*E*)-2-Hexen-1-ol	108 ± 8a	103 ± 8a	82 ± 6a	96 ± 6a
(*Z*)-3-Hexen-1-ol	644 ± 23a	528 ± 21b	556 ± 16b	540 ± 13b
Sum of C_5 and C_6 alcohols	**2104 ± 63a**	**1738 ± 27b**	**1480 ± 39c**	**1414 ± 30c**
Esters				
Ethyl acetate	94 ± 9a	0 ± 0b	0 ± 0b	0 ± 0b
Hexyl acetate	21 ± 6a	14 ± 2a	12 ± 3a	10 ± 1a
(*Z*)-3-Hexenyl acetate	102 ± 8a	82 ± 6ab	68 ± 1bc	58 ± 4c
Sum of esters	**123 ± 10a**	**96 ± 6b**	**80 ± 3bc**	**68 ± 4c**
Ketones				
3-Pentanone + 2-Pentanone	178 ± 21a	133 ± 12a	139 ± 13a	43 ± 10b
1-Penten-3-one	136 ± 21a	87 ± 33ab	82 ± 8ab	33 ± 7b
6-Methyl-5-hepten-2-one	10 ± 4a	10 ± 1a	14 ± 6a	13 ± 0a
Sum of ketones	**324 ± 30a**	**230 ± 35a**	**235 ± 16a**	**89 ± 12b**
Acetic acid	731 ± 2a	625 ± 25b	606 ± 23b	620 ± 29b

*The data are the mean values of two independent extractions, ± standard deviation. For each different cultivar, the values in each row having different letters (a-d) are significantly different from one another ($p < 0.05$).

2.4. Cryo-scanning electron microscopy (Cryo-SEM) analysis of the structure of frozen-hydrated olive paste during the olive oil extraction process.

Portions of crushed olive paste, malaxed olive paste and malaxed olive paste obtained under high vacuum were sampled during the olive oil extraction process, quickly cryo-fixed in liquid nitrogen and stored frozen-hydrated until subsequent analyses by Cryo-SEM to study the olive paste structure. The frozen-hydrated samples were freeze-fractured and mounted under liquid nitrogen on an aluminum stub using Tissue-Tek (O.C.T. Compound, Miles Inc., Elkhart, IN, USA). The mounted samples were transferred into a dedicated cryo-preparation chamber (SEM cryo-unit, SCU 020, Bal-Tech, Balzers, Liechtenstein). The surfaces were etched for 3 min at −80 °C under high vacuum (lower than 2×10^{-4} Pa) and sputter-coated with 10 nm of platinum in an argon atmosphere to produce an electrically conductive surface. The frozen-hydrated samples were then transferred inside the scanning electron microscope (Philips SEM 515, Eindhoven, The Netherlands) equipped with a SEM cryo-unit SCU 020, observed at −180 °C, and the images were digitized and elaborated by AnalySIS 2.0 software (Soft-Imaging Software Gmbh, Munster, Germany.

2.5. Statistical analysis

The data obtained in the analysis of the VOO controls and VOOs extracted with a high vacuum system were statistically analyzed using one-way analysis of variance (ANOVA) with the support of SigmaPlot Software 12.3 (Systat Software Inc., San Jose, CA, USA).

3. Results and discussion

Preliminary tests on the impact of vacuum on the VOOs quality were conducted using a high malaxation temperature. The trials were performed using olives from the Moraiolo cultivar at three different temperatures, 40 °C, 50 °C and 60 °C, to evaluate the effects on VOO quality and to assess the potential stripping of volatile compounds related to the level of vapor and gas condensation induced by high vacuum at temperatures over 40 °C.

The content of phenolic compounds notably increased, with values of 33.2%, 40.1% and 50.1% for VOOs obtained at 40 °C, 50 °C and 60 °C, respectively (data not shown). These significant results were probably achieved thanks to the combined effects of the high vacuum and the high temperature, which both cause the release of abundant phenols from fruit tissues. The combined effect of pressure and high temperature increased the liquid mass transfer improved the solubility of phenolic compounds into the oily phase. On the other hand, the absence of oxygen, due to the high vacuum, inhibited the degradation of phenolic compounds by the enzymatic activity of PPO and POD.

In contrast, the concentration of volatile compounds responsible for VOO flavor changed substantially relative to the control, with notable decreases in the contents of aldehydes (67.4%, 68.4%, and 71.3%), alcohols (51.9%, 47.9% and 49.0%) and esters (36.7%, 28.9% and 64.4%) at 40 °C, 50 °C and 60 °C, respectively. The high stripping effect of volatile molecules, because of the use of high vacuum combined with high temperature, was due to the intense evaporation and condensation process, resulting in abundant extraction of the aqueous phase from the olive paste. The significant reduction in VOO volatile compounds suggested a substantial reduction in malaxation temperatures was necessary to preserve VOO aroma.

Therefore, the experimental trials were carried out using processing temperatures no higher than 30 °C.

On the basis of the preliminary data, another batch of Moraiolo olives was processed using lower kneading temperatures, and the phenolic composition showed the same increasing trend. The

Table 5
Volatile composition (μg/kg) of VOOs control (C) and VOOs extracted under high vacuum conditions (V) processing Peranzana olives at different malaxation temperatures.

	Cv Peranzana			
Malaxation temperature	**C (25 °C)**	**V (20 °C)**	**V (25 °C)**	**V (30 °C)**
*Aldehydes**				
Pentanal	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
(*E*)-2-Pentenal	119 ± 12a	74 ± 4b	73 ± 6b	75 ± 8b
Hexanal	1427 ± 151a	737 ± 22b	448 ± 62bc	352 ± 39c
(*E*)-2-Hexenal	30211 ± 3039a	27305 ± 269ab	22065 ± 667b	20264 ± 1804b
(*E*,*E*)-2,4-Hexadienal	265 ± 29a	197 ± 3ab	171 ± 14b	163 ± 19b
Sum of C_5 and C_6 aldehydes	**32023 ± 3043a**	**28313 ± 270ab**	**22757 ± 670bc**	**20854 ± 1804c**
Alcohols				
Ethanol	1939 ± 41a	1761 ± 38b	1701 ± 47bc	1584 ± 44c
1-Pentanol	18 ± 5a	0 ± 0b	0 ± 0b	16 ± 1a
1-Penten-3-ol	778 ± 2a	680 ± 25b	531 ± 33c	403 ± 20d
(*E*)-2-Penten-1-ol	344 ± 30a	264 ± 28a	284 ± 2a	327 ± 5a
(*Z*)-2-Penten-1-ol	848 ± 75a	743 ± 54ab	689 ± 15ab	583 ± 20b
1-Hexanol	583 ± 56a	469 ± 49a	401 ± 85a	420 ± 3a
(*E*)-2-Hexen-1-ol	222 ± 29a	247 ± 62a	285 ± 68a	324 ± 33a
(*Z*)-3-Hexen-1-ol	850 ± 81a	663 ± 65a	689 ± 72a	694 ± 5a
Sum of C_5 and C_6 alcohols	**3645 ± 131a**	**3066 ± 122b**	**2879 ± 135b**	**2765 ± 44b**
Esters				
Ethyl acetate	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
Hexyl acetate	2416 ± 205a	2342 ± 20a	2001 ± 42ab	1769 ± 94b
(*Z*)-3-Hexenyl acetate	4783 ± 376a	4424 ± 51a	4015 ± 21a	3856 ± 425a
Sum of esters	**7199 ± 428a**	**6766 ± 54ab**	**6016 ± 47ab**	**5625 ± 436b**
Ketones				
3-Pentanone + 2-Pentanone	47 ± 8ab	69 ± 8a	45 ± 5ab	35 ± 6b
1-Penten-3-one	684 ± 64a	348 ± 25b	299 ± 11b	284 ± 27b
6-Methyl-5-hepten-2-one	7 ± 3a	9 ± 1a	8 ± 1a	11 ± 3a
Sum of ketones	**738 ± 65a**	**425 ± 26b**	**352 ± 12b**	**330 ± 28b**
Acetic acid	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a

*The data are the mean values of two independent extractions, ± standard deviation. For each different cultivar, the values in each row having different letters (a-d) are significantly different from one another ($p < 0.05$).

enhancements reached 25.2%, 33.6% and 32.7% at 20 °C, 25 °C and 30 °C, respectively (Table 1), which demonstrates how the impact of high vacuum seems to be greater than the impact of temperature with a significant increase in the phenolic content even at 20 °C. The improvement in the VOO phenolic profile is also due to the rapid removal of oxygen from the olive paste during the vacuum treatment (Miho, Moral, López-González, Díez, & Priego-Capote, 2020), which inhibits the activities of polyphenoloxydase (PPO) and peroxidase (POD). Moreover, the increases in the phenolic content were probably underestimated as a consequence of the use of a discontinuous lab-scale extraction system that probably promoted oxidation at the end of the malaxation process and during the subsequent steps until the oil separation phase with swinging-bucket centrifugation.

Extraction at a low malaxation temperature with high vacuum was also tested using the two other cultivars, Peranzana and Coratina. The phenolic compositions of both Peranzana and Coratina VOOs also increased with increasing processing temperature, and the different genetic origins of the fruits influenced the percentage by which the phenolic fractions of the VOOs increased. The phenolic contents of Peranzana VOOs were enhanced by 34.6%, 36.1% and 38.4% and in Coratina VOOs by 31.6%, 40.0% and 48.6% at 20, 25 and 30 °C, respectively (Table 1). Even if the increase in hydrophilic phenols was cultivar dependent, the effect of extraction and potential solubilization of the phenolic fraction induced by the application of high vacuum during the malaxation phase was very significant with increases in the range of 202 mg/kg to 407 mg/kg.

Regarding the main legally regulated quality parameters, no differences in free fatty acids, peroxide values, K_{232}, K_{270} and ΔK were detected in the experimental VOO samples compared to the corresponding VOO controls of the Peranzana, Coratina and Moraiolo cultivars (Table 2).

In contrast, as shown in the preliminary tests carried out at high temperatures, the volatile content tended to decrease with increasing malaxation temperature. In addition, the range of variability seems to be cultivar dependent with a minimum decrease of 6% relative to the sum of the esters obtained at 20 °C from cv Peranzana (Table 5) and a maximum of 47% of the sum of aldehydes of Moraiolo VOO extracted at 30 °C (Table 4). Smaller reductions in the aldehyde, alcohol and ester concentrations (20%, 17% and 22% in Moraiolo VOO; 12%, 16% and 6% in Peranzana VOO; and 21%, 15% and 16% in Coratina VOO, respectively) were detected in all the samples subjected to malaxation at 20 °C with high vacuum compared to the control trials (Tables 3–5). The results were also confirmed by analyses of the volatile composition of the aqueous extracts obtained by evaporation and condensation of the olive paste moisture through the tubular condenser after treatment with high vacuum. All the classes of VOO volatile compounds (aldehydes, alcohols and esters) increased in the aqueous extract with increasing extraction temperature (data not shown), confirming the reduction in the VOO volatile fraction. In addition, the aqueous extract, condensed during high vacuum treatment, was added back into the olive paste as is commonly done for other food processes, such as fruit and vegetable juices extracted with vacuum technology. The results did not demonstrate any improvement in the VOO volatile fraction, highlighting a limit of the VOO high vacuum-assisted extraction process.

The same decreasing trend in the main volatile compounds, responsible for the positive sensory notes of VOO, was also seen in some volatile compounds not involved in the determination of VOO flavor, such as ethanol, ethyl acetate and acetic acid. Large amounts of these molecules are usually developed as a consequence of the fermentation of olives during their storage before oil extraction. However, the ethanol content can also be influenced by other variables, such as genetic origin, ripening stage, growing area, environmental factors and agronomic practices (García-Vico et al., 2018, Di Serio, Giansante, Di Loreto, Faberi, Ricchetti, & Di Giacinto, 2017).

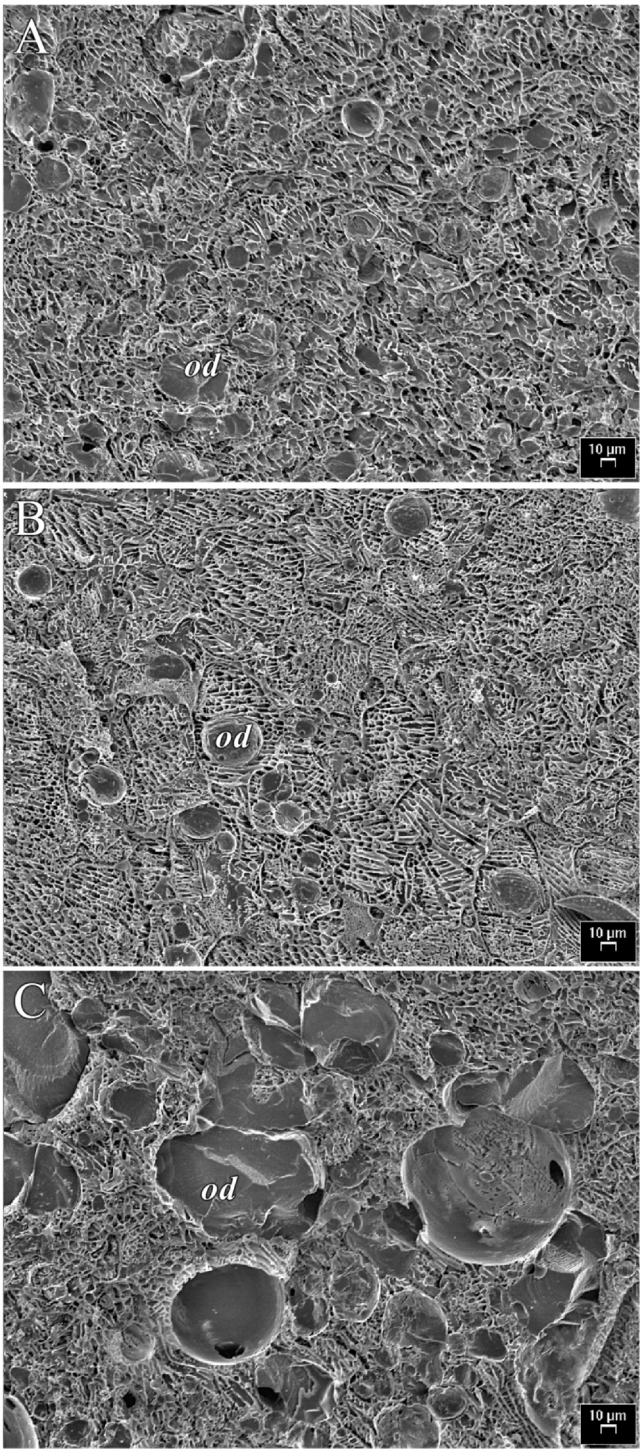

Fig. 1. Representative cryo-scanning electron microscopy images of frozen-hydrated/freeze-fractured crushed olive paste (A), malaxed olive paste (B) and malaxed olive paste obtained under high vacuum conditions (C) from Moraiolo olives processed at 25 °C; ***od***, oil droplets.

Ethyl acetate and acetic acid can generate negative sensory notes related to a vinegary off-flavor (García-González and Aparicio, 2002), whereas a high content of ethanol can cause an increase in fatty acid ethyl esters (FAEEs) during the storage of VOO. Both effects significantly alter the quality characteristics of the olive oil.

During the treatment with high vacuum at a malaxation temperature of 20 °C, the concentrations of ethyl acetate and acetic acid in the VOOs significantly decreased. Ethyl acetate and acetic acid, which were not detected in Peranzana VOOs, decreased by 80.4% and 62.9% for Coratina VOO and 100% and 14.5% for Moraiolo VOO (Tables 3 and 4). Neither molecule was influenced by increases in the malaxation temperature. The ethanol content also decreased during the vacuum treatment of VOOs at 20 °C during kneading (9.2%, 41.2% and 44.8% for Peranzana, Coratina and Moraiolo, respectively) but was mostly influenced by higher malaxation temperatures, with decreases of 18.3%, 57.2% and 70.9% at 30 °C for Peranzana, Coratina and Moraiolo, respectively (Tables 3–5). The decreasing trend in the volatile compounds due to the stripping process induced by high vacuum treatment seems to be more dramatic for ethanol, ethyl acetate and acetic acid compared to the other molecules extracted at the same malaxation temperature.

This new technique also substantially changed the physicochemical structure of the olive paste tissues, enhancing the porosity and capillarity, increasing the cellular liquid flow (Betoret et al., 2015). The high volume of liquid mass transfer induced by the high vacuum resulted in higher mobility of the oily phase into the cellular and intracellular space during the kneading of the olive paste with a resulting alteration of the physicochemical characteristics of the VOO. The process seems to improve the coalescence of the oil droplets that rapidly flow into the olive paste from different parts of the fruit mass, increasing their volume. Crushed olive paste, malaxed olive paste and malaxed olive paste obtained under high vacuum conditions were analyzed in a frozen-hydrated state with a cryo-scanning electron microscope (Fig. 1) to better understand the different physical impacts of this new technique on the olive cell structure and on the dispersion of oil droplets into the vegetable matrix. Fig. 1A, related to Moraiolo olives extracted at 25 °C, shows the cells of the crushed olive paste partially damaged with some oil droplets (**od**) still located inside of the vacuoles of the cells of the mesocarp. In contrast, malaxed olive paste (Fig. 1B, **od**) and malaxed olive paste processed under high vacuum conditions (Fig. 1C, **od**) showed collapsed cellular membranes and walls and larger free oil drops in the aqueous and vegetal intra/intercellular matrices. However, comparing the two malaxed olive pastes, it was very clear that the oil drops (***od***) obtained after high vacuum treatment were much larger (Fig. 1C, ***od***), highlighting the major increase in the coalescence of the oil drops and the subsequent olive oil extractability.

4. Conclusions

The use of this new extraction technique involving a high vacuum system had a positive impact on VOO quality related to the content of phenolic compounds, as this parameter increased in all trials; this parameter was closely related to the extraction method and to a lesser extent to the processing temperature and genetic origin of the olives.

In contrast, vacuum treatment significantly reduced the volatile compounds as a function of processing temperature. The extraction process carried out at the lowest malaxation temperature (20 °C) showed a limited reduction in the volatile compounds, minimizing the negative impact on the sensory quality of the VOO due to the removal of C_5 and C_6 saturated and unsaturated aldehydes, alcohols and esters. The high vacuum treatment also cause significant stripping of molecules not involved with positive sensory notes (ethanol, ethyl acetate and acetic acid) but in contrast may be potentially deleterious to the quality characteristics of VOO. This effect should be confirmed and further studied to evaluate the possibility of a remarkable reduction of the volatile compounds responsible for alterations and decreases in the sensory qualities and stability of VOO.

The high vacuum technique needs further investigation with a VOO industrial plant to better understand the real impacts on the extraction yield and VOO quality mainly related to the volatile and phenolic fractions.

CRediT authorship contribution statement

Agnese Taticchi: Conceptualization, Methodology. **Sonia Esposto:** Conceptualization, Methodology. **Gianluca Veneziani:** Conceptualization, Methodology, Validation, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Antonio Minnocci:** Validation, Formal analysis, Investigation. **Stefania Urbani:** Validation, Formal analysis, Investigation. **Roberto Selvaggini:** Validation, Formal analysis, Investigation. **Beatrice Sordini:** Validation, Formal analysis, Investigation. **Luigi Daidone:** Validation, Formal analysis, Investigation. **Luca Sebastiani:** Conceptualization, Visualization. **Maurizio Servili:** Supervision, Project administration, Funding acquisition.

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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