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ORIGINAL PAPER

Effect of crushing on olive paste and virgin olive oil minor components

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Abstract The present study focuses on the influence of the olive crushing technique on the minor composition of olive pastes and their corresponding virgin olive oils since these compounds are strongly related to their quality and characteristics. Two different cultivars, Arbequina and Cornicabra—known for their different minor component composition—were processed at laboratory scale using hammer mills at various breakage forces and grid hole diameters, a blade cutter and a mortar. Crushing and kneading produce a profound change in the composition of the phenolic compounds in the olive paste and in the final oil. Hydroxytyrosol derivatives in virgin olive oil were most affected by the crushing conditions. The stronger the crushing conditions (i.e. hammer crushers using smaller grid holes and a higher rotation speed), the higher the phenolic content in both olive paste and oil in both varieties. Interestingly, the effect on volatile compounds of milder or stronger crushing conditions was opposite to that described for the phenolic compounds.

Keywords Crushing · Kneading · Olive paste · Virgin olive oil · Minor components

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Introduction

Crushing of olives is not only a simple physical process used to break the fruit's tissues and release the oil drops contained in the vegetal cell vacuoles, but it is also a critical step that affects the quality of the final virgin olive oil (VOO) produced. Indeed, upon olive crushing, several enzymes that are involved in the generation and transformation of polar phenols and volatile compounds are triggered. Depending on crushing conditions, the concentration of these minor components, which are intimately related to the taste, aroma and stability of VOO, are therefore modified [1, 2].

According to [3], olive fossils date from about one million years ago, whereas the first mortars employed to obtain olive oil date from 5,000 years B.C. The extraction of olive oil initially consisted of crushing olives with a mortar and placing the olive paste produced in contact with hot water in a vessel to allow separation of the oily phase from the top. Later, wood mats were introduced into the process in order to press the olive paste, and at about 50 years B.C., the Romans improved the technique with the "trapetum" and pressing system, which was used until 1795 when Joseh Grahan invented the hydraulic press, still in use nowadays in some small oil mill industries.

In recent decades, the most relevant advance in virgin olive oil processing technology has been the appearance of a continuous process that uses a metal crusher, a malaxer and a horizontal centrifuge called a decanter, which has improved not only the oil yield because of the more complete breakage of the fruit, but also productivity due to the higher process capacity that has in turn reduced the preceding storage of the olives and therefore the fermentation processes, thus leading to higher quality oils [4].

Different types of olive crushers are currently available for industrial application, such as hammer mills, toothed

crushers or blade cutters, which claim improvements in the oil yield or quality. Of the different crushing techniques, the hammer crusher is generally recognized as the strongest and generally produces more bitter oils [1, 5, 6]. For these reasons, some manufacturers still believe in the advantages of using the traditional stone mills, since apart from softly crushing, the fruit it is also slightly kneaded [7], producing less bitter and more aromatic VOOs.

Hammer crushers usually possess fixed or mobile grids with different hole diameters in order to control the intensity of crushing depending on the maturity of the fruit, the variety and the desired oil characteristics. Grid hole size and rotation speed are therefore technological parameters that should be taken into account during the crushing process in order to modulate the composition and the quality of the final virgin olive oil produced. To this end, the present study focuses on the influence of the olive crushing technique on the minor composition of olive pastes and their corresponding VOOs since these compounds are strongly related to the quality and characteristics of virgin olive oil. Two different cultivars, Arbequina and Cornicabra—known for their different minor component composition—were processed using (1) hammer mills with various applied breakage forces and grid hole diameters (2) a blade cutter and (3) a mortar.

Materials and methods

Olive fruit sampling and crushing

The study was carried out using Arbequina and Cornicabra cv. olives cultivated in the Ciudad Real area in the 2008/2009 crop season. Fruit ripeness was determined according to the method proposed by the International Olive Council [8], based on the evaluation of the olive skin and pulp colours of the fruit. Ripeness index values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin).

The olive samples (800 g)—from homogeneous batches of 40–50 kg—were crushed at laboratory scale using two hammer crushers working at 1,500 rpm (H1) and 3,000 rpm (H3; average crushing time of 20 and 30 s, respectively), each one equipped with fixed grids with different hole diameters (5, 6 and 7 mm). A blade cutter was also employed to obtain pastes composed of the uncrushed olive stone together with the pulp. The rotation speed of the cutter was set at 1,500 rpm (C1) or 3,000 rpm (C3), as for the hammer crushers and the crushing time was set at 30 s. To complete this study, the pressure produced by a manual mortar (P; 30 s crushing time) was also used, this being the oldest crushing technique, as previously mentioned.

The paste obtained from the different techniques was kneaded according to the Abencor procedure [9] under the same conditions of temperature (28 °C) and malaxation time (45 min). The kneaded paste was then centrifuged at 3,500 rpm in a basket centrifuge, and the oil was recovered. Olive oil samples were filtered with anhydrous Na_2SO_4 as a drying agent to preserve the samples from the oxidation and stored at 4 °C in darkness using topaz glass bottles without head space prior to analysis. Olive paste samples were obtained just after crushing and kneading and were frozen at −70 °C until analysis.

Analytical determinations in olive fruit and paste

The water and fat content of the olive fruit in both cultivars was assessed according to the UNE Spanish Standard method [10]. The fat content was determined by Soxtec (2055 Foss, Höganäs, Sweden) extraction and was expressed as a percentage of fresh olive paste weight.

The phenolic composition of the olive fruit and paste was determined via the following procedure: 4.0 g ± 0.0001 g of sample was homogenized together with 4-hydroxyphenylacetic acid as the internal standard in a mixture of methanol:water (80:20 v/v) (40 mL) for 2 min with an Ultraturrax homogenizer (14,000 rpm). The suspension obtained was shaken (20 min, 150 rpm, <4 °C in darkness) and then centrifuged (10 min, 4 °C and 3,850 g). The hydromethanolic phase was recovered and filtered through a 0.45-μm nylon syringe filter. The phenolic fraction extracted was analysed by high-performance liquid chromatography (HPLC) using an Agilent Technologies 1100 series system equipped with an automatic injector, a column oven and a diode array UV detector. A ZORBAX SB-C18 column (250 × 4.6 id mm, 5-μm particle size) (Agilent Technologies, USA), maintained at 30 °C, was used with an injection volume of 20 μL and a flow rate of 1.0 mL/min. The mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A) and methanol (Solvent B). The gradient changed as follows: 95% A/5% B for 2 min, 75% A/25% B for 8 min, 60% A/40% B for 10 min, 50% A/50% B in 16 min, and 0% A/100% B for 14 min, maintained for 10 min, and return to initial conditions for 13 min according to [11].

The identification of phenolic compounds was carried out by comparison to their retention times, UV–visible characteristics and MS spectra with their standard substances. The mass detector used was an LCQ Deca XP Plus (Thermo Electron Corp., Waltham, MA) equipped with an electrospray ionization system. Nitrogen was used as a nebulizing gas at a flow rate of 14 units. The temperature and voltage of the capillary were 250 °C and 4.50 kV, respectively. Data were acquired in the negative ionization mode. Fragmentation experiments were performed using helium as the collision gas with collision energy between 30 and 40%.

Volatile compounds. According to [12], solid-phase microextraction (SPME) followed by GC-FID were used to analyse the volatile compounds in the fruit and olive paste samples studied. A sample of 1.5 g of olive material was placed in a 10-mL vial fitted with a silicone septum. SPME sampling was performed by exposing the DVB/Carboxen/PDMS fibre (50/30 μm, 2 cm long from Supelco Inc., Bellefonte, USA) for 30 min in the head space of the sample maintained at 40 °C, which was then retracted into the needle and immediately transferred and desorbed for 5 min in the injection port of a gas chromatograph equipped with a FID detector. Compounds were resolved on a Supelcowax-10 column (30 m × 0.25 mm × 0.25 μm, Supelco Inc., Bellefonte, USA) under the following conditions: injection port temperature 260 °C; helium flow 0.8 mL/min; oven temperature ramp 35 °C for 10 min, 3 °C/min up to 160 °C and then 15 °C/min up to 200 °C (maintained for 5 min). Volatile compounds were identified by comparison to the retention times and mass spectra of standard substances (Sigma–Aldrich) that are added to refined olive oil. The GC–MS used was an Agilent 5975C Series mass spectrometer (Agilent Technologies, USA) equipped with an electron ionization (EI +) detector and coupled to an Agilent 6850 Series gas chromatograph. The capillary column used was a DB-Wax (30 m × 0.25 mm × 0.25 μm, J&W Scientific, USA). Helium was employed as the carrier gas at a flow rate of 0.8 mL/min. The transfer line temperature was 280 °C, and the temperature of the ionization source and the quadrupole was 230 and 150 °C, respectively, with an electromultiplier voltage of +941 eV. Their quantification was achieved by a minimum of a five-point calibration curve on the basis of the corresponding standard substances.

Analytical determinations in virgin olive oil

The free acidity, given as the percentage of oleic acid, peroxide value (PV) expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg), and the K_{232} and K_{270} extinction coefficients calculated from absorption at 232 and 270 nm were measured following the analytical methods described in the European Union standard methods and subsequent amendments [13].

Fatty acid composition was determined according to European Regulations and subsequent amendments, corresponding to the AOCS method Ch 2–91 [13]. The methyl-esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 mol/L methanolic potassium hydroxide and were analysed by GC with a FID detector. A fused silica column (50 m length × 0.25 mm i.d.) coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor, Spain) was used. The carrier gas was helium, at a flow through the column of 1 mL/min. The injector and detector temperature was set at 250 °C and the oven temperature at 210 °C. The injection volume was 1 μL.

Tocopherols. The content of tocopherols in the VOOs was evaluated following the AOCS method Ce8-89 [14]. A solution of oil in hexane was analysed on an Agilent Technologies HPLC (1100 series) on a silica gel Lichrosorb Si-60 column (particle size 5 μm, 250 mm × 4.6 mm i.d.; Sugerlabor, Madrid, Spain) which was eluted with hexane/2-propanol (98.5:1.5) at a flow rate of 1 mL/min. A fluorescence detector (Waters 470) was used with the excitation and emission wavelengths set at 290 and 330 nm.

Phenolic compounds. A solution of the internal standard (250 μL of 15 mg/kg of syringic acid in methanol) was added to a sample of virgin olive oil (2.5 g), and the solvent was evaporated with a rotary evaporator at 35 °C under vacuum. The oil was then dissolved in 6 mL of hexane, and a diol-bonded phase cartridge (Supelco Co., Bellefonte, USA) was used to extract the phenolic fraction. The cartridge was conditioned with methanol (6 mL) and hexane (6 mL), the oil solution was then applied, and the SPE column was washed with hexane (2 × 3 mL) and with hexane/ethyl acetate (85:15, v/v; 4 mL). Finally, the phenols were eluted with methanol (15 mL), and the solvent was removed with a rotary evaporator at 35 °C under vacuum until dryness. The phenolic residue was dissolved in methanol/water (1:1 v/v; 250 μL) and analysed by HPLC. A Chromolith performance RP-18 endcapped column (100 × 4.6 id mm) was used, maintained at 30 °C, with an injection volume of 20 μL and a flow rate of 2.0 mL/min. The mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A), methanol (B) and acetonitrile (C): from 2.5% (B)/2.5% (C) to 25%(B)/25%(C) in 25 min, to 100% (C) in 3 min maintained for 6 min, to 2.5%(B) and 2.5%(C) in 3 min at 1.2 mL/min flow and kept for 40 min at normal flow. Phenolic compounds were quantified at 280 nm using syringic acid as the internal standard, and the response factors determined by [15].

Volatile compounds. Solid-phase microextraction (SPME) followed by GC-FID were used to analyse the volatile compounds in the virgin olive oil samples studied. One and a half grams of olive oil together with 2-methyl-4-pentanol as the internal standard were placed in a 10-mL vial fitted with a silicone septum. The SPME sampling and CG-FID/MS conditions, as well as identification of volatiles, were the same as those used with the olive paste volatile fraction.

The oleuropein score (OlS) was determined by the method described by [16], which consists of the extraction of the bitter components from a sample of 1.0 g ± 0.01 g of oil dissolved in 5 mL of n-hexane with 5 mL MeOH/H_2O

(60:40, v/v). The mixture was vortexed and centrifuged at 3500 rpm for 10 min. The polar fraction was transferred in a 10-mL volumetric flask, and the volume was made up to 10 mL with MeOH/H_2O (60:40, v/v) (stock solution, C0); an aliquot (1.25 mL) was diluted to 5 mL with the same solvent (C1). The absorbance of C1 was recorded at 225 nm against methanol/water (1:1) in a 1-cm cuvette by means of a spectrophotometer (Agilent 8453). Oleuropein was used as an external standard dissolved in MeOH/water, 60:40, v/v.

Chlorophyll and carotenoid compounds were analysed at 472 and 670 nm in cyclohexane using specific extinction values, by the method described by [17].

Standards, reagents and solvents

Oleuropein (98% purity) was purchased from Extrasynthese (Genay, France); 4-hydroxyphenylacetic acid (98% purity), syringic acid (98% purity) and 4-methyl-2-pentanol (99%) were from Sigma–Aldrich (Steinheim, Germany). All the others common reagents were of the appropriate purity from various suppliers. HPLC grade methanol, acetonitrile and *n*-hexane were from Merk KgaA (Darmstadt, Germany). Ultra purity water was produced using a Millipore Milli-Q system.

All experiments and analytical determinations were carried out at least in duplicate.

Statistical analysis

Data were analysed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL) to perform one-way and two-way analysis of variance (ANOVA) at a 95% confidence level ($p \leq 0.05$) to identify significant differences between the mean values of the minor components of the olive paste and VOO obtained in the different crushing conditions, followed by the multiple Duncan test ($p \leq 0.05$). Furthermore, two-way analysis of variance was carried out to study the effect of the main variables involved during crushing in the minor composition of the samples.

Results and discussion

Initial composition, oil yield and quality indices

The initial composition of the Arbequina and Cornicabra olive fruits used in this study is shown in Table 1. Arbequina and Cornicabra olives were harvested for this study at a ripeness index of 2.8 and 4.5, respectively (the Arbequina cultivar is processed at a lower maturation index than Cornicabra), and their oil content was 31.4 and 44.7% as dry weight, respectively.

Table 1 Initial composition of the olive fruits

		Arbequina	Cornicabra
	Ripeness index	2.8	4.5
	Humidity (%)	47.5 ± 0.0	39.0 ± 0.1
	Oil yield (%, f.w.)	16.5 ± 0.2	26.7 ± 0.6
	Oil yield (%, d.w.)	31.4 ± 0.4	44.7 ± 1.2
Phenols (mg/kg IS)[a]			
Simple phenols	Hydroxytyrosol	351 ± 4	575 ± 183
Secoiridoids	Oleuropein	224 ± 99	3,587 ± 103
	Demethyloleuropein	820 ± 278	109 ± 5
Volatiles (mg/kg)			
C6 Aldehydes	Hexanal	2.9 ± 0.8	0.3 ± 0.4
	E-2-hexenal	17.0 ± 1.5	3.7 ± 0.2
C6 Esters	Hexyl acetate	0.1 ± 0.0	0.0 ± 0.0
	Z-3-hexenyl acetate	1.5 ± 0.9	0.8 ± 0.2
C6 Alcohols	Hexan-1-ol	0.0 ± 0.0	1.9 ± 0.0
	Z-3-hexen-1-ol	0.5 ± 0.1	11.6 ± 1.5
	E-2-hexen-1-ol	0.9 ± 0.2	0.1 ± 0.1

[a] Quantification of phenolic compounds performed by HPLC–UV employing 4-hydroxyphenylacetic acid as internal standard

These olive cultivars were chosen for this study due to the known differences in their minor component profiles [18, 19]. Indeed, Cornicabra olives possessed a much higher oleuropein content (3,587 mg IS/kg) than Arbequina (224 mg IS/kg; Table 1). On the contrary, Arbequina olives showed much higher levels of demethyloleuropein (820 mg IS/kg) than Cornicabra. Arbequina virgin olive oil is known to possess a higher content of volatile compounds. In the olives, analysed the major component in the Arbequina variety was the C6 aldehyde *E*-2-hexenal (17.0 mg/kg; Table 1), followed by the C6 aldehyde hexanal (2.9 mg/kg) and the C6 ester Z-3-hexenyl acetate (1.5 mg/kg). In Cornicabra olives, the major component was the C6 alcohol Z-3-hexen-1-ol (11.6 mg/kg), followed by the C6 aldehyde *E*-2-hexenal (3.7 mg/kg).

The effect of crushing conditions on the obtained oil yield, as well as on the temperature reached by the crushed olive paste in both cultivars, is depicted Fig. 1. The oil yield obtained significantly depended on the crushing conditions employed. Hammer crushers (H) using a higher grid rotation rate (3,000 rpm) and a smaller grid diameter (5 mm; H35) produced a higher oil yield (i.e. 26% under H35 conditions, compared to 22% in H37 or 19% in C3 or C1) probably due to the stronger and therefore more complete breakage of the fruit tissue, which also generated a higher temperature in the olive paste (35 °C) due to the higher release of energy. The higher olive paste temperature diminished the viscosity of the oil, making the release of the oil from the fruit tissue easier [20]. The diameter of the grid holes had a greater effect on the oil yield than the rotation

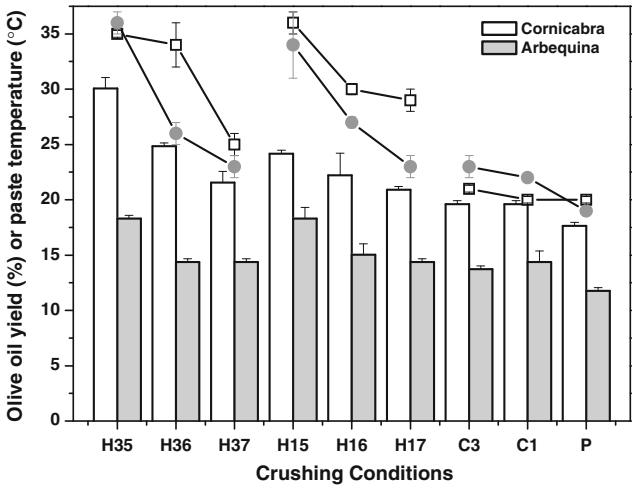

Fig. 1 Effect of crushing conditions on olive oil yield (% fresh weight). *H* hammer crusher, *C* blade cutter, *P* pressure by a mortar

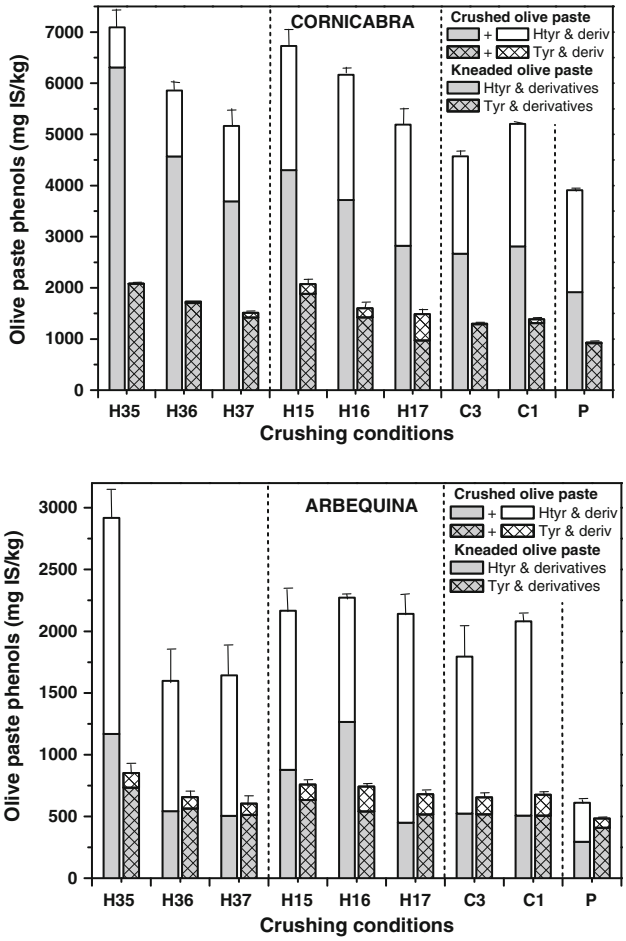

Fig. 2 Effect of crushing and kneading on main phenolic families in olive paste

rate, as shown in Fig. 1 and confirmed by statistical analysis (see later). The use of a blade cutter (C) produced lower olive paste temperatures (20–22 °C) and oil yield, with similar results to those observed using hammer crushers with a bigger grid diameter (H37 or H17). The temperature of the olive paste obtained by applying pressure (P) using a mortar was almost the same as the room temperature (19 °C), resulting in the lowest oil yield. Cornicabra olive paste temperature reached using hammer crushers was higher than that of Arbequina (Fig. 1), probably due to the bigger size of the Cornicabra kernel that produces a higher resistance by passing through the grid holes.

The quality indices of the virgin olive oils (VOO) produced in this study were far below the limits established by the European Commission Regulation [21] for the extra virgin category since the olives employed were fresh and healthy, and careful processing of the raw material was carried out. Furthermore, no statistically significant differences were observed in the quality indices such as the free acidity, the peroxide value and the ultraviolet absorption characteristics at 232 and 270 nm or in the fatty acid composition of the triacylglycerols matrix in any of the VOOs obtained using the different crushing conditions (crushing type, rotation rate and grid diameter) in either cultivar (data not shown).

Effect on phenolic compounds

The effect of crushing and kneading on olive paste biophenols is reported in Fig. 2; and Table 2. These operations produce a profound change in the composition of the phenolic compounds of the olive fruit and the olive paste. This is apparently due to the activity of enzymes related to the biogeneration of phenols like β-glucosidase and esterase [22] and of oxidative enzymes such as polyphenol oxidase, peroxidase and lipooxygenase during kneading [11, 23].

Indeed, oleuropein diminished considerably, i.e. from 3,587 down to 127–794 mg/kg (expressed as internal standard, IS) in the case of Cornicabra (Table 1 and 2) depending on the crushing conditions employed. Oleuropein is transformed during crushing into complex aglycone secoiridoid derivatives that are present in both the olive paste and the olive oil (i.e. DOA 3,453–5,986 mg/kg and DLA 412–1,119 mg/kg; Table 2), but are not detected in the olive fruit.

In both olive varieties, there was an important effect of the crushing type and conditions on the two main phenol families, hydroxytyrosol (Htyr) and its derivatives (mainly DOA, dialdehydic form of elenolic acid linked to Htyr and AOA, aldehydic form of elenolic acid linked to Htyr) and tyrosol (Tyr) and its derivatives (mainly DLA, dialdehydic form of elenolic acid linked to Tyr and ALA, aldehydic form of elenolic acid linked to Tyr; Fig. 2).

Similarly to what was observed for the oil yield (Fig. 1), the stronger the crushing conditions (i.e. smaller grid holes

Table 2 Effect of crushing and kneading on the phenolic compounds (mg/kg IS) in the Cornicabra olive paste

	Hydroxytyrosol		DOA		AOA		Oleuropein	
	Crushed	Kneaded	Crushed	Kneaded	Crushed	Kneaded	Crushed	Kneaded
H35	509 ± 11^{bc}	595 ± 14^{b}	$5{,}986 \pm 852^{e}$	$5{,}183 \pm 232^{e}$	595 ± 126^{cd}	528 ± 60^{b}	794 ± 328^{b}	894 ± 229^{c}
H36	518 ± 88^{bc}	604 ± 113^{b}	$4{,}776 \pm 271^{bcd}$	$3{,}446 \pm 480^{c}$	562 ± 59^{bcd}	518 ± 182^{b}	332 ± 85^{a}	354 ± 99^{ab}
H37	442 ± 15^{b}	449 ± 35^{ab}	$4{,}258 \pm 645^{abc}$	$2{,}761 \pm 866^{abc}$	468 ± 23^{b}	432 ± 16^{ab}	197 ± 16^{a}	241 ± 22^{a}
H15	570 ± 10^{c}	591 ± 108^{b}	$5{,}530 \pm 642^{de}$	$3{,}275 \pm 1{,}739^{bc}$	630 ± 12^{d}	438 ± 10^{ab}	339 ± 127^{a}	342 ± 102^{ab}
H16	527 ± 15^{bc}	433 ± 239^{ab}	$5{,}027 \pm 36^{cde}$	$2{,}889 \pm 692^{abc}$	612 ± 7^{d}	395 ± 12^{ab}	175 ± 2^{a}	195 ± 103^{a}
H17	469 ± 35^{bc}	543 ± 11^{ab}	$4{,}263 \pm 627^{abc}$	$1{,}988 \pm 100^{abc}$	459 ± 26^{b}	292 ± 155^{a}	127 ± 2^{a}	179 ± 26^{a}
C3	318 ± 7^{a}	672 ± 101^{b}	$3{,}776 \pm 65^{ab}$	$1{,}637 \pm 245^{ab}$	477 ± 29^{bc}	359 ± 54^{ab}	145 ± 16^{a}	295 ± 44^{a}
C1	458 ± 79^{b}	558 ± 77^{b}	$4{,}200 \pm 129^{abc}$	$1{,}998 \pm 277^{abc}$	545 ± 30^{bcd}	256 ± 36^{a}	184 ± 10^{a}	343 ± 48^{ab}
P	241 ± 3^{a}	286 ± 4^{a}	$3{,}453 \pm 20^{a}$	$1{,}285 \pm 13^{a}$	215 ± 3^{a}	344 ± 5^{ab}	214 ± 3^{a}	552 ± 2^{b}

	Tyrosol		DLA		ALA	
	Crushed	Kneaded	Crushed	Kneaded	Crushed	Kneaded
H35	19 ± 1^{a}	22 ± 14^{ab}	$1{,}064 \pm 87^{d}$	$1{,}029 \pm 21^{c}$	$1{,}000 \pm 119^{e}$	$1{,}026 \pm 54^{b}$
H36	29 ± 14^{ab}	34 ± 13^{ab}	808 ± 72^{c}	703 ± 65^{abc}	794 ± 69^{d}	967 ± 210^{b}
H37	51 ± 6^{c}	35 ± 0^{ab}	741 ± 165^{bc}	544 ± 0^{ab}	720 ± 12^{cd}	843 ± 194^{b}
H15	51 ± 4^{c}	47 ± 22^{bc}	$1{,}119 \pm 19^{d}$	965 ± 510^{bc}	952 ± 15^{e}	872 ± 12^{b}
H16	27 ± 3^{ab}	19 ± 8^{a}	804 ± 32^{c}	596 ± 23^{abc}	771 ± 50^{d}	809 ± 30^{b}
H17	24 ± 2^{a}	32 ± 3^{ab}	760 ± 38^{bc}	558 ± 6^{ab}	702 ± 50^{cd}	383 ± 203^{a}
C3	39 ± 9^{bc}	65 ± 10^{c}	600 ± 3^{b}	458 ± 69^{a}	551 ± 46^{b}	767 ± 115^{b}
C1	44 ± 2^{c}	28 ± 4^{ab}	709 ± 36^{bc}	624 ± 87^{abc}	632 ± 51^{bc}	758 ± 105^{b}
P	24 ± 1^{a}	32 ± 2^{ab}	412 ± 3^{a}	636 ± 2^{abc}	296 ± 5^{a}	252 ± 5^{a}

Different letters within crushing conditions (a–e) indicate significant differences ($p < 0.05$). Concentration expressed as ppm of p-hydroxyphenylacetic acid, the internal standard used. *DOA* dialdehydic form of elenolic acid linked to hydroxytyrosol, *DLA* dialdehydic form of elenolic acid linked to tyrosol, *AOA* aldehydic form of elenolic acid linked to hydroxytyrosol, *ALA* aldehydic form of elenolic acid linked to tyrosol. The quantification of phenolic compounds was performed by HPLC–UV

and higher rotation speed), the higher the phenolic content in the olive paste in both varieties. This may be explained by the better breakage of the fruit tissue and also by the increased activity of enzymes related to the biogeneration of phenolic compounds such as β-glucosidase [24]. This effect is clearly visible in Fig. 2; Table 2 and was confirmed by the statistical analysis. This effect was particularly clear in the case of the hydroxytyrosol family. Indeed, its content decreased from about 7,090 mg/kg down to 5,170 mg/kg (as IS) and from 2,920 mg/kg down to 1,640 mg/kg, when changing the crushing conditions from H35 (5 mm diameter grid holes and 3,000 rpm hammer mill) to H37 (7 mm diameter holes) for Cornicabra and Arbequina, respectively (Fig. 2). In general, a less relevant crushing effect was observed in the case of Arbequina olive paste, especially under H1 conditions. When a blade cutter (C) was used, the content of hydroxytyrosol family phenolics in the olive paste was lower (4,600–5,100 mg/kg) and similar to that in the H37 and H17 conditions, especially in the case of the Cornicabra variety. The mortar produced an olive paste that was even poorer in phenolic compounds, especially in the case of the Arbequina variety (Fig. 2).

Apart from crushing, the kneading operation also has an important effect on the content of phenols in the olive paste. After 45 min malaxation (the conditions used in this study and in general in the industry), the content of the main phenolic secoiridoid compounds was significantly reduced (i.e. DOA from 4,776 down to 3,446 mg/kg under H36 conditions, or DLA from 804 to 596 mg/kg under H16 in Cornicabra; Table 2) in all conditions and types of crushing studied. This effect was especially clear once again for the hydroxytyrosol family and in the Arbequina olive paste (Fig. 2). This is probably due to their orthodiphenolic structure that is more sensible to the enzymatic oxidation [25].

On the contrary, the effect on AOA, ALA, oleuropein and simple phenols (Htyr and Tyr) was less clear, and their contents remained similar during kneading (Table 2).

The effect of crushing conditions on the main phenolics in virgin olive oil (VOO) is reported in Fig. 3; Table 3. A similar pattern to that observed for the olive paste was seen in the VOO (Fig. 3; Table 3). Thus, the hydroxytyrosol derivatives in VOO were the most affected by crushing conditions, due to both their different stability and their partition coefficients between the oil and water phases, and

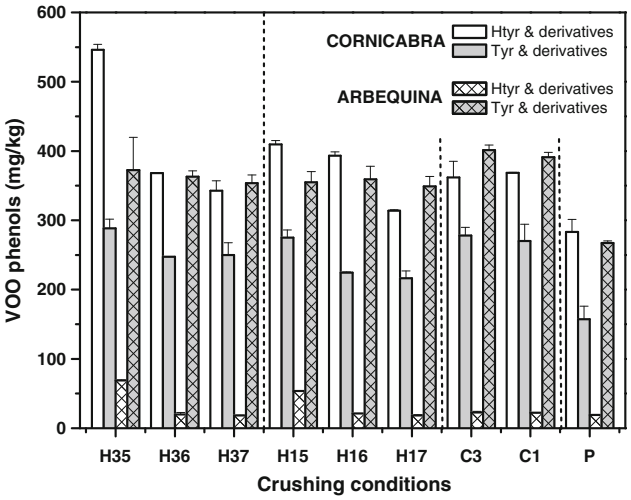

Fig. 3 Effect of crushing conditions on phenolic compounds in VOOs. *H* hammer crusher, *C* blade cutter, *P* pressure by a mortar

therefore, the sensory attributes of the oil would also be affected due to the known direct correlation between their concentration and bitterness [26].

Tyrosol derivatives are mainly associated with the pungent sensory notes in VOO, chiefly the deacetoxy form of ligstroside (DLA) [26], recently renamed Oleocanthal.

Furthermore, the only partial solubility of the phenolic compounds in the oil greatly reduces the amount of phenols contained in the final product. Thus, in Cornicabra, only 1.4 mg/kg of Htyr (under H35 conditions) or 210 mg/kg of DOA (H17) was found in the virgin oil after the decanter separation (Table 3). Moreover, oleuropein was not found in VOO since it is almost completely soluble in the aqueous phase.

Two-factor ANOVA (grid hole diameter and rotation speed for the H3 and H1 crushing conditions) confirmed what can clearly be seen in Fig. 3. The diameter of the grid holes was the main source of variation in the phenolic compositions in the olive paste and oil. For example, an *F*-value of 415 and 118 was obtained for the grid hole diameter and rotation speed, respectively, for hydroxytyrosol secoiridoid derivatives in the Cornicabra variety and 1709 and 48 for Arbequina. In the case of tyrosol secoiridoids, an *F*-value of 25 and 14 for grid hole diameter and rotation speed was observed for Cornicabra, whereas no statistically significant differences were observed for tyrosol secoiridoids in Arbequina. The diameter–rotation speed ANOVA interaction was also generally statistically significant, with the exception again of the tyrosol secoiridoid family. As indicated earlier, the effect of crushing conditions and kneading was higher for hydroxytyrosol derivatives than for tyrosol.

Table 3 Main phenolic compounds (mg/kg) found in VOOs processed using different crushing techniques

	Hydroxytyrosol	DOA	AOA	Tyrosol	DLA	ALA	Pinoresinol	Acetoxipinoresinol
ARBEQUINA								
H35	0.4 ± 0.0^{c}	56 ± 1^{c}	13 ± 1^{d}	0.7 ± 0.0^{cd}	47.2 ± 3.2^{c}	325.1 ± 44.3^{b}	6.7 ± 0.6^{a}	0.9 ± 0.1^{a}
H36	0.1 ± 0.0^{a}	11 ± 2^{a}	10 ± 1^{ab}	0.6 ± 0.1^{ab}	37.1 ± 2.1^{ab}	325.9 ± 10.4^{b}	8.4 ± 0.2^{b}	1.2 ± 0.1^{b}
H37	0.2 ± 0.0^{ab}	9 ± 1^{a}	9 ± 1^{a}	0.8 ± 0.1^{cd}	37.2 ± 3.4^{ab}	316.5 ± 8.3^{b}	10.2 ± 0.3^{c}	1.4 ± 0.1^{bc}
H15	0.4 ± 0.0^{c}	44 ± 1^{b}	10 ± 1^{abc}	0.7 ± 0.1^{bc}	40.0 ± 0.4^{bc}	315.2 ± 14.8^{b}	6.3 ± 0.7^{a}	1.2 ± 0.1^{b}
H16	0.2 ± 0.0^{b}	11 ± 1^{a}	10 ± 1^{abc}	0.8 ± 0.1^{cd}	36.5 ± 1.2^{ab}	322.5 ± 17.6^{b}	8.9 ± 0.3^{b}	1.3 ± 0.1^{bc}
H17	0.2 ± 0.1^{ab}	9 ± 1^{a}	9 ± 1^{a}	0.9 ± 0.1^{d}	36.8 ± 5.4^{ab}	312.2 ± 19.7^{b}	10.3 ± 0.1^{c}	1.2 ± 0.0^{b}
C3	0.2 ± 0.0^{ab}	11 ± 1^{a}	12 ± 1^{cd}	0.9 ± 0.1^{d}	42.8 ± 4.2^{bc}	358.5 ± 3.1^{b}	11.0 ± 0.4^{cd}	1.4 ± 0.0^{c}
C1	0.2 ± 0.0^{ab}	11 ± 1^{a}	12 ± 1^{bcd}	1.1 ± 0.1^{e}	42.7 ± 3.0^{bc}	348.6 ± 3.8^{b}	11.4 ± 0.1^{c}	1.4 ± 0.0^{c}
P	0.2 ± 0.0^{ab}	10 ± 2^{a}	9 ± 1^{a}	0.5 ± 0.0^{a}	30.6 ± 2.1^{a}	236.8 ± 5.2^{a}	8.1 ± 0.2^{b}	1.0 ± 0.1^{a}
CORNICABRA								
H35	1.4 ± 0.1^{c}	393 ± 7^{e}	153 ± 1^{d}	2.1 ± 0.3^{bc}	217.3 ± 12.2^{d}	71.2 ± 0.9^{cd}	1.7 ± 0.5^{b}	2.8 ± 0.5^{ef}
H36	0.9 ± 0.0^{a}	258 ± 1^{c}	111 ± 1^{b}	1.7 ± 0.0^{ab}	184.8 ± 0.2^{bc}	62.5 ± 0.2^{bc}	2.2 ± 0.0^{bc}	0.3 ± 0.1^{a}
H37	1.0 ± 0.2^{ab}	222 ± 11^{b}	121 ± 3^{bc}	2.4 ± 0.1^{cd}	188.3 ± 13.9^{bc}	61.7 ± 3.6^{bc}	3.4 ± 0.1^{d}	1.9 ± 0.2^{cd}
H15	1.4 ± 0.1^{c}	281 ± 4^{d}	129 ± 1^{c}	3.0 ± 0.1^{d}	210.0 ± 6.2^{cd}	65.0 ± 5.0^{bcd}	3.5 ± 0.1^{de}	0.9 ± 0.4^{b}
H16	1.3 ± 0.0^{c}	275 ± 5^{cd}	118 ± 1^{bc}	2.0 ± 0.1^{bc}	166.8 ± 0.9^{b}	57.6 ± 0.3^{b}	0.7 ± 0.0^{a}	1.5 ± 0.0^{c}
H17	1.2 ± 0.2^{bc}	210 ± 8^{a}	112 ± 4^{b}	2.6 ± 0.6^{cd}	163.0 ± 8.7^{b}	53.6 ± 1.6^{b}	2.5 ± 0.3^{c}	2.5 ± 0.0^{de}
C3	1.2 ± 0.1^{bc}	230 ± 15^{b}	132 ± 8^{c}	3.1 ± 0.2^{d}	203.1 ± 15.5^{cd}	74.9 ± 3.5^{d}	3.9 ± 0.1^{e}	3.2 ± 0.1^{f}
C1	0.8 ± 0.3^{a}	237 ± 11^{b}	132 ± 12^{c}	2.4 ± 0.0^{cd}	199.8 ± 14.0^{cd}	70.4 ± 10.2^{cd}	4.0 ± 0.2^{e}	3.2 ± 0.4^{f}
P	0.7 ± 0.1^{a}	188 ± 9^{a}	96 ± 9^{a}	1.4 ± 0.3^{a}	119.3 ± 12.2^{a}	38.2 ± 6.5^{a}	1.1 ± 0.2^{a}	$0.5 \pm 0,3^{ab}$

Different letters within crushing conditions (a–f) indicate significant differences ($p < 0.05$). *DOA* dialdehydic form of elenolic acid linked to hydroxytyrosol, *DLA* dialdehydic form of elenolic acid linked to tyrosol, *AOA* aldehydic form of elenolic acid linked to hydroxytyrosol, *ALA* aldehydic form of elenolic acid linked to tyrosol. The quantification of phenolic compounds was performed by HPLC–UV

Table 4 Pigments and tocopherol composition (mg/kg) in VOOs processed using different crushing techniques

	ARBEQUINA			CORNICABRA		
	α-Tocopherol	Carotenoids	Chlorophylls	α-Tocopherol	Carotenoids	Chlorophylls
H35	209 ± 2.7^{cd}	16.4 ± 0.6^{f}	28.6 ± 0.9^{f}	197 ± 5.8^{cde}	9.7 ± 0.2^{e}	16.0 ± 0.7^{d}
H36	208 ± 1.6^{cd}	9.9 ± 1.3^{d}	10.7 ± 1.2^{d}	190 ± 6.6^{bc}	9.1 ± 0.4^{d}	13.3 ± 0.5^{c}
H37	199 ± 4.0^{bc}	8.4 ± 0.8^{c}	5.9 ± 0.8^{bc}	188 ± 2.1^{bc}	7.5 ± 0.2^{c}	12.1 ± 0.4^{c}
H15	213 ± 3.9^{d}	14.7 ± 0.9^{e}	25.4 ± 0.4^{e}	205 ± 2.6^{e}	9.6 ± 0.4^{f}	12.7 ± 1.9^{c}
H16	211 ± 4.3^{d}	7.8 ± 0.2^{bc}	6.8 ± 0.4^{c}	201 ± 6.1^{cd}	7.7 ± 0.2^{c}	9.2 ± 0.1^{b}
H17	205 ± 6.1^{bcd}	7.4 ± 0.1^{abc}	5.7 ± 0.7^{bc}	191 ± 0.2^{bcd}	4.7 ± 0.1^{a}	6.9 ± 0.3^{a}
C3	200 ± 7.5^{bc}	7.4 ± 0.4^{abc}	4.9 ± 0.5^{ab}	186 ± 2.3^{b}	6.4 ± 0.2^{b}	5.5 ± 0.5^{a}
C1	196 ± 3.5^{b}	6.7 ± 0.2^{ab}	4.3 ± 0.2^{ab}	185 ± 3.2^{b}	6.3 ± 0.0^{b}	5.5 ± 0.6^{a}
P	178 ± 2.5^{a}	5.8 ± 0.5^{a}	3.8 ± 0.4^{a}	175 ± 2.9^{a}	4.8 ± 0.7^{a}	5.1 ± 0.3^{a}

Different letters within crushing conditions (a–f) indicate significant differences ($p < 0.05$)

Table 5 Effect of crushing and kneading on volatiles compounds (mg/kg) in the Arbequina olive paste

C6 aldehydes and C6 esters	Hexanal		*E*-2-hexenal		Hexyl acetate		Z-3-hexenyl acetate	
	Crushed	Kneaded	Crushed	Kneaded	Crushed	Kneaded	Crushed	Kneaded
H35	42 ± 16^{ab}	13 ± 2^{ab}	44 ± 7^{a}	20 ± 2^{a}	0.3 ± 0.1^{ab}	0.3 ± 0.0^{ab}	7 ± 0^{a}	3 ± 0^{a}
H36	48 ± 13^{abc}	13 ± 1^{ab}	42 ± 20^{a}	25 ± 2^{a}	0.1 ± 0.0^{a}	0.3 ± 0.0^{b}	4 ± 3^{a}	3 ± 0^{a}
H37	75 ± 47^{bc}	42 ± 24^{bc}	73 ± 36^{ab}	46 ± 23^{a}	0.9 ± 0.7^{ab}	0.2 ± 0.0^{ab}	8 ± 2^{a}	5 ± 3^{a}
H15	18 ± 5^{a}	15 ± 3^{ab}	33 ± 25^{a}	26 ± 3^{a}	0.4 ± 0.0^{ab}	0.2 ± 0.1^{ab}	6 ± 3^{a}	3 ± 1^{a}
H16	20 ± 9^{a}	38 ± 24^{abc}	39 ± 13^{a}	24 ± 4^{a}	0.3 ± 0.1^{ab}	0.2 ± 0.1^{ab}	5 ± 3^{a}	4 ± 1^{a}
H17	98 ± 28^{c}	36 ± 9^{abc}	81 ± 31^{a}	38 ± 22^{a}	0.8 ± 0.5^{ab}	0.1 ± 0.2^{ab}	10 ± 4^{a}	6 ± 4^{a}
C3	29 ± 18^{ab}	64 ± 15^{c}	56 ± 32^{a}	70 ± 9^{ab}	0.3 ± 0.2^{ab}	0.1 ± 0.0^{ab}	5 ± 3^{a}	7 ± 0^{a}
C1	17 ± 17^{a}	9 ± 1^{a}	64 ± 30^{a}	32 ± 5^{a}	0.6 ± 0.1^{ab}	0.1 ± 0.0^{a}	6 ± 3^{a}	4 ± 1^{a}
P	60 ± 4^{abc}	57 ± 4^{c}	46 ± 4^{a}	30 ± 2^{a}	0.9 ± 0.1^{b}	0.1 ± 0.0^{ab}	5 ± 1^{a}	4 ± 1^{a}

C6 alcohols	Hexan-1-ol		Z-3-hexanol		*E*-3-hexenol	
	Crushed	Kneaded	Crushed	Kneaded	Crushed	Kneaded
H35	4 ± 1^{ab}	1 ± 0^{a}	4 ± 1^{a}	1 ± 0^{a}	4 ± 0^{a}	2 ± 0^{a}
H36	3 ± 2^{ab}	1 ± 0^{b}	3 ± 3^{a}	1 ± 0^{a}	3 ± 1^{a}	3 ± 0^{a}
H37	7 ± 4^{bc}	2 ± 2^{ab}	7 ± 4^{a}	2 ± 2^{a}	8 ± 4^{ab}	7 ± 5^{a}
H15	2 ± 0^{a}	1 ± 4^{a}	2 ± 1^{a}	2 ± 1^{a}	3 ± 2^{a}	3 ± 1^{a}
H16	2 ± 2^{ab}	3 ± 3^{ab}	3 ± 2^{a}	3 ± 3^{ab}	3 ± 2^{a}	6 ± 4^{a}
H17	11 ± 2^{c}	1 ± 0^{a}	8 ± 4^{a}	1 ± 0^{a}	9 ± 3^{ab}	4 ± 2^{a}
C3	3 ± 2^{ab}	5 ± 1^{b}	4 ± 3^{a}	6 ± 1^{b}	5 ± 4^{ab}	8 ± 1^{a}
C1	3 ± 1^{ab}	1 ± 0^{a}	4 ± 3^{a}	2 ± 0^{a}	6 ± 4^{ab}	3 ± 0^{a}
P	11 ± 1^{c}	8 ± 1^{c}	6 ± 0^{a}	6 ± 0^{b}	11 ± 2^{b}	9 ± 1^{b}

Different letters within crushing conditions (a–g) indicate significant differences ($p < 0.05$)

The major lignans found in VOO are acetoxypinoresinol and pinoresinol, which generally followed an opposite trend to the rest of the phenolic compounds especially in the case of Arbequina VOO (Table 3). For example, the pinoresinol content in Arbequina VOOs increased from the H35 to H37 conditions (6.7–10.2 mg/kg) and from the H15 to H17 conditions (6.3–10.3 mg/kg) and moreover was higher using a blade cutter (11.0–11.4 and 3.9–4.0 mg/kg in Cornicabra). A similar trend was observed in the case of acetoxypinoresinol.

Oleuropein score (OlS)

Bitterness in VOO is a typical positive taste [27] that is associated with oil processed from green or turning colour olives, and its intensity is directly related to the phenol concentration. As expected, the oleuropein score (OlS) [16] followed a similar trend to total phenol content, and therefore, stronger extraction conditions produced more bitter VOOs. Thus, VOOs produced under the H35 conditions were significantly more bitter than those under H37

Table 6 Volatiles compounds (mg/kg IS) in VOOs processed using different crushing techniques

	Hexanal	*E*-2-hexenal	Hexyl acetate	Z-3-hexenyl acetate	Hexanol	Z-3-hexen-1-ol	*E*-2-hexen-1-ol
ARBEQUINA							
H35	1.3 ± 0.6^{abc}	15.1 ± 2.5^{bc}	0.1 ± 0.0^{ab}	0.4 ± 0.1^{ab}	0.4 ± 0.0^{a}	0.6 ± 0.1^{ab}	0.3 ± 0.1^{a}
H36	2.9 ± 0.7^{d}	26.1 ± 0.5^{d}	0.1 ± 0.0^{ab}	0.5 ± 0.0^{bc}	0.5 ± 0.0^{a}	0.7 ± 0.0^{bc}	0.9 ± 0.1^{bc}
H37	2.9 ± 0.2^{d}	34.1 ± 1.3^{ef}	0.1 ± 0.0^{b}	0.6 ± 0.1^{cd}	0.5 ± 0.1^{a}	0.8 ± 0.1^{cd}	1.2 ± 0.1^{bc}
H15	1.0 ± 0.1^{ab}	12.0 ± 8.1^{ab}	0.1 ± 0.0^{ab}	0.4 ± 0.0^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	0.3 ± 0.3^{a}
H16	1.9 ± 0.0^{bcd}	22.0 ± 1.3^{cd}	0.1 ± 0.0^{ab}	0.5 ± 0.0^{abc}	0.5 ± 0.2^{a}	0.7 ± 0.1^{abc}	0.7 ± 0.7^{ab}
H17	2.3 ± 0.0^{cd}	29.3 ± 1.3^{de}	0.1 ± 0.0^{ab}	0.5 ± 0.0^{abc}	0.4 ± 0.1^{a}	0.6 ± 0.1^{abc}	1.1 ± 0.2^{bc}
C3	1.9 ± 0.0^{bcd}	33.6 ± 2.8^{ef}	0.0 ± 0.0^{ab}	0.5 ± 0.0^{bcd}	0.5 ± 0.0^{a}	0.9 ± 0.1^{de}	1.5 ± 0.0^{c}
C1	1.5 ± 0.0^{abc}	37.5 ± 1.4^{f}	0.0 ± 0.0^{ab}	0.6 ± 0.1^{d}	0.6 ± 0.0^{a}	1.1 ± 0.0^{e}	2.0 ± 0.7^{d}
P	0.8 ± 0.0^{a}	7.0 ± 0.2^{a}	0.0 ± 0.0^{a}	0.4 ± 0.1^{ab}	0.4 ± 0.0^{a}	0.6 ± 0.0^{ab}	1.1 ± 0.1^{bc}
CORNICABRA							
H35	0.3 ± 0.0^{ab}	1.1 ± 0.1^{ab}	<0.01	0.0 ± 0.0^{a}	0.7 ± 0.0^{a}	3.0 ± 0.2^{ab}	0.1 ± 0.0^{a}
H36	0.4 ± 0.0^{bcd}	1.6 ± 0.1^{bc}	<0.01	0.0 ± 0.0^{ab}	0.7 ± 0.0^{a}	3.2 ± 0.3^{ab}	0.1 ± 0.0^{abc}
H37	0.6 ± 0.1^{e}	2.5 ± 0.3^{d}	<0.01	0.1 ± 0.0^{bc}	0.8 ± 0.1^{a}	3.3 ± 0.7^{ab}	0.1 ± 0.0^{abc}
H15	0.2 ± 0.0^{a}	0.6 ± 0.0^{a}	<0.01	0.0 ± 0.0^{a}	0.6 ± 0.0^{a}	2.3 ± 0.1^{a}	0.1 ± 0.0^{abc}
H16	0.3 ± 0.0^{abc}	1.5 ± 0.0^{b}	<0.01	0.0 ± 0.0^{a}	0.7 ± 0.0^{a}	2.7 ± 0.9^{ab}	0.1 ± 0.0^{ab}
H17	0.5 ± 0.1^{cde}	2.3 ± 0.1^{cd}	<0.01	0.0 ± 0.0^{ab}	0.7 ± 0.2^{a}	3.0 ± 0.7^{ab}	0.1 ± 0.0^{abc}
C3	0.6 ± 0.1^{e}	3.8 ± 0.8^{e}	<0.01	0.1 ± 0.0^{c}	0.7 ± 0.0^{a}	3.4 ± 0.3^{b}	0.2 ± 0.1^{cd}
C1	0.6 ± 0.1^{de}	3.6 ± 0.2^{e}	<0.01	0.1 ± 0.0^{c}	0.7 ± 0.0^{a}	3.3 ± 0.1^{ab}	0.2 ± 0.0^{bcd}
P	0.5 ± 0.0^{cde}	5.2 ± 0.2^{f}	<0.01	0.1 ± 0.0^{d}	0.7 ± 0.0^{a}	2.6 ± 0.2^{ab}	0.2 ± 0.0^{d}

Different letters within crushing conditions (a–f) indicate significant differences ($p < 0.05$). Concentration expressed as ppm of 2-methyl-4-pentanol, the internal standard used

(744–622 mg/kg OlS in Arbequina and 1,888–1,619 mg/kg OlS in Cornicabra). However, rotation speed did not significantly influence bitterness. The blade cutter produced VOOs with a similar OlS (611 and 564 mg/kg OlS in Arbequina and 1,578–1,593 mg/kg OlS in Cornicabra, for the C1 and C3 conditions, respectively) to those oils produced under the H7 conditions, and the pressure treatment (P) apparently produced even less bitter VOOs (499 mg/kg OlS in Arbequina and 1,519 mg/kg OlS in Cornicabra).

Tocopherols and pigments

α-tocopherol is the major tocopherol found in VOO, representing about 90% of all the isomers [28]; in Arbequina and Cornicabra VOOs, the α-tocopherol content was an average of 203 and 192 mg/kg, respectively. As reported in Table 4, VOOs produced by hammer crushers showed a slightly higher concentration, sometimes statistically significant, of α-tocopherol than the rest of the techniques studied (blade cutter and mortar), therefore following a similar trend to that in the phenolic compounds although the intensity of the effect was much lower (i.e. 213 mg/kg in H15, 205 mg/kg in H17, 196 mg/kg in C1 and 178 mg/kg using a mortar in the case of Arbequina).

VOOs processed under stronger extraction conditions showed a significantly higher content of carotenoids and chlorophyll pigments (Table 4) probably due to the better breakage of the olive tissue and the higher paste temperature reached that also could induce the inactivation of the enzymes responsible for the pigment degradation during processing [29]. Indeed, this effect on the pigment concentration was even more pronounced than that in the case of the hydroxytyrosol phenols family and therefore may be used as an index of the degree of extraction produced by different crushing conditions (i.e. 12.7 mg/kg in H15, 6.9 mg/kg in H17, 5.5 mg/kg in C1 and 5.1 mg/kg using a mortar, for chlorophylls in Cornicabra). The already discussed much greater effect of the hammer crusher grid hole diameter rather than the rotation speed, which was confirmed by ANOVA, is clearly visible in the data reported in Table 4.

Volatile compounds

Volatile compounds formed by the lipooxygenase (LOX) pathway represent about 80% of the total volatiles in VOO and are responsible for the desirable green and fruity sensory notes [30]. As in the case of the other minor components, volatile compounds were studied and analysed from their initial content in the olive fruit, through the crushing and kneading operation in the corresponding olive pastes, and finally in the VOO produced.

In both olive cultivars, volatile compounds are elicited once the fruit is cut. A substantial increase in the LOX volatile content just after fruit crushing (Table 5) compared to their initial concentration in the olives (Table 1) was observed, especially in the Arbequina variety. The C6 aldehydes showed the greatest increase (i.e. from 3 up to 17–98 mg/kg for hexanal, depending on the crushing conditions employed), whereas a more moderate increase was observed for C6 esters and alcohols (i.e. from 1.5 to 4–10 mg/kg for Z-3-hexenyl acetate and from 0 to 2–11 mg/kg for hexan-1-ol).

A relevant and interesting difference in the volatile contents in the crushed olive paste was observed depending on the type and conditions of the crushing operation used. Milder crushing conditions, obtained mainly using bigger grid diameters (7 mm) as already indicated, produced olive pastes richer in LOX volatiles, especially in the case of the C6 aldehydes (i.e. from 44 up to 73 mg/kg for *E*-2-hexenal using the H35 or H37 conditions). This could probably due to the higher activity of some enzymes related to the biogeneration of volatiles at lower crushing temperature [31]. Smaller differences, though statistically significant in many cases, were observed for the C6 esters and alcohol contents in the crushed paste (Table 5).

Kneading always reduced the volatile content in the olive paste (for example from 48 down to 13 mg/kg in the case of hexanal under the H36 conditions or from 8 to 5 mg/kg in Z-3-hexenyl acetate (H37) and from 7 to 2 mg/kg for Z-3-hexanol (H37; Table 5). This may be due simply to their evaporation during malaxation. Therefore, as far as the content of volatile components in the final VOO is concerned, a shorter malaxation time would apparently reduce the loss of these important and desirable aromatic compounds from the final product.

The effect of the different crushing conditions studied on the volatile content in the final VOO was large, as shown in Fig. 4. As previously observed in the case of the olive paste, the use of milder crushing conditions using bigger grid holes (7 mm) or employing a blade cutter (C) allowed the production of a VOO with a significantly higher amount of total LOX volatiles and especially C6 aldehydes (i.e. from 17 up to 40 mg/kg (as IS) of total volatiles using the H35 or H37 conditions in the case of Arbequina). A similar positive effect on aromatic compounds was obtained by employing a blade cutter to smash the olive fruit (38–42 mg/kg). The behaviour of Cornicabra volatiles was similar to that observed in Arbequina, although the content of these compounds in this VOO variety was much lower (i.e. a maximum value of 8–9 mg/kg of total LOX volatiles; Table 5). The use of a blade cutter to mill the olives is therefore apparently very beneficial as far as the content of aromatic compounds is concerned.

The individual volatile compounds found in the final VOO according to the crushing conditions employed are

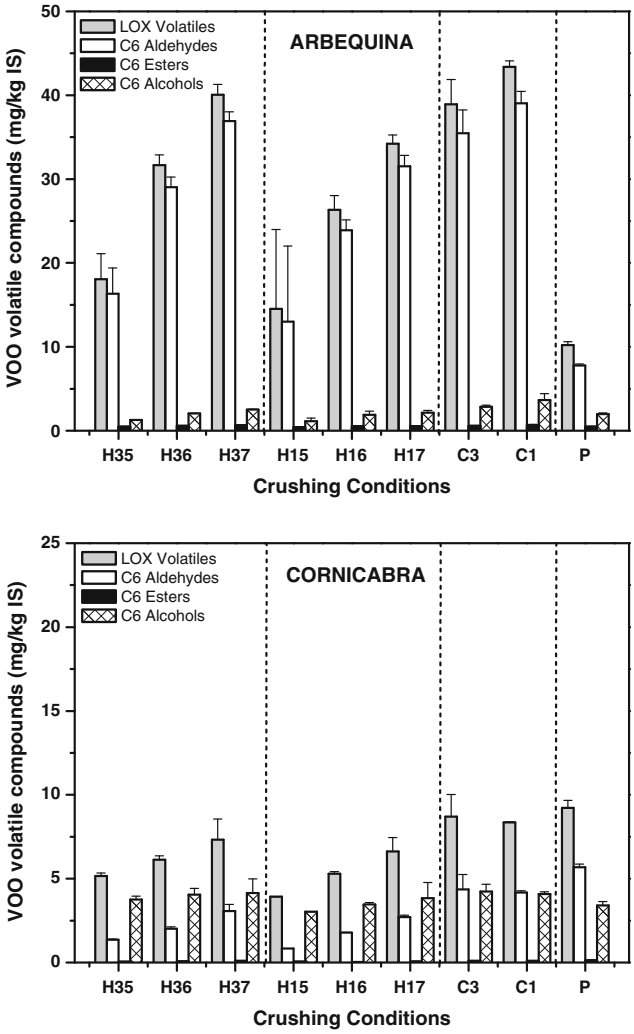

Fig. 4 Effect of crushing conditions on volatile compounds in VOOs

reported in Table 6. It is noteworthy that the main volatile compound in Arbequina VOO was *E*-2-hexenal (from 7 up to 38 mg/kg, depending on the crushing conditions used), whereas the concentration of the other individual volatiles was below 1–3 mg/kg. On the contrary, in Cornicabra VOO, a variety with a much lower amount of volatile compounds, the main component was Z-3-hexen-1-ol (between 2.3 to 3.4 mg/kg depending on crushing conditions), followed by *E*-2-hexenal (from 0.6 to 3.8 or 5.2 mg/kg) and hexanol (0.6–0.8 mg/kg). The effect of the different crushing conditions studied on individual volatiles was very similar to that described for their families in Fig. 4. The positive effect of using a blade cutter was very clear in the case of *E*-2-hexenal in both varieties (Table 6).

It is interesting that the effect on the content of volatile compounds of milder or stronger crushing conditions was the opposite to that described for the phenolic compounds. This is particularly beneficial in the case of very rich phenol olive varieties, like Cornicabra, that as a consequence are

often too bitter and generally contain a low amount of volatiles; milder extraction conditions (H7 or C) allow the volatile content to be increased and at the same time reduce the excessive bitterness related to excessive phenols. On the other hand, stronger crushing conditions should enhance the phenolic content of varieties such as Arbequina normally characterized by a high volatile composition but by a low oxidative stability.

The use of the different crushing conditions may be therefore very useful to modulate the VOO content in minor compounds related to its overall quality.

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