JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Olive Stoning on the Volatile and Phenolic Composition of Virgin Olive Oil

MAURIZIO SERVILI,* AGNESE TATICCHI, SONIA ESPOSTO, STEFANIA URBANI, ROBERTO SELVAGGINI, AND GIANFRANCESCO MONTEDORO

Dipartimento di Scienze Economico-Estimative e degli Alimenti, Sezione di Tecnologie e Biotecnologie degli Alimenti, Università degli Studi di Perugia, Via S. Costanzo, 06126 Perugia, Italy

Olive stoning during the virgin olive oil (VOO) mechanical extraction process was studied to show the effect on the phenolic and volatile composition of the oil. To study the impact of the constitutive parts of the fruit in the composition of olive pastes during processing, the phenolic compounds and several enzymatic activities such as polyphenoloxidase (PPO), peroxidase (POD), and lipoxygenase (LPO) of the olive pulp, stone, and seed were also studied. The olive pulp showed large amounts of oleuropein, demethyloleuropein, and lignans, while the contribution of the stone and the seed in the overall phenolic composition of the fruit was very low. The occurrence of crushed stone in the pastes, during malaxation, increased the peroxidase activity in the pastes, reducing the phenolic concentration in VOO and, at the same time, modifying the composition of volatile compounds produced by the lipoxygenase pathway. The oil obtained from stoned olive pastes contained higher amounts of secoiridoid derivatives such as the dialdehydic forms of elenolic acid linked to (3,4-dihydroxyphenyl)-ethanol and (*p*-hydroxyphenyl)ethanol (3,4-DHPEA-EDA and p-HPEA-EDA, respectively) and the isomer of the oleuropein aglycon (3,4-DHPEA-EA) and, at the same time, did not show significant variations of lignans. The stoning process modified the volatile profile of VOO by increasing the C_6 unsaturated aldehydes that are strictly related to the cut-grass sensory notes of the oil.

KEYWORDS: Olive stone; oil mechanical extraction process; phenols; secoiridoids; volatile compounds; polyphenoloxidase; peroxidase; lipoxygenase

INTRODUCTION

Phenolic compounds affect healthy properties of virgin olive oil (VOO) and, in combination with the volatile compounds, define the peculiar flavor of VOO (*1*−*6*). A new approach to the oil mechanical extraction process, aimed at producing high-quality oils, can be oriented to increase the phenolic concentration while, at the same time, promoting the production, during processing, of volatile compounds responsible for the VOO aroma. So far, however, the occurrence of volatile and phenolic compounds in VOO is strictly related to the activity of various endogenous enzymes that are activated during processing (*7*−*11*), such as polyphenoloxidase (PPO), peroxidase (POD), and lipoxygenase (LPO) (*12*, *13*). The PPO and POD catalyze the oxidation of phenols during malaxation, reducing their concentration in the pastes and in the oil. The oil-processing operating conditions also affect the LPO pathway that, producing C_5 and C_6 saturated and unsaturated aldehydes, alcohols, and esters, regulates the intensity in VOO of some typical sensory notes [such as "cut grass" and "haylike" and "floral" (*5*)]. In this context, the definition of the operative conditions that allow a selective control of these enzymes is a crucial point of the oil mechanical extraction process, which is strictly related to the sensory and health quality of VOO.

The potential strategies that can be applied to improve the phenolic and volatile composition of VOO could include, therefore, the control of the PPO, POD, and LPO pathway. To this end, the oxygen concentration control in the pastes, during malaxation, can be used as a technological parameter, in combination with the duration and the temperature of malaxation, to optimize phenolic and volatile composition in VOO (*14*−*20*). Another opportunity that can be evaluated is the oil extraction from stoned olive pastes. The relationships between the volatile and the phenolic composition of VOO and this new technological approach to the oil processing are based on the distribution of PPO, LPO, and POD in the constitutive parts of the olive fruit. A previous paper reporting preliminary results on the distribution of endogenous oxidoreductases in the seed, pulp, and skin described differential levels of PPO, POD, and LPO activities in the constitutive parts of the olive fruit (*11*). The high POD activity, observed in the seed, indicates the possibility of reducing the phenolic oxidative degradation, during processing, through the olive stone removal before malaxation. Several results reported in previous papers seem to support this hypothesis (*21*, *22*).

* To whom correspondence should be addressed. Tel: +39 075 5857942. Fax: +39 075 5857916. E-mail: servimau@unipg.it.

10.1021/jf070600i CCC: $37.00
Published on Web 08/01/2007

Table 1. Phenolic Composition (mg/100 g d.w.) of the Constitutive Parts of Olive Fruits from Coratina and Frantoio Cultivars

	Coratina cv.			Frantoio cv.		
	pulp	stone	seed	pulp	stone	seed
3,4-DHPEA[a]	24.3 (1.2)	10.4 (0.6)	3.1 (0.2)	11.7 (0.6)	8.0 (0.4)	5.9 (0.3)
p-HPEA	15.0 (0.7)	3.1 (0.2)	25.6 (1.3)	nd	nd	13.7 (0.7)
nüzhenide	nd[b]	nd	871.1 (43.6)	nd	nd	645.5 (32.6)
verbascoside	2563.0 (139.4)	14.8 (0.7)	nd	274.6 (13.7)	61.8 (3.1)	nd
ligstroside glucoside	83.6 (0.3)	nd	nd	75.0 (0.2)	nd	nd
oleuropein	1745.5 (42.3)	110.0 (5.5)	nd	1893.9 (160.1)	152.9 (7.6)	nd
demethyloleuropein	1210.3 (50.6)	41.8 (2.1)	nd		nd	nd
(+)-1-acetoxypinoresinol	28.10 (0.1)	13.60 (0.1)	nd	36.20 (0.2)	8.40 (0)	nd
(+)-pinoresinol	1.40 (0.01)	38.20 (0.2)	nd	3.40 (0.1)	45.00 (0.2)	nd

[a] The phenolic content is the mean value of three independent determinations; the standard deviation is reported in parentheses. [b] Not detected.

Table 2. Total Activities (U/mg d.w.) and Corresponding Contribution (%) of the Constitutive Parts of Olive Fruits from Coratina and Frantoio Cultivars

	pulp	% contribution	seed	% contribution
		Frantoio Cultivar		
PPO[a]	15.45 (1.2)	100.0	nd[b]	nd
POD	27.35 (1.72)	59.20	210.40 (12.81)	40.80
LPO	2.26 (0.20)	81.95	6.01 (0.80)	18.05
		Coratina Cultivar		
PPO	4.80 (0.10)	100.00	nd	nd
POD	13.10 (1.13)	41.48	209.45 (8.41)	58.16
LPO	2.67 (0.32)	81.80	7.16 (0.87)	18.20

[a] The enzymatic activity is the mean value of three independent determinations; the standard deviation is reported in parentheses. [b] Not detected.

In this work, the effects of the stoning process, applied to the oil mechanical extraction process, on the volatile and phenolic composition of VOO were investigated.

MATERIALS AND METHODS

Olives. Drupes of the Coratina and Frantoio cultivars, which are two of the most diffused Italian cultivars, harvested during the year 2005, were used. The ripening stage of the green olives [evaluated as the pigmentation index, according to Pannelli et al. (*23*)] was 0.90 and 0.95 for Coratina and Frantoio cultivars, respectively. The moisture content in the fruit was 53.2% for the Frantoio cultivar and 55.8% for Coratina, while the oil contents were 39.5 and 48.8%, respectively. For the phenolic composition and the enzymatic activity evaluation, the fresh fruits were frozen in liquid nitrogen and freeze-dried immediately after freezing. The freeze-drying conditions were reported as follows: the process began at −40 °C for 24 h, after which the temperature was increased up to −5 °C and maintained for 72 h, and the temperature was then set at 5 °C and maintained for 48 h. Freeze-dried materials were stored at −30 °C in dry conditions less than 30 days before enzymatic assays.

Reference Compounds. (3,4-Dihydroxyphenyl)ethanol (3,4-DHPEA) was obtained from Cayman Chemicals Ltd. (United States), while the (*p*-hydroxyphenyl)ethanol (*p*-HPEA) was obtained from Janssen Chemical Co. (Beerse, Belgium). Oleuropein glucoside was purchased from Extrasynthèse (France). Demethyloleuropein verbascoside and nüzhenide were extracted from olive fruit according to the procedure reported in the previous paper (*24*). Briefly, the phenols were extracted from the (5 g) freeze-dried olive pulps and seeds using a mixture of methanol:water 80:20 v/v at low temperature (50 mL); the extraction procedure was performed three times. The dialdehydic forms of elenolic acid linked to 3,4-DHPEA and *p*-HPEA (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively), the isomer of oleuropein aglycon (3,4-DHPEA-EA), the (+)-1-acetoxypinoresinol, and (+)-pinoresinol were extracted from VOO using a procedure previously reported (*25*). In short, the phenols were extracted from the oil using methanol:water 80:20 v/v; after solvent evaporation and partial purification of the crude extract obtained from the olive fruit and VOO, the phenolic separation was performed by semipreparative high-performance liquid chromatography (HPLC) analysis using a 9.4 mm i.d. × 500 mm Whatman Partisil 10 ODS-2 semipreparative column; the mobile phase was 0.2% acetic acid in water (pH 3.1) (A)/methanol (B) at a flow rate of 6.5 mL/min while the phenols detection was performed using a diode array detector (DAD) (*25*). The purity of all of the substances obtained from direct extraction was tested by HPLC, and their chemical structures were verified by nuclear magnetic resonance (NMR) using the same operative conditions reported in previous papers (*24*, *25*). Pure analytical standards of volatile compounds were purchased from Fluka and Aldrich (Milan, Italy).

VOO Mechanical Extraction Process. The experiments were performed on an industrial scale using a Rapanelli SPA industrial implant. The crushing operation, for the traditional process, was carried out by a hammer crusher model GR 32 (Rapanelli Fioravante S.p.a., Foligno, Italy) while, for the olive stoning, a stoning machine (Agrivision S.r.l., Firenze, Italy) was employed. The malaxation was carried out at 25 °C for 30 min, and the oil separation was obtained using a three phase decanter at low water addition (0.2:1 v:w), RAMEF model 400 ECO-G (Rapanelli Fioravante S.p.a., Foligno, Italy). The VOO samples were filtered and stored in the dark at 13 °C until analysis; for the phenolic composition and the enzymatic activity evaluations, the crushed and malaxed pastes were immediately frozen in liquid nitrogen and freeze-dried as reported above.

Analytical Methods. *Extraction and HPLC Analysis of Phenolic Compounds of the Constitutive Parts of Fruit and Olive Pastes*. The phenolic extraction from the pulp, stone, and seed of the olive fruit was carried out according to the procedure previously published by Servili et al. (*24*), modified according to the reported. Three grams of freeze-dried olive pulp, stone, seed, and olive paste was homogenized with 100 mL of 80% methanol containing 20 mg/L of sodium diethyldithiocarbamate (DIECA); the extraction was performed in triplicate. After methanol removal, the aqueous extract was used for solid-phase extraction (SPE) phenol separation. The SPE procedure was applied, for all of the phenolic extracts, by loading with 2 mL of the sample a 5 g/25 mL Extraclean highload C18 cartridge (Alltech Italia S.r.l., Sedriano, Italy) using, as the eluting solvent, 200 mL of methanol. After solvent removal under vacuum at 30 °C, the phenolic extract was recovered and then dissolved in methanol (1 mL). The reversed-phase HPLC analyses of phenolic extracts were conducted with an Agilent Technologies system model 1100 composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, a DAD, and a fluorescence detector (FLD). For the evaluation of all of the phenolic compounds (*24*), except lignans, a C18 Inertsil ODS-3 column (150 mm × 4.6 mm i.d.) (Alltech Co.) was employed, and the injected sample volume was 20 μL. The mobile phase was 0.2% acetic acid (pH 3.1) in water (A)/methanol (B) at a flow rate of 1.5 mL/min. The total running time was 55 min, and the gradient changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 10 min, and 0% A/100% B in 10 min, maintained for 5 min, return to initial conditions in 10 min; the total running time was 55 min. For the detection, a DAD was employed as follows: The wavelengths used were 278 and 339 nm. For the evaluation of the lignans, to better separate them (*27*),

Table 3. Phenolic Composition (mg/100 g d.w.) of Crushed and Malaxed Pastes Obtained from Whole and Stoned Frantoio and Coratina Olive Fruits

	crushed paste		malaxed paste	
	traditional	stoned	traditional	stoned
	Frantoio Cultivar			
3,4-DHPEA[a]	19.1 (0.8) a	30.9 (2.3) b	33.3 (2.3) b	40.1 (3.1) c
p-HPEA	nd[b]	nd	nd	nd
verbascoside	95.8 (5.2) a	125.9 (4.8) b	90.1 (5.6) a	110.8 (6.5) c
oleuropein	115.5 (8.4) a	104.5 (5.3) ab	95.6 (7.1) bc	85.0 (3.0) c
3,4-DHPEA-EDA	482.8 (10.5) a	950.4 (12.1) b	342.5 (11.6) c	720.4 (8.4) d
(+)-1-acetoxypinoresinol	12.2 (0.7) a	21.5 (1.5) b	14.0 (1.0) c	20.5 (1.1) a
(+)-pinoresinol	9.4 (0.4) a	28.0 (1.8) b	34.3 (2.6) c	24.3 (0.9) b
ligstroside aglycone	9.5 (0.6) a	12.1 (0.7) b	10.9 (0.5) ab	11.2 (0.7) b
	Coratina Cultivar			
3,4-DHPEA[a]	35.7 (1.7) a	39.6 (3.3) ab	48.9 (6.6) b	17.9 (1.2) c
p-HPEA	11.8 (1.7) a	17.4 (1.1) b	12.9 (1.4) a	19.3 (2.0) b
verbascoside	1350.2 (26.0) a	1583.1 (32.0) b	1378.4 (25.0) a	1411.1 (41.0) a
oleuropein	106.4 (10.1) a	120.3 (12.3) a	76.3 (10.2) a	42.9 (3.6) b
3,4-DHPEA-EDA	1023.9 (22.0) a	1338.3 (39.0) b	876.9 (24.0) a	1144.1 (23.0) c
(+)-1-acetoxypinoresinol	14.5 (0.7) a	14.8 (0.6) ab	16.8 (0.7) b	20.2 (1.1) c
(+)-pinoresinol	20.9 (1.1) a	20.2 (0.8) a	28.3 (0.8) b	33.6 (1.5) c
ligstroside aglycone	13.2 (0.5) a	18.4 (0.5) b	16.1 (0.6) c	21.2 (1.4) d

[a] The phenolic content is the mean value of three independent experiments; the standard deviation is reported in parentheses. Values in each row having different letters (a–d) are significantly different from one another at $p < 0.01$. [b] Not detected.

Table 4. Enzymatic Activities (U/mg d.w.) in Crushed and Malaxed Pastes Obtained from Whole and Stoned Frantoio and Coratina Olive Fruits

	crushed paste		malaxed paste	
	traditional	stoned	traditional	stoned
	Frantoio Cultivar			
POD[a]	37.75 (2.05) a	12.45 (1.06) b	14.05 (0.35) b	6.40 (0.28) c
PPO	17.00 (0.28) a	17.35 (1.48) a	10.30 (0.70) b	10.70 (1.41) b
	Coratina Cultivar			
POD	16.05 (0.80) a	5.35 (0.40) b	7.25 (0.21) c	2.60 (0.14) d
PPO	5.35 (0.49) a	5.95 (0.21) a	4.20 (0.42) b	5.20 (0.42) ab

[a] The enzymatic activity is the mean value of three independent experiments; the standard deviation is reported in parentheses. Values in each row having different letters (a–d) are significantly different from one another at $p < 0.01$.

the C18 column used was a Spherisorb ODS-1 250 mm × 4.6 mm with a particle size of 5 μm (Phase Separation Ltd., Deeside, United Kingdom); the injected sample volume was 20 μL. The mobile phase was composed of 0.2% acetic acid (pH 3.1) in water (solvent A)/methanol (solvent B) at a flow rate of 1 mL/min. The gradient changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 16 min, and 0% A/100% B in 14 min, and this composition was maintained for 10 min and then was returned to the initial conditions and equilibration in 13 min; the total running time was 73 min. Lignans were detected by FLD operated at an excitation wavelength set at 280 nm and emissions at 339 nm (*27*).

Extraction and HPLC Analysis of Phenolic Compounds of VOO. The extraction of VOO phenols was performed as reported in a previous paper by Montedoro et al. (*26*). The HPLC analyses of phenolic extracts were conducted with an Agilent Technologies system model 1100 composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, a DAD, and a FLD. For the HPLC analysis of phenolic extracts, the C18 column used was a Spherisorb ODS-1 250 mm × 4.6 mm with a particle size of 5 μm (Phase Separation Ltd., Deeside, United Kingdom); the injected sample volume was 20 μL. The operating conditions of the chromatographic analysis were identical to those reported above, in the previous subparagraph, for the same column. For the detection of all of the phenolic compounds, a DAD was employed as follows: The wavelengths used were 278 and 339 nm (*27*).

Volatile Compounds. To evaluate the volatile compounds production by the LPO pathway from the constitutive parts of the fruit, 10 g of pulp and 5 g of seeds were crushed using a porcelain mortar: The operation was performed for 5 min at room temperature. Two grams of the crushed material (olive pulp or seed) was placed in a 10 mL vial and maintained at 25 °C for 5 min to develop LPO pathway compounds, and then, we added 2 mL of a $CaCl_2$-saturated solution as an enzymatic inhibitor and stored it at −20 °C until the analysis. To study the volatile composition of stoned and traditional olive pastes after crushing and malaxation, 2 g of the pastes was placed in a 10 mL vial; this was then added to 2 mL of a $CaCl_2$-saturated solution as an enzymatic inhibitor and stored as reported above. To evaluate the volatile compound in VOO, 3 g of oil was put into a 10 mL vial. For the sampling of the head space (HS) volatile compounds, solid-phase microextraction (SPME) was applied as follows: All of the vials were thermostatted at 35 °C, and then, the fiber (a 50/30 μm DVB/Carboxen/PDMS of 1 cm of length, stableflex) (Supelco, Inc., Bellefonte, PA) was exposed to the vapor phase for 30 min to sample the volatile compounds. Afterward, the fiber was inserted into the gas chromatograph (GC) injector set in splitless mode using a splitless inlet liner of 0.75 mm i.d. for thermal desorption where it was left for 10 min. All of the SPME operations were automated using the Varian CP 8410 Autoinjector (Varian, Walnut Creek, CA).

Gas Chromatography−Mass Spectrometry (GC-MS) Analysis of Volatile Compounds. A GC-MS Varian 4000 equipped with a 1079 split/splitless injector (Varian) was used. A fused-silica capillary column DB-Wax-ETR, 50 m, 0.32 mm i.d., 1 μm film thickness (J&W Scientific, Folsom, CA) was employed. The column was operated with helium with a flow rate of 1.7 mL/min that was kept constant during the analysis using an electronic flow controller (EFC). The GC oven heating was started at 35 °C. This temperature was maintained for 8 min, then increased to 45 °C at a rate of 1.5 °C/min, increased to 150 °C at a rate of 3 °C/min, increased to 180 °C at a rate of 4 °C/min, and finally increased to 210 °C at a rate of 3.6 °C/min where it was kept for 14.50 min. The total time of analysis was 80 min. The injector temperature was maintained at 250 °C. The temperature for the transfer line was fixed at 170 °C. The mass spectrometer was operated in the electron ionization (EI) mode at an ionization energy of 70 eV in the mass range of 25−350 amu at a scan rate of 0.79 s/scan and a manifold temperature of 150 °C. The GC-MS was operated through the software Varian MS Workstation version 6.6 (Varian). The volatile compounds were identified by comparison of their mass spectra and retention times

Table 5. Phenolic Composition (mg/kg) in Oils Obtained by Mechanical Extraction with Traditional Process and with Stoning

	traditional	stoned
	Frantoio Cultivar	
3,4-DHPEA[a]	1.9 (0.3) a	1.8 (0.2) a
p-HPEA	4.6 (0.4) a	4.9 (0.4) a
3,4-DHPEA-EDA	88.8 (7.2) a	112.4 (10.1) b
p-HPEA-EDA	43.8 (3.1) a	54.8 (3.1) b
3,4-DHPEA-EA	30.3 (2.7) a	42.8 (3.6) b
(+)-1-acetoxypinoresinol	40.7 (2.3) a	42.8 (1.1) a
(+)-pinoresinol	4.2 (0.4) a	4.5 (0.5) a
	Coratina Cultivar	
3,4-DHPEA	3.1 (0.2) a	2.8 (0.3) a
p-HPEA	14.9 (1.1) a	15.0 (0.8) a
3,4-DHPEA-EDA	365.2 (18.6) a	507.1 (19.9) b
p-HPEA-EDA	92.8 (7.8) a	115.9 (6.2) b
3,4-DHPEA-EA	101.2 (5.8) a	125.9 (7.1) b
(+)-1-acetoxypinoresinol	41.1 (1.1) a	44.0 (1.8) a
(+)-pinoresinol	8.4 (0.6) a	10.4 (0.6) b

[a] The phenolic content is the mean value of three independent experiments; the standard deviation is reported in parentheses. Values in each row having different letters (a–b) are significantly different from one another at $p < 0.01$.

Table 6. Volatile Compounds, Produced by the LPO Pathway, from Crushed Olive Pulp and Seed from Frantoio and Coratina Cultivars (μg/g f.w.)

	seed	pulp
	Frantoio Cultivar	
aldehydes		
2-pentenal (*E*)[a]	0.28 (0.03) a	0.74 (0.06) b
hexanal	6.39 (0.55) a	2.97 (0.04) b
2-hexenal (*E*)	1.22 (0.46) a	51.35 (1.94) b
2,4-hexadienal (*E*,*E*)	0.10 (0.02) a	0.49 (0.02) b
alcohols		
1-pentanol	0.54 (0.01) a	0.57 (0.08) a
2-penten-1-ol (*E*)	0.03 (0.00) a	0.19 (0.00) b
1-penten-3-ol	0.42 (0.04) a	2.07 (0.03) b
1-hexanol	0.68 (0.02) a	0.34 (0.02) b
3-hexen-1-ol (*E*)	0.01 (0.00) a	0.01 (0.00) a
3-hexen-1-ol (*Z*)	0.22 (0.00) a	0.79 (0.03) b
2-hexen-1-ol (*Z*)	37.40 (0.18) a	24.93 (1.24) b
	Coratina Cultivar	
aldehydes		
2-pentenal (*E*)	0.16 (0.02) a	0.78 (0.01) b
hexanal	4.38 (1.4) a	3.22 (1.51) a
2-hexenal (*E*)	0.13 (0.03) a	44.48 (0.16) b
2,4-hexadienal (*E*,*E*)	nd[b]	0.52 (0.07)
alcohols		
1-pentanol	0.23 (0.01) a	0.39 (0.06) b
2-penten-1-ol (*E*)	0.63 (0.04) a	0.02 (0.00) b
1-penten-3-ol	0.75 (0.15) a	0.50 (0.03) a
1-hexanol	0.74 (0.04) a	0.31 (0.06) b
3-hexen-1-ol (*E*)	0.03 (0.00) a	0.01 (0.00) b
3-hexen-1-ol (*Z*)	1.93 (0.10) a	0.16 (0.02) b
2-hexen-1-ol (*Z*)	33.82 (3.35) a	14.21 (2.64) b

[a] Data are the mean values of three independent determinations; the standard deviation is reported in parentheses. Values in each row having different letters (a–b) are significantly different from one another at $p < 0.01$. [b] Not detected.

with those of authentic reference compounds. Integration of all of the chromatographic peaks was performed choosing the three masses, among those specific for each compound, with the highest intensities as to selectively discriminate them from the nearest neighbors. The results of the peak areas were calculated on the basis of the relative calibration curve for each compound and expressed in μg/kg of oil or μg/g of fresh weight (*28*).

Enzymatic Activities. *Acetone Powders.* Eight hundred milliliters of 3:1 acetone:water (v:v) + polyethylene glycol (PEG) 2000 + PEG 20000 (0.5 g + 0.5 g) at −35 °C was added to the freeze-dried pulp (20 g), seed (10 g), and paste (30 g) (*29*). The mixture was homogenized for 2 min at 10000 rpm in an Omni-mixer (Ultra-Turrax T50) and filtered through filter paper in a Büchner funnel. The ground pulp was washed with 400 mL of ethyl ether (−35 °C), 400 mL of 1:1 methanol:acetone (v:v), 400 mL of 3:1 acetone:water (v:v) four times, and then with 400 mL of acetone; each washing step involved the homogenization in an Omni-mixer (Ultra-Turrax T50). The powder was recovered, dried under nitrogen flow for 1 h, and stored at −30 °C (*29*).

LPO. Acetonic powder (0.4 g) was suspended in 15 mL of extraction buffer (0.2 M sodium phosphate buffer at pH 7.0 containing 0.10% NaCl) and stirred for 20 min at 4 °C; this suspension was centrifuged at 12000 rpm for 20 min at 4 °C and filtered (0.45 μm). The crude extract was used for the enzymatic assays. The LPO activity was determined by measuring the increase in absorbance due to the hydroperoxides at 234 nm (*30*).

PPO. Acetonic powder (0.4 g) was suspended in 15 mL of extraction buffer (0.05 M potassium phosphate buffer at pH 6.2 containing 1 M KCl) and stirred for 30 min at 4 °C; the suspension was centrifuged at 11000 rpm for 15 min and filtered (0.45 μm). The crude extract was used for the enzymatic assays. The PPO activity was determined spectrophotometrically (*10*). POD was carried out according to the procedures reported in a previous paper (*31*).

Statistical Analysis. A priori one-way analysis of variance, using the Tukey's honest significant differences test, was performed (*32*).

RESULTS AND DISCUSSION

In the first step of the work, the distribution of phenols and the enzymatic activities of olive fruit were evaluated in the constitutive parts to study the specific contribution of each part of the drupe on the phenolic and volatile composition of the olive pastes and corresponding VOOs. As displayed in **Table 1**, the phenolic compositions of Coratina and Frantoio cultivars were strongly different in relation to the cultivar and the constitutive part of the fruit. The main differences due to the cultivar were related to the phenolic composition of the pulp; in fact, demethyloleuropein occurred exclusively in Coratina where higher concentrations in verbascoside were also observed, while the concentration of oleuropein was higher in Frantoio. Ligstroside was found only in the pulp, and it showed low concentrations, in comparison with the other secoiridoids, in the two cultivars. The pulp, in both of the cultivars, contained, as the main phenolic compounds, secoiridoids such as oleuropein, demethyloleuropein, and cinnamyl derivatives such as verbascoside. A low concentration of oleuropein and demethyloleuropein was, on the contrary, found in the stone and in the seed for both of the cultivars studied. Flavonoids such as luteolin-7-glycoside and rutin, as also reported in a previous paper, were found only in the pulp (data not shown) (*24*). The lignans such as (+)-1-acetoxypinoresinol and (+)-1-pinoresinol were detected in the pulp and in the stone. The concentration of (+)-1-acetoxypinoresinol was higher in the pulp; nevertheless, (+)-1-pinoresinol was the prevalent lignan in the stone. As shown in a previous study (*24*), the main phenolic compound of the seed was the nüzhenide, which occurred only in this part of the fruit. Anyhow, the pulp is the richest part in terms of phenols that are involved in the VOO phenolic composition (such as secoiridoids and lignans). In fact, as reported in the literature, the main secoiridoids of VOO are oleuropein and demethyoleuropein derivatives (*6*), whereas nüzhenide does not take part in the VOO phenolic composition. Furthermore, the pulp contained 93 and 97% of the total phenolic concentration of olive fruit in Frantoio and Coratina, respectively.

The distribution of PPO, POD, and LPO was analyzed in the pulp and in the seed. The results displayed in **Table 2** evidence that the PPO activity was found only in the pulp while the seed was characterized by high levels of POD activity in

Table 7. Volatile Compounds, Produced by the LPO Pathway, from Crushed and Malaxed Pastes Obtained from Whole and Stoned Frantoio and Coratina Olive Cultivars (μg/g f.w.)

	crushed paste		malaxed paste	
	traditional	stoned	traditional	stoned
		Frantoio Cultivar		
aldehydes				
2-pentenal (*E*)[a]	0.60 (0.060) a	0.79 (0.081) b	0.43 (0.045) a	0.85 (0.86) b
hexanal	5.30 (0.55) a	3.50 (0.35) b	6.79 (0.70) c	3.68 (0.41) b
2-hexenal (*E*)	37.84 (3.5) a	48.72 (4.1) a	39.80 (3.4) a	67.26 (6.2) b
2,4-hexadienal (*E*,*E*)	0.31 (0.041) a	0.60 (0.061) b	0.28 (0.026) a	0.34 (0.039) a
alcohols				
1-pentanol	0.41 (0.050) a	0.43 (0.040) a	0.81 (0.083) b	0.44 (0.043) a
2-penten-1-ol (*E*)	0.24 (0.024) a	0.31 (0.032) b	0.32 (0.036) b	0.32 (0.041) b
1-penten-3-ol	2.53 (0.25) a	3.08 (0.31) a	2.93 (0.28) a	5.86 (0.59) b
1-hexanol	0.97 (0.095) a	0.51 (0.056) b	1.04 (0.12) a	0.57 (0.060) b
3-hexen-1-ol (*E*)	0.03 (0.0040) a	0.02 (0.0023) b	0.04 (0.0044) c	0.03 (0.0038) a
3-hexen-1-ol (*Z*)	1.10 (0.15) a	0.50 (0.052) b	1.19 (0.20) a	0.77 (0.071) b
2-hexen-1-ol (*Z*)	89.30 (9.1) a	28.80 (2.6) b	141.44 (13.9) c	78.86 (7.1) a
		Coratina Cultivar		
aldehydes				
2-pentenal (*E*)[a]	1.61 (0.16) a	1.09 (0.10) b	0.81 (0.076) b	1.06 (0.13) b
hexanal	4.62 (0.46) a	4.29 (0.41) a	5.62 (0.58) a	10.69 (1.16) b
2-hexenal (*E*)	34.04 (2.0) a	41.60 (2.5) b	40.77 (2.2) b	50.84 (3.0) c
2,4-hexadienal (*E*,*E*)	0.78 (0.088) a	0.41 (0.051) b	0.28 (0.031) a	0.48 (0.051) b
alcohols				
1-pentanol	0.52 (0.047) a	0.31 (0.028) b	0.99 (0.089) c	0.42 (0.039) ab
2-penten-1-ol (*E*)	0.58 (0.061) a	0.11 (0.014) b	1.31 (0.140) c	0.10 (0.019) b
1-penten-3-ol	4.38 (0.34) ab	3.10 (0.41) a	4.87 (0.42) b	7.64 (0.81) c
1-hexanol	0.47 (0.057) a	0.70 (0.080) ab	1.71 (0.18) c	0.80 (0.082) b
3-hexen-1-ol (*E*)	0.02 (0.0016) a	0.07 (0.0072) b	0.10 (0.016) c	0.05 (0.0055) b
3-hexen-1-ol (*Z*)	1.52 (0.15) a	0.25 (0.033) b	3.00 (0.35) c	0.67 (0.069) b
2-hexen-1-ol (*Z*)	45.19 (4.1) a	18.73 (1.6) b	106.3 (9.4) c	65.87 (5.6) d

[a] Data are the mean values of three independent experiments; the standard deviation is reported in parentheses. Values in each row having different letters (a–d) are significantly different from one another at $p < 0.01$.

both of the cultivars studied. The activity of the LPO, evaluated spectrophotometrically, was found to be greater in the seed than in the pulp.

According to these results and considering the dry weight of each constitutive part of the fruit, the seed contributed 40.8 ad 58.2% of the overall the POD activity of the olive fruit, respectively, for Frantoio and Coratina, while the contribution of the seed in terms of LPO activity was lower than 20% for both of the cultivars studied. Moreover, the stone removal, before malaxation, could reduce the overall activity of POD and LPO in the pastes during processing and, as a consequence, may modify the phenolic and volatile composition of VOO. To well define this aspect, phenols and the enzymatic activity of pastes, during malaxation, in traditional and stoned pastes were studied. As reported in **Table 3**, the stoning process affects the phenolic composition of pastes in both of the cultivars analyzed, with a higher influence on the secoiridoid derivatives (such as 3,4-DHPEA-EDA) that showed a strong reduction in the malaxed pastes for both of the cultivars studied. Lignans and ligstroside aglycon, on the contrary, were less conditioned by the stone removal. These results may be explained with the selective activity of the endogenous PPO and POD of olive fruit that seem to use, as an elective substrate, the aglycon derivatives of oleuropein, such as 3,4-DHPEA-EDA, while demonstrating a lower affinity not only for the lignans but also for phenols occurring in the pastes as glucosidic forms (such as oleuropein and verbascoside). The hydrolysis of the ester bond between the 3,4-DHPEA and the elenolic acid occurring in the aglycon derivatives of oleuropein, which might be catalyzed by the endogenous esterases, can explain the increase of the 3,4-DHPEA concentration in the pastes after malaxation.

As shown in **Table 4**, the stoning process reduced the POD activity in both of the cultivars, while no significant differences were found for the PPO activity. As observed for the distribution of PPO in the constitutive parts of fruit, results for the Coratina confirm lower PPO and POD activities in comparison to Frantoio. After malaxation, a partial reduction of PPO and POD was found in the pastes of both of the cultivars studied. It is possible to suppose that the tanning reactions, happening between proteins and phenols, which can involve structural and enzymatic proteins, may explain the partial inactivation of PPO and POD observed in the malaxed pastes (*33*). The analysis of the phenolic composition of the oil samples (**Table 5**) pointed out that the VOO extracted from the stoned fruits contained higher concentrations of secoiridoid derivatives such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA, mainly in the Coratina cultivar, while no significant differences were observed for the lignans in both of the cultivars studied. The aglycon derivative of ligustroside (*p*-HPEA-EDA) also shows significant modifications. These experimental data stress that the phenolic composition of VOO is strongly affected by the activity of the PPO and POD and confirm the selective activity of these enzymes for the aglycon derivatives of oleuropein and the demethyl-oleuropein. The insignificant modifications observed for the lignan's concentration in VOO can be due to the lower affinity of the PPO and POD for those substances but, at the same time, prove that the stone has a minimal contribution in the lignan's concentration of VOO. This result is in agreement with the low level of lignan's release from the stone detected in the pastes during malaxation (**Table 3**) but, at the same time, seems to be in contrast to the high concentration of (+)-pinoresinol found in this part of the olive fruit (**Table 1**). With regard to this, it is possible to suppose that lignans occurring in the stone may be linked to the lignin and cellulose structures of the stone and, consequently, are not able to be released in pastes and in the corresponding oil during malaxation. The results related to the

Table 8. Volatile Compounds (μg/kg) in Oils Obtained by Mechanical Extraction with Traditional Process and with Stoning from Frantoio and Coratina Cultivars

	traditional	stoned
	Frantoio Cultivar	
aldehydes		
2-pentenal (*E*)[a]	39.6 (2.5) a	35.7 (2.7) a
hexanal	834.0 (37.9) a	748.4 (27.9) b
2-hexenal (*E*)	24006.0 (650.5) a	27866.2 (705.5) b
2,4-hexadienal (*E*,*E*)	200.3 (22.3) a	256.5 (12.1) b
alcohols		
1-pentanol	95.5 (4.9) a	20.8 (1.7) b
2-penten-1-ol (*E*)	36.3 (1.5) a	12.8 (2.1) b
1-penten-3-ol	285.9 (10.7) a	114.5 (4.6) b
1-hexanol	1457.0 (50.4) a	405.0 (18.4) b
3-hexen-1-ol (*E*)	44.7 (1.8) a	21.5 (2.3) b
3-hexen-1-ol (*Z*)	243.1 (18.8) a	163.7 (8.0) b
2-hexen-1-ol (*Z*)	19912.0 (705.9) a	6446.1 (205.9) b
	Coratina Cultivar	
aldehydes		
2-pentenal (*E*)	33.1 (1.0) a	49.4 (1.7) b
hexanal	877.5 (68.5) a	698.6 (57.0) b
2-hexenal (*E*)	24083.5 (834.1) a	33362.1 (964.3) b
2,4-hexadienal (*E*,*E*)	258.4 (33.0) a	385.4 (17.9) b
alcohols		
1-pentanol	98.6 (4.4) a	17.2 (1.1) b
2-penten-1-ol (*E*)	22.8 (0.8) a	23.0 (2.4) a
1-penten-3-ol	335.8 (15.5) a	324.7 (20.4) a
1-hexanol	1495.1 (66.5) a	348.5 (10.5) b
3-hexen-1-ol (*E*)	37.2 (2.2) a	7.1 (0.4) b
3-hexen-1-ol (*Z*)	252.4 (14.3) a	212.9 (16.5) b
2-hexen-1-ol (*Z*)	16913.9 (674.8) a	3529.4 (742.6) b

[a] Data are the mean values of three independent experiments; the standard deviation is reported in parentheses. Values in each row having different letters (a–b) are significantly different from one another at $p < 0.01$.

phenolic modifications of VOO with the olive stoning are in agreement with other authors that studied the positive effect of the stoning process in the overall phenolic composition of VOO evaluated colorimetrically (*22*, *34*) or in the secoiridois derivatives (*21*, *35*), but it is the first time that all of the main classes of phenols occurring in VOO, which includes not only the secoiridoids but also the lignans, were studied together to explain why this new technology can affect the occurrence of these compounds in the oil.

The stone removal, in conclusion, produces a selective increase of the phenolic content of VOO affecting mainly the concentration of secoiridoid derivatives such as 3,4-DHPEA-EDA, *p*-HPEA-EDA, and 3,4-DHPEA-EA. For that reason, because of the biological properties of those compounds, the stoning process could be considered as an opportunity, from the technological standpoint, to improve health-related properties of VOO. In fact, as reported in many papers, the aglycon derivatives of oleuropein, demethyoleuropein, and ligstroside are involved in cancer prevention and in the reduction of the risk of cardiovascular diseases (*6*, *36*).

To investigate the effect of the stoning removal on the genesis of volatile compounds in the olive pastes and in VOO, the HS analysis of the volatile composition of crushed seed and pulp was performed by HS-SPME/GC-MS. The outcome was that the seed produced lower concentrations of volatile compounds coming from the LPO pathway in comparison to the pulp (**Table 6**). Furthermore, the most interesting results were related to the relationship between the composition of C_6 unsaturated aldehydes and C_6 alcohols found in the crushed seed and pulp. In fact, as shown in **Table 6**, the concentrations of C_6 unsaturated aldehydes such as 2-hexenal (*E*) were higher in the HS of the crushed pulp, in both of the cultivars studied; the crushed seed HS, on the contrary, was richer in C_6 alcohols. These results are particularly important because they would demonstrate that the enzymes involved in the LPO pathway have a different activity in the pulp and in the seed. In particular, the seed seems to have a lower hydroperoxide lyase activity and a higher alcohol dehydrogenase activity in comparison to the pulp, confirming the results obtained by Luaces et al. (*37*) where the presence of the stone during processing significantly affected the C_6 compound concentrations. To study the effect of the stoning process in the aroma production by the LPO pathway during malaxation, the HS compositions of crushed and malaxed pastes obtained by traditional and stoned olives were evaluated. The results reported in **Table 7** confirm the previous findings: In fact, the concentration of the C_6 unsaturated aldehydes was higher in the stoned crushed and malaxed pastes for both of the cultivars studied, while the concentration of the C_6 unsaturated alcohols [such the 2-hexen-1-ol (*Z*), 3-hexen-1-ol (*Z*), and 1-hexen 1-ol] was significantly higher in the crushed and malaxed traditional pastes in Frantoio and Coratina cultivars. The malaxation, moreover, increases the volatile concentration in the pastes, showing that the enzymes involved in the LPO pathway remain active during the process. The HS composition of the VOOs produced by the extraction process from traditional and stoned olive pastes, reported in **Table 8**, was in agreement with the results reported above. The amount of C_6 unsaturated aldehydes was greater in the VOOs obtained from the stoning process, while the seed presence in the traditional extraction process, in both of the cultivars studied, increased the concentration of C_6 alcohols. These results are in agreement with Angerosa et al. (*38*) who, working on the Coratina cultivar and considering three subsequent malaxation times, concluded that the concentration of the C_6 saturated and unsaturated aldehydes was higher in VOO extracted from stoned olives. Similar results in terms of a higher amount of C_6 unsaturated aldehydes in the stoned oil were obtained by Amirante et al. (*22*), while the data reported by Patumi et al. (*39*) seem to be in contrast with all of these findings. These authors, in fact, working on five Sicilian olive cultivars, concluded that the stone removal does not modify the volatile and phenolic composition of VOO. These conflicting results could be explained with the cultivar effect. In fact, as observed by Lavelli and Bondesan (*21*) for the phenolic composition and, as confirmed by our results that also include the volatile compounds, the stoning impact in VOO composition is strongly dependent on the cultivars that are genetically differentiated in terms of the phenolic concentrations and enzymatic activities of fruit. The modification of C_6 unsaturated aldehydes and alcohols can affect the sensory properties of VOO since, as reported in the literature (*5*), the C_6 unsaturated aldehydes are potent odorants of VOO strictly related to the cut-grass sensory note.

In conclusion, the overall results reported in this paper show that the stoning process modifies the activity of PPO, POD, and LPO in the olive pastes during malaxation, thus affecting volatile and phenolic compositions of VOO.

ABBREVIATIONS USED

VOO, virgin olive oil; PPO, polyphenoloxidase; POD, peroxidase; LPO, lipoxygenase; 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (3,4-dihydroxyphenyl)ethanol; *p*-HPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (*p*-hydroxypheny1)ethanol; 3,4-DHPEA-EA, isomer of the oleuropein aglycon; 3,4-DHPEA, (3,4-dihydroxyphenyl)ethanol; *p*-HPEA, (*p*-hydroxyphenyl)-ethanol; HPLC, high-performance liquid chromatography; NMR,

nuclear magnetic resonance; DIECA, sodium diethylditiocarbamate; SPE, solid-phase extraction; DAD, diode array detector; FLD, fluorescence detector; SPME, solid-phase microextraction; GC-MS, gas chromatography−mass spectrometry; EFC, electronic flow controller; EI, electron ionization; PEG, polyethylene glycol; HS, head space.

ACKNOWLEDGMENT

We thank Roberto Santibacci, Sergio Falaluna, and Michele Giglioni for the technical contribution.

LITERATURE CITED

(1) Guth, H.; Grosch, W. Quantitation of potent odorants of virgin olive oil by stable-isotope dilution assays. *J. Am. Oil Chem. Soc*. **1993**, *70*, 513−518.

(2) Reiners, J.; Grosch, W. Odorants of virgin olive oils with different flavor profiles. *J. Agric. Food Chem*. **1998**, *46*, 2754−2763.

(3) Aparicio, R.; Morales, M. T. Characterization of olive ripeness by green aroma compounds in virgin olive oil. *J. Agric. Food Chem*. **1998**, *46*, 1116−1122.

(4) Servili, M.; Conner, J. M.; Piggott, J. R.; Withers, S. J.; Paterson, A. Sensory characterization of virgin olive oil and relationship with headspace composition. *J. Sci. Food Agric*. **1995**, *67*, 61−70.

(5) Angerosa, F.; Servili, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G. Volatile compounds in virgin olive oil: Occurrence and their relationship with the quality. *J. Chromatogr. A* **2004**, *1054*, 17−31.

(6) Servili, M.; Selvaggini, R.; Esposto, S.; Taticchi, A.; Montedoro, G.; Morozzi, G. Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil. *J. Chromatogr. A* **2004**, *1054*, 113−127.

(7) Angerosa, F.; d'Alessandro, N.; Basti, C.; Vito, R. Biogeneration of volatile compounds in virgin olive oil: Their evolution in relation to malaxation time. *J. Agric. Food Chem*. **1998**, *46*, 2940−2944.

(8) Angerosa, F.; d'Alessandro, N.; Di Girolamo, M.; Vito, R.; Serraiocco, A. Relationship between aroma components and malaxation time. *Acta Hortic*. **1999**, *474*, 621−625.

(9) Di Giovacchino, L.; Costantini, N.; Ferrante, M. L.; Serraiocco, A. Influence of malaxation time of olive paste on oil extraction yields and chemicals and organoleptic characteristics of virgin olive oil obtained by a centrifugal decanter at water saving. *Grasas Aceites* **2002**, *53*, 179−186.

(10) Sciancalepore, V. Enzymatic browning in five olive varieties. *J. Food Sci.* **1988**, *50*, 1194−1195.

(11) Servili, M.; Baldioli, M.; Begliomini, A. L.; Selvaggini, R.; Montedoro, G. F. The phenolic and volatile compounds of virgin olive oil: Relationships with the endogenous oxidoreductases during the mechanical oil extraction process. In *Flavour and Fragrance Chemistry*; Proceedings of the Phytochemical Society of Europe, Campobasso, Italy, January 13−16 2000; Lanzotti, V., Taglialatela-Scafati, O., Eds.; Kluwer Academic: Dordrecht, The Netherlands, 2000; pp 163−173.

(12) Olías, J. M.; Perez, A. G.; Rios, J. J.; Sanz, L. C. Aroma of virgin olive oil: Biogenesis of the "green" odour notes. *J. Agric. Food Chem*. **1993**, *4*, 2368−2373.

(13) Morales, M. T.; Angerosa, F.; Aparicio, R. Effect of the extraction conditions of virgin olive oil on the lipoxygenase cascade: Chemical and sensory implications. *Grasas Aceites* **1999**, *50*, 114−121.

(14) Servili, M.; Baldioli, M.; Montedoro, G. F. Phenolic composition of virgin olive oil in relationship to some chemical and physical aspects of malaxation. *Acta Hortic*. **1994**, *1*, 331−336.

(15) Angerosa, F.; Mostallino, R.; Basti, C.; Vito, R. Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chem.* **2001**, *72*, 19−28.

(16) Di Giovacchino, L.; Sestili, S.; Di Vincenzo, D. Influence of olive processing on virgin olive oil quality. *Eur. J. Lipid Sci. Technol*. **2002**, *104*, 587−601.

(17) Servili, M.; Selvaggini, R.; Baldioli, M.; Begliomini, A. L.; Taticchi, A.; Esposto, S.; Montedoro, G. F. Effect of the time of exposure to the air contact of olive pastes during mechanical extraction process on the volatile and phenolic compounds of virgin olive oil. *Riv. Ital. Sostanze Grasse* **2002**, *79*, 439−441.

(18) García, A.; Brenes, M.; Martínez, F.; Alba, J.; García, P.; Garrido, A. High-performance liquid chromatography evaluation of phenols in virgin olive oil during extraction at laboratory and industrial scale. *J. Am. Oil Chem. Soc.* **2001**, *78*, 625−629.

(19) Servili, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G. F. Air exposure time of olive pastes during the extraction process and phenolic and volatile composition of virgin olive oil. *J. Am. Oil Chem. Soc*. **2003**, *80*, 685−695.

(20) Servili, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G. Volatile compounds and phenolic composition of virgin olive oil: Optimization of temperature and time of exposure of olive pastes to air contact during the mechanical extraction process. *J. Agric. Food Chem*. **2003**, *51*, 7980−7988.

(21) Lavelli, V.; Bondesan, L. Secoiridoids, tocopherols, and antioxidant activity of monovarietal extra virgin olive oils extracted from destoned fruits. *J. Agric. Food Chem.* **2005**, *53*, 1102−1107.

(22) Amirante, P.; Clodoveo, M. L.; Dugo, G.; Leone, A.; Tamborrino, A. Advance technology in virgin olive oil production from traditional and de-stoned pastes: Influence of the introduction of a heat exchanger on oil quality. *Food Chem.* **2006**, *98*, 797−805.

(23) Pannelli, G.; Servili, M.; Selvaggini, R.; Baldioli, M.; Montedoro, G. F. Effect of agronomic and seasonal factors on olive (*Olea europaea* L.). Production and the qualitative characterization of the oil. *Acta Hortic.* **1994**, *356*, 239−243.

(24) Servili, M.; Baldioli, M.; Selvaggini, R.; Macchioni, A.; Montedoro, G. F. Phenolic compounds of olive fruit: One- and two-dimensional nuclear magnetic resonance characterization of nüzhenide and its distribution in the constitutive parts of fruit. *J. Agric. Food Chem.* **1999**, *47*, 12−18.

(25) Montedoro, G. F.; Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterization of the secoiridoids derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228−2234.

(26) Montedoro, G. F.; Servili, M.; Baldioli, M.; Miniati, E. Simple and hydrolyzable compounds in virgin olive oil. 1. Their extraction, separation and quantitative and semiquantitative evaluation by HPLC. *J. Agric. Food Chem*. **1992**, *40*, 1571−1576.

(27) Selvaggini, R.; Servili, M.; Urbani, S.; Esposto, S.; Taticchi, A.; Montedoro, G. F. Evaluation of phenolic compounds in virgin olive oil by direct injection in high-performance liquid chromatography with fluorometric detection. *J. Agric. Food Chem*. **2006**, *54*, 2832−2838.

(28) Servili, M.; Selvaggini, R.; Taticchi, A.; Montedoro, G. F. Headspace composition of virgin olive oil evaluated by solid phase microextraction: Relationships with the oil sensory characteristics. In *Food Flavors and Chemistry*; Spanier, A. H., Shahidi, F., Parliament, T. H., Mussiman, C., Ho, C. T., Tratras, E., Contis, Eds.; The Royal Society of Chemistry: Cambridge, United Kingdom, 2001; pp 236−247.

(29) Montedoro, G. F.; Baldioli, M.; Selvaggini, R.; Begliomini, A. L.; Taticchi, A.; Servili, M. Relationships between phenolic composition of olive fruit and olive oil: The importance of the endogenous enzymes. *Acta Hortic*. **2002**, *586*, 551−556.

(30) Bonnet, J. L.; Crouzet, J. Lipoxygenase from tomato fruit: Partial purification and study of some properties. *J. Food Sci*. **1977**, *42* (3), 625−628.

(31) Begliomini, A. L.; Montedoro, G. F.; Servili, M.; Petruccioli, M.; Federici, F. Oxidoreductases from tomato fruit: Inhibitory effect of fungal glucose oxidase. *J. Food Biochem*. **1995**, *19*, 161−173.
(32) *Statgraphics*, version 6; Manugistics, Inc.: Rockville, MA, 1992.
(33) Shahidi, F.; Naczk, M. Nutritional and pharmacological effects of food phenolics. In *Food Phenolics*; Tecnomic Publishing Co.: Lancaster, 1995; pp 171−179.
(34) Del Caro, A.; Vacca, V.; Poiana, M.; Fenu, P.; Piga, A. Influence of technology, storage and exposure on components of extra virgin oil (Bosana *cv*) from whole and de-stoned fruits. *Food Chem.* **2006**, *98*, 311−316.
(35) Mulinacci, N.; Giaccherini, C.; Innocenti, M.; Romani, A.; Vincieri, F. F.; Marotta, F.; Mattei, A. Analysis of extra virgin olive oils from stoned olives. *J. Sci. Food Agric.* **2005**, *85*, 662−670.
(36) Fitó, M.; Covas, M. I.; Lamuela-Raventos, R. M.; Vila, J.; Torrents, J.; de la Torre, C.; Marrugat, J. Protective effect of olive oil and its phenolic compounds against low density lipoprotein oxidation. *Lipids* **2000**, 35, 633−638.
(37) Luaces, P.; Pérez, A. G.; Sanz, C. Role of olive seed in the biogenesis of virgin olive oil aroma. *J. Agric. Food Chem.* **2003**, *51*, 4741−4745.
(38) Angerosa, F.; Basti, C.; Vito, R.; Lanza, B. Effect of fruit stone removal on the production of virgin olive oil volatile compounds. *Food Chem*. **1999**, *67*, 295−299.
(39) Patumi, M.; Terenziani, S.; Ridolfi, M.; Fontanazza, G. Effect of fruit stoning on olive oil quality. *J. Am. Oil Chem. Soc.* **2003**, *80*, 249−255.

Received for review March 1, 2007. Revised manuscript received June 11, 2007. Accepted June 13, 2007. This research was supported by VALOROLIO and RIOM projects founded by the Italian MIPAF (Ministry of Agriculture and Forestry).

JF070600I