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Influence of the Decrease in Oxygen during Malaxation of Olive Paste on the Composition of Volatiles and Phenolic Compounds in Virgin Olive Oil

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The sensory and health properties of virgin olive oil (VOO) are highly related to its volatile and phenolic composition. Oxygen control in the pastes during malaxation may be a new technological parameter to regulate enzymatic activities, such as polyphenoloxidase, peroxidase, and lipoxygenase, which affect the phenolic and volatile composition of VOO. In this work, we monitored CO_2 and O_2 concentrations during industrial-scale olive paste malaxation with various initial O_2 concentrations within the malaxer headspace. Results show that the O_2 concentration in the malaxer headspace did not affect CO_2 production during processing, whereas a strong influence was observed on the changes of the phenolic composition of olive pastes and VOOs, with high correlation coefficient for the total phenols ($R = 0.94$), especially for oleuropein and demethyloleuropein derivatives ($R = 0.81$). In contrast, aroma production during malaxation was minimally affected by the O_2 concentration in the malaxer headspace.

KEYWORDS: Virgin olive oil; malaxation; CO_2; O_2; phenolic compounds; volatile compounds

INTRODUCTION

Phenolic and volatile compounds can be considered important markers of virgin olive oil (VOO) quality. The main class of phenols is composed of derivatives of secoiridoids and lignans other than phenolic alcohols, phenolic acids, and flavonoids. Secoiridoids have biological activity, particularly in cardiovascular diseases and cancer prevention (*1–5*) and in the improvement of the shelf life of the oil. In addition, secoiridoids (including the aglycon derivatives of oleuropein, demethyloleuropein, and ligustroside) are the impact compounds that define the "pungent" and "bitter" taste of VOO. The concentration of those compounds in VOO is largely affected by the enzymatic degradative activity of polyphenoloxydases (PPOs) and peroxidases (PODs) during the mechanical extraction process; in particular, one of the most critical points is malaxation (*1*), the operation of slow mixing of the olive crushed pastes in order to promote the oil droplets' coalescence and improve the separation efficiency by the subsequent centrifugation. Malaxation conditions also affect the lipoxygenase (LPO) pathway that produces C_5 and C_6 saturated and unsaturated aldehydes, alcohols, and esters and therefore regulate the intensity of some typical sensory notes, such as "cut grass," "haylike", and "floral" (*6*).

During the last ten years, the scientific research has contributed to the understanding of the technological parameters implicated in the PPO, POD, and LPO activities. The role of the time and temperature of malaxation on the degradation of secoiridoid derivatives has been well investigated (*7–10*). Subsequent works have demonstrated that inhibition of PPO and POD may be achieved by reducing the O_2 level in the paste by the introduction of an inert gas during malaxation (*11, 12*). So far, however, only a few papers have reported on the relationship between oxygen control during malaxation and the phenolic and volatile composition of VOO (*13*).

Technological innovations built on those findings through the introduction of new malaxers covered with a top, sometimes equipped with a valve for an inert gas entry. Recent studies (*14, 15*) have demonstrated that, in laboratory scale and in sealed malaxing conditions, CO_2 is emitted from olive pastes during malaxation while O_2 is depleted. Such behavior is attributed to respiratory metabolism and is recognized in fruits during the postharvest period; furthermore, the respiration rate is affect by several factors, such as the olive variety and its ripening stage other than storage conditions (*16, 17*). This effect is enhanced by tissue damage (*18, 19*) but is relatively unknown in olive pastes.

With respect to the sensory and health properties of VOO, understanding the role of O_2 availability in the production of CO_2 and on the control of oxidoreductase activity would

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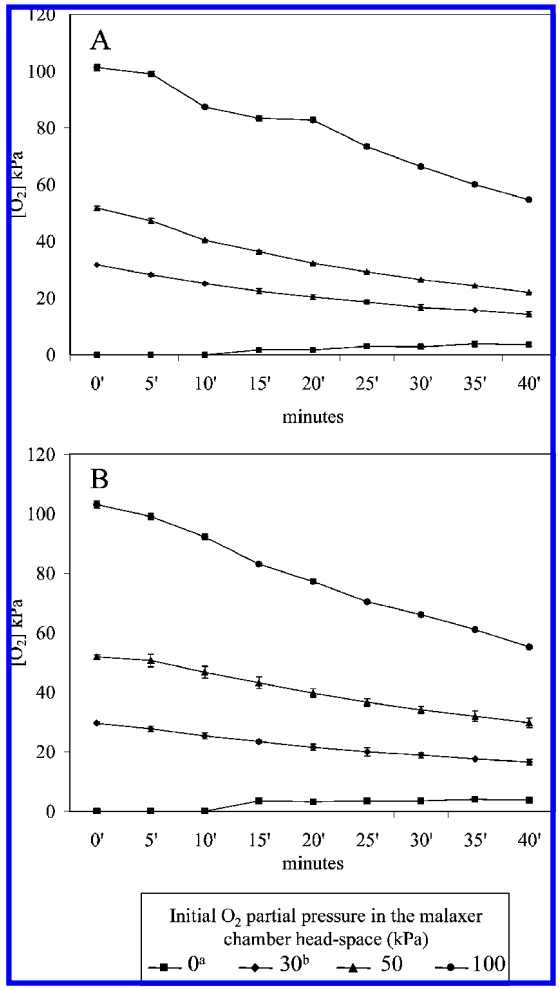

Figure 1. Change in the O_2 concentration in the malaxer chamber headspace during olive paste malaxation in different initial atmosphere composition, in Ogliarola (**A**) and Coratina (**B**) cultivars. Results are the mean values of three independent experiments ([a]saturated with N_2; [b]corresponding to the air composition).

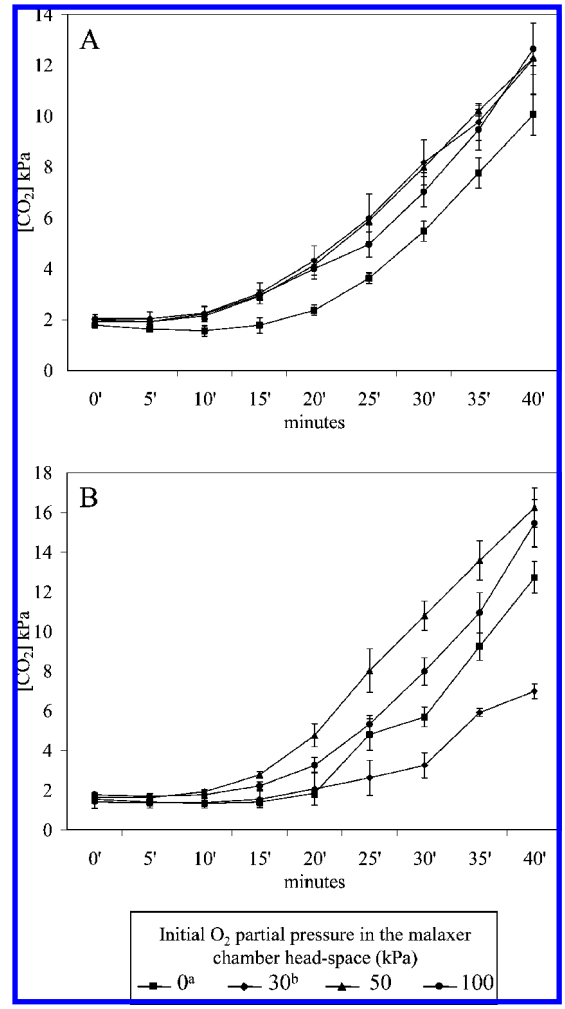

Figure 2. Change in the CO_2 concentration in the malaxer chamber headspace during the olive paste malaxation in different initial atmosphere composition, in Ogliarola (**A**) and Coratina (**B**) cultivars. Results are the mean values of three independent experiments ([a]saturated with N_2; [b]corresponding to the air composition).

represent an important step in defining the technological parameters aimed at improving VOO quality. In this paper, the effects of oxygen levels in the malaxer chamber headspace on the CO_2 production and on the phenolic and volatile composition of olive pastes and VOOs were investigated on an industrial scale.

MATERIALS AND METHODS

Olives. Drupes of the Coratina and Ogliarola cultivars, harvested during the year 2006, were used. The ripening stages of the green olives (evaluated by the pigmentation index, according to Pannelli et al. *20*) were 0.93 and 0.98 for Coratina and Ogliarola cultivars, respectively.

Reference Compounds. (3,4-Dihydroxyphenyl)ethanol (3,4-DHPEA) was obtained from Cayman Chemicals Ltd. (Ann Arbor, MI), and the (*p*-hydroxyphenyl)ethanol (*p*-HPEA) was obtained from Janssen Chemical Co. (Beerse, Belgium). Oleuropein glucoside was purchased from Extrasynthèse (France). Demethyloleuropein and verbascoside were extracted from olive fruit according to the procedure reported in a previous paper (*21*). Briefly, the phenols were extracted from the freeze-dried olive pulps (5 g) 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), (+)-1-acetoxypinoresinol, and (+)-pinoresinol were extracted from VOO using a procedure previously reported (*22*). 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 separation of phenols was carried out 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. Phenol detection was performed using a diode array detector (DAD) (*22*). The purity of all the substances obtained from direct extraction was tested by HPLC, and their chemical structures were verified by NMR using the same operative conditions reported in previous papers (*21, 22*). Pure analytical standards of volatile compounds were purchased from Fluka and Aldrich (Milan, Italy).

Virgin Olive Oil Mechanical Extraction Process. The experiments were conducted on an industrial scale using a Rapanelli SPA industrial implant. Each extraction was performed on a sample of 150 kg of olives. For the crushing operation, a hammer crusher mod. GR 32 (Rapanelli Fioravante S.p.a., Foligno, Italy) was employed. Malaxation was carried out at 25 °C for 40 min. The malaxing machine (Rapanelli S.p.A, Foligno, Italy) was top-covered, equipped with two valves for O_2 and N_2 entry and two sensors for the measurement of oxygen and carbon dioxide in the malaxer headspace (METTLER TOLEDO mod. O_2 4100; METTLER TOLEDO mod. CO_2 5100). Four trials of sealed malaxation conditions were performed: trial 1, N_2 atmosphere without oxygen; trial 2, control, with normal atmosphere composition (O_2 = 30 kPa); and trials 3 and 4, with initial oxygen partial pressures of 50 and 100 kPa, respectively, in the headspace of the malaxer chamber. For each trial, the filling volume in the malaxer was identical, leaving 68 L of

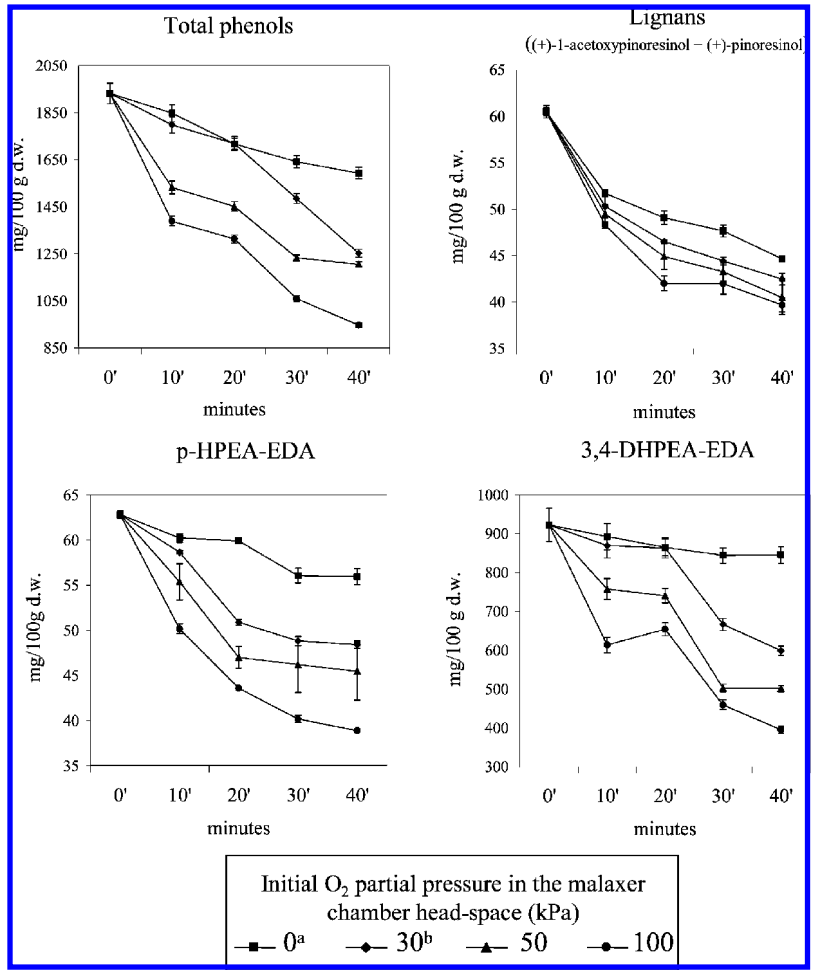

Figure 3. Change in the phenolic composition of the olive pastes during malaxation in different initial atmosphere composition, in the Ogliarola cultivar. Total phenols are reported as the sum of the phenolic fractions (3,4-DHPEA; *p*-HPEA; demethyloleuropein; verbascoside; 3,4-DHPEA-EDA; oleuropein; *p*-HPEA-EDA; (+)-1-acetoxypinoresinol; (+)-pinoresinol). Results are the mean values of three independent experiments ([a]saturated with N_2; [b]corresponding to the air composition).

headspace. The oxygen and carbon dioxide concentrations were measured in the malaxer chamber headspace and recorded every 5 min. Oil separation was obtained using a three phase decanter at low water addition (0.2:1 v:w), RAMEF mod. 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. To evaluate the phenolic composition of the olive pastes and their modifications during malaxation, paste samples at the beginning (after crushing), every 10 min, and at the end of the malaxing process were collected, immediately frozen in liquid nitrogen, and stored at - 80 °C until analysis.

Analytical Methods. *Extraction and HPLC Analysis of Phenolic Compounds of Olive Pastes.* The phenolic extraction from crushed and malaxed olive pastes was carried out according to a modification of the procedure previously published by Servili et al. (*21*). Olive paste (5 g) was homogenized with 100 mL of 80% methanol containing 20 mg/L sodium diethyldithiocarbamate (DIECA); the extraction was performed in triplicate. After methanol removal, the aqueous extract was used for SPE phenol separation. The SPE procedure was applied, loading with 2 mL of the aqueous extract a 5 g/25 mL Extraclean highload C18 cartridge (Alltech Italia S.r.l., Sedriano, Italy). Methanol (200 mL) was used as the eluting solvent. After removing the solvent 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 mod. 1100 composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, a DAD, and a FLD. For evaluation of the phenolic compounds (*23*), a Spherisorb column ODS-1 250 mm × 4.6 mm with a particle size of 5 μm (Phase Separation Ltd., Deeside, U.K.) thermostatted at 25 °C was employed, and 20 μL of sample volume was injected. 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 for 8 min, 60% A/40% B for 10 min, 50% A/50% B for 16 min, and 0% A/100% B for 14 min. This composition was maintained for 10 min, then was returned to the initial conditions and equilibration for 13 min; the total running time was 73 min. Lignans were detected by FLD operated at an excitation wavelength of 280 nm and emission at 339 nm (*23*), while the other compounds were detected by DAD with a wavelength of 278 nm.

Extraction and HPLC Analysis of Phenolic Compounds of Virgin Olive Oil. The extraction of VOO phenols was performed, as reported by Montedoro et al. (*24*). The HPLC analyses of phenolic extracts were conducted with the same equipment reported above. 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, U.K.); 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 detection of all the phenolic compounds, a DAD was employed with a wavelength of 278 nm (*23*).

Volatile Compounds. To evaluate the production of volatile compounds by LPO pathway in crushed and malaxed pastes, 2 g of the pastes were placed in a 10 mL vial containing 2 mL of a $CaCl_2$ saturated solution as enzymatic inhibitor and stored at −20 °C. To evaluate the volatile compounds in VOO, 3 g of oil were placed into a 10 mL vial. For the sampling of the headspace volatile compounds, solid-phase microextraction (SPME) was applied as follows: all 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

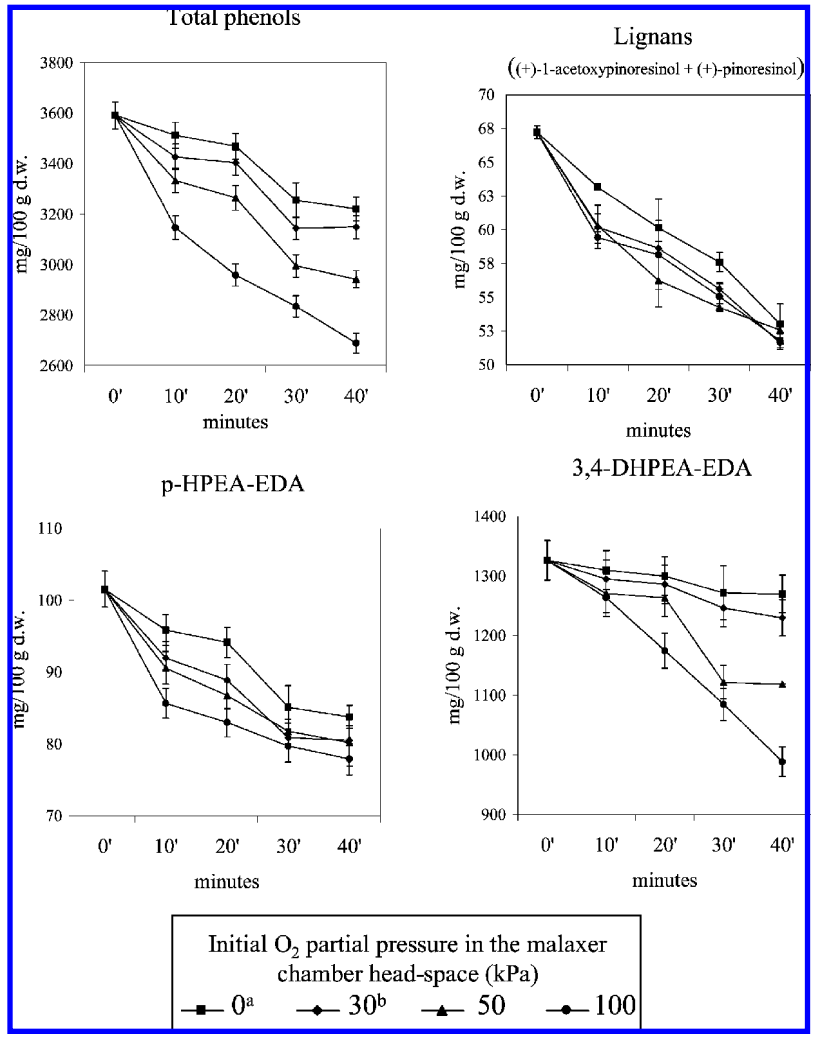

Figure 4. Change in the phenolic composition of the olive pastes during malaxation in different initial atmosphere composition, in the Coratina cultivar. Total phenols are reported as the sum of the phenolic fractions (3,4-DHPEA; *p*-HPEA; demethyloleuropein; verbascoside; 3,4-DHPEA-EDA; oleuropein; *p*-HPEA-EDA; (+)-1-acetoxypinoresinol; (+)-pinoresinol). Results are the mean values of three independent experiments ([a]saturated with N_2; [b]corresponding to the air composition).

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 through the Varian CP 8410 Autoinjector (Varian, Walnut Creek, CA).

GC-MS Analysis. A GC-MS Varian 4000 equipped with a 1079 split/splitless injector (Varian, Walnut Creek, CA) was used. A fused-silica capillary column DB-Wax-ETR, 50 m, 0.32 mm ID, 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 all 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; this temperature was maintained 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 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, Walnut Creek, CA). The volatile compounds were identified by comparison of their mass spectra and retention times with those of authentic reference compounds. Integration of all of the chromatographic peaks was performed choosing the three masses with the highest intensities from among those specific for each compound 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 micrograms per kilogram of oil or micrograms per gram of fresh weight (*25*).

Statistical Analysis. A priori one way ANOVA, using the Tukey's honest significant differences test was run. Correlation coefficients (R) and significance levels (p) were calculated between the change in O_2 concentration in the malaxer headspace during malaxation (difference between the final and the initial concentration) and the changes of the concentration of the CO_2 or phenols or volatile compounds in the olive pastes, with respect to all four trials performed in triplicate for both the olive cultivars (number of observations = 24). All the above-reported statistical tests were run using Statgraphics software, version 6 (Manugistics, Inc., Rockville, MA) (*26*).

RESULTS AND DISCUSSION

As shown in a previous paper (*14*), O_2 consumption may be related to CO_2 production during malaxation. For this reason, the concentrations of O_2 and CO_2 were analyzed. As displayed in **Figure 1**, the O_2 concentration decreases according to its initial level during malaxation, with the only exception for the trial at 0 kPa of O_2, where there is a slight increase, probably due to the partial release of oxygen absorbed by the pastes during the crushing operation. However, the CO_2 production was independent from the O_2 consumption. In fact, as shown

Table 1. Correlation Coefficients (*R*) and Significance Levels (*p*) between the Change in O_2 Concentration (Difference between the Final and the Initial Concentration) in the Malaxer Headspace during Malaxation and the Change in the Concentration of Phenolic and Volatile Compounds in the Olive Pastes

	R	*p*[a]
phenolic compd		
3,4-DHPEA	−0.705	0.051
p-HPEA	−0.671	0.068
demethyloleuropein	0.181	0.668
verbascoside	0.749	0.033
3,4-DHPEA-EDA	0.811	0.000
oleuropein	0.618	0.102
p-HPEA-EDA	0.596	0.024
(+)-1-acetoxypinoresinol	0.580	0.131
(+)-pinoresinol	0.453	0.260
sum of phenols	*0.941*	*0.000*
volatile compd		
(*E*)-2-pentenal	−0.057	0.846
hexanal	−0.013	0.964
(*E*)-2-hexenal	−0.104	0.722
sum of aldehydes	*−0.105*	*0.721*
1-pentanol	−0.513	0.061
(*E*)-2-penten-1-ol	−0.393	0.165
1-penten-3-ol	−0.417	0.138
1-hexanol	0.152	0.598
(*E*)-3-hexen-1-ol	0.130	0.657
(*Z*)-3-hexen-1-ol	−0.079	0.789
(*E*)-2-hexen-1-ol	−0.024	0.935
sum of alcohols	*−0.071*	*0.810*
$n = 24$		

[a] *p* Values below 0.05 indicate a statistically significant correlation at the 95% confidence level.

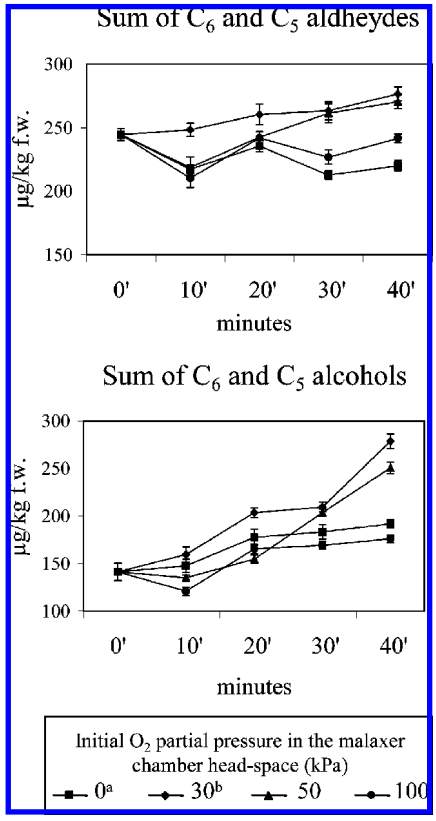

Figure 5. Change in the composition of volatile compounds in the malaxer headspace during olive paste malaxation under different initial atmosphere composition, in the Ogliarola cultivar. C_6 and C_5 aldheydes: hexanal, (*E*)-2-hexenal, and (*E*)-2-pentenal. C_6 and C_5 alcohols: 1-hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, 1-pentanol, (*E*)-2-penten-1-ol, and 1-penten-3-ol. Results are the mean values of three independent experiments ([a]saturated with N_2; [b]corresponding to the air composition).

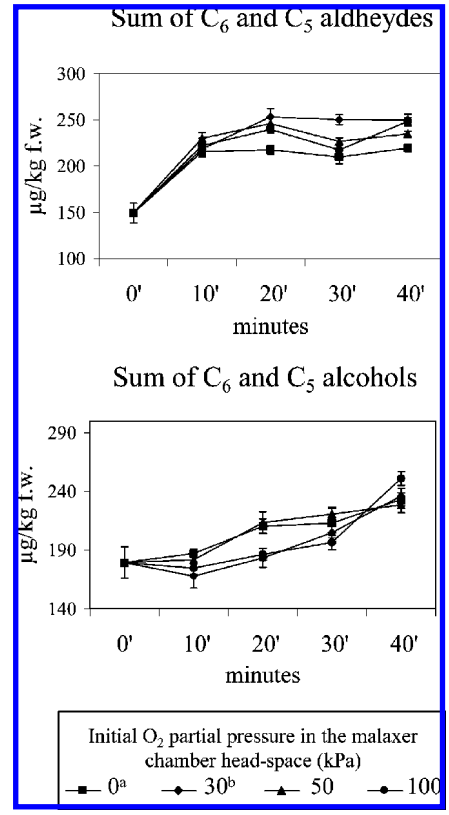

Figure 6. Change in the composition of volatile compounds in the malaxer headspace during olive paste malaxation under different initial atmosphere composition, in the Coratina cultivar. C_6 and C_5 aldheydes: hexanal, (*E*)-2-hexenal, and (*E*)-2-pentenal. C_6 and C_5 alcohols: 1-hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, 1-pentanol, (*E*)-2-penten-1-ol, and 1-penten-3-ol. Results are the mean values of three independent experiments ([a]saturated with N_2; [b]corresponding to the air composition).

in **Figure 2**, while the absolute values of CO_2 concentration are slightly different, the kinetics of CO_2 increase during malaxation; in terms of trend among the four trials, kinetics were quite similar for all the O_2 concentrations tested, including the assay performed under N_2. In this context, no significant differences in terms of trend were observed between Ogliarola and Coratina cultivars. These results seem to be in contrast with the observations of Parenti et al. (*15*) that show a correlation between O_2 consumption and CO_2 production in laboratory-scale experiments. That paper reports a correlation between the O_2 and CO_2 concentrations during malaxation, expressed as percentages in the overall headspace, but this study evaluated only one oxygen level. The present research represents the first study of CO_2 emission evaluated with different initial O_2 concentrations. Respiration or a fermentation process performed by cells of the different tissues constituting the olive fruit may explain the CO_2 production during processing. It is more likely however that this gas accumulated in the intercellular of the olive during respiration and was released when the tissues were disrupted during the crushing operation (*19*). Observed for the first time, the most important evidence for that hypothesis is that CO_2 emission is not related to O_2 consumption; in fact, the quantities of CO_2 emitted seem not to be proportional to the O_2 availability and depletion during malaxation, and thus, the correlation coefficient (*R*) between the changes in O_2 and CO_2 concentrations in the malaxer headspace during malaxation was -0.409 ($p > 0.05$). However, the exhaustive comprehension of the phoenomena at the basis of the CO_2 emission from the

Table 2. Phenolic Composition (mg/kg) of the Oils Obtained after Malaxation in Different Initial Atmosphere Compositions[c]

	initial O_2 partial pressure in the malaxer chamber headspace (kPa)			
	0[a]	30[b]	50	100
		Ogliarola cv.		
3,4-DHPEA	1.00 (0.02) a	0.84 (0.05) b	0.64 (0.004) c	0.75 (0.01) d
p-HPEA	3.11 (0.03) a	3.12 (0.7) a	4.05 (0.001) b	4.18 (0.03) b
3,4-DHPEA-EDA	247.68 (1.9) a	235.16 (5.5) b	117.80 (0.8) c	118.09 (0.03) c
p-HPEA-EDA	126.41 (0.4) a	118.61 (5.9) b	86.28 (0.3) c	85.43 (0.62) c
(+)-1-acetoxypinoresinol	21.00 (0.4) a	25.39 (1.5) b	22.30 (0.3) ac	24.07 (0.09) bc
(+)-pinoresinol	6.83 (0.07) a	7.57 (0.3) b	7.01 (0.04) a	7.12 (0.03) a
3,4-DHPEA-EA	212.21 (0.1) a	186.40 (4.8) b	100.88 (1.1) c	98.19 (0.2) c
		Coratina cv.		
3,4-DHPEA	6.79 (0.7) a	3.15 (0.8) b	4.40 (0.7) b	1.38 (0.2) c
p-HPEA	10.00 (1.1) a	5.88 (0.5) bc	7.80 (0.9) b	4.35 (0.4) c
3,4-DHPEA-EDA	478.87 (16.2) a	437.70 (14.3) b	343.08 (11.5) c	229.86 (9.2) d
p-HPEA-EDA	144.24 (1.8) a	135.30 (1.59) b	126.16 (1.4) c	125.11 (3.1) c
(+)-1-acetoxypinoresinol	30.81 (0.94) a	25.83 (2.8) b	29.19 (0.4) ab	27.14 (0.5) ab
(+)-pinoresinol	8.12 (0.03) ab	7.96 (0.04) a	8.64 (0.4) b	7.93 (0.1) a
3,4-DHPEA-EA	475.59 (13.9) a	361.91 (14.1) b	339.15 (6.9) b	170.61 (2.3) c

[a] Saturated with N_2. [b] Corresponding to the air composition. [c] Data are the mean values of three independent experiments; standard deviation is reported in parentheses. Values in each row having different letters (a−d) are significantly different from one another at $p < 0.01$.

Table 3. Volatile Composition (μg/kg) of the Oils Obtained after Malaxation in Different Initial Atmosphere Compositions[c]

	initial O_2 partial pressure in the malaxer chamber headspace (kPa)			
	0[a]	30[b]	50	100
		Ogliarola cv.		
aldehydes				
2-pentenal (*E*)	291.5 (31.8) ab	343.0 (31.1) a	247.5 (11.7) b	269.5 (13.5) b
hexanal	939.5 (9.2) a	1546.0 (200.8) b	1011.5 (27.6) a	1499.5 (16.3) b
2-hexenal (*E*)	43645.0 (912.2) a	39130.0 (1054.7) b	37315.0 (233.3) b	38170.0 (1258.7) b
alcohols				
1-pentanol	28.5 (2.1) a	128.0 (6.8) b	122.5 (3.5) b	158.0 (1.4) c
2-penten-1-ol (*E*)	55.5 (3.5) a	63.0 (4.6) a	50.5 (9.2) ab	38.5 (6.4) b
1-penten-3-ol	567.0 (17) a	871.0 (4.7) b	690.0 (1.4) c	809.5 (3.5) d
1-hexanol	8357.0 (102.6) a	9699.0 (106.1) b	11660.0 (99) c	13675.0 (63.6) d
3-hexen-1-ol (*E*)	35.0 (1.2) a	41.0 (3.5) a	47.5 (2.1) b	61.5 (2.1) c
3-hexen-1-ol (*Z*)	286.5 (4.9) a	434.0 (20.6) b	341.0 (11.3) c	400.5 (7.8) d
2-hexen-1-ol (*E*)	7662.5 (75.7) a	8616.0 (87.9) b	9355.0 (353.6) c	9780.0 (60.8) c
		Coratina cv.		
aldehydes				
2-pentenal (*E*)	548.5 (16.3) ab	509.7 (5.8) b	636.7 (17.9) c	613.0 (51.2) ac
hexanal	1187.0 (9.9) a	1624.3 (30) bc	1532.1 (27.3) b	1744.0 (121.2) c
2-hexenal (*E*)	51565.0 (827.3) a	52900.0 (565.7) ab	54340.5 (355.7) b	53920.0 (332.1) b
alcohols				
1-pentanol	40.0 (5.7) a	54.3 (5) b	39.4 (5) a	48.0 (3.2) ab
2-penten-1-ol (*E*)	87.5 (0.7) a	67.0 (0.2) b	105.8 (5.7) c	105.0 (8.3) c
1-penten-3-ol	890.0 (2.8) a	820.0 (1.2) b	1093.5 (33.7) c	1185.0 (91.2) c
1-hexanol	2326.0 (49.5) a	3694.2 (2) b	1788.0 (57.2) c	2170.0 (123.1) a
3-hexen-1-ol (*E*)	25.5 (0.7) ab	31.6 (3.8) a	20.0 (1.9) b	21.0 (1.9) b
3-hexen-1-ol (*Z*)	561.0 (4.2) a	513.6 (9.6) b	486.3 (11.1) b	498.0 (31.2) b
2-hexen-1-ol (*E*)	3654.5 (30.4) a	5905.0 (321) b	3350.1 (80.5) a	4185.0 (35.6) c

[a] Saturated with N_2. [b] Corresponding to the air composition. [c] Data are the mean values of three independent experiments; standard deviation is reported in parentheses. Values in each row having different letters (a−d) are significantly different from one another at $p < 0.01$.

olive pastes during malaxation requires further appropriately aimed studies.

As shown in **Figures 3** and **4**, the oxygen concentration in the malaxer headspace strongly affects the phenolic composition of the olive paste. The compounds most affected by the oxidative process were oleuropein and the ligstroside derivatives such as 3,4-DHPEA-EDA, and *p*-HPEA-EDA, other then verbascoside. Other compounds such as oleuropein, demethyloleuropein, and the lignans were hardly affected by O_2 concentrations in the malaxer, showing similar modifications in all the four trials and as confirmed by the correlation coefficients comparing O_2 depletion with phenolic changes in the pastes (**Table 1**). The enzymatic oxidation of derivatives of secoiridoids catalyzed by PPO and POD can explain the relationship between the oxygen decrease and phenolic loss during processing. In fact, as shown elsewhere (*1, 27–29*), the activity of these enzymes are strongly affected by the O_2 concentration in the pastes, and the derivatives of secoiridoids can be considered to be elective substrates for the above-reported enzymes. The lack of correlation between O_2 concentration and the glucosidic forms of secoiridoids (oleuropein and demethyloleuropein) may be due to the concomitant reactions, catalyzed by endogenous β-glucosidases and independent of oxygen, that contribute to the decrease in those compounds (*1*). On the contrary, the modest negative correlation coefficients for the phenyl alcohols (3,4-DHPEA and *p*-HPEA) are attributable to their increase in the olive pastes during the malaxation time due to release from secoiridoid hydrolysis.

The evolution of volatile compounds in the olive pastes during malaxation was also studied to show the effect of O_2 concentration on aroma formation due to the lipoxygenase activity. As shown in **Figures 5** and **6**, the volatile compounds are modified during the malaxation time, but their production seems to be hardly affected by oxygen availability in the malaxer. In fact, no significant correlations were observed (**Table 1**) between decreasing O_2 and the formation of volatile compounds due to lipoxygenase activity, as expressed as the sum of those saturated and unsaturated C_5 and C_6 aldehydes and alcohols responsible for the most important sensory notes of VOO, such as the "cut grass" odor.

The analytical results obtained in the VOOs produced with different O_2 levels during malaxation confirmed the observations reported on the basis of the pastes' composition. The phenols' concentration in the oils, reported in **Table 2**, was strongly modified by O_2 availability, in both of the cultivars studied. The oleuropein, demethyloleuropein, and ligstroside derivatives such as the 3,4-DHPEA-EDA, 3,4-DHPEA-EA, and *p*-HPEA-EDA were highly affected by the O_2 concentrations during malaxation, whereas lignans, such as pinoresinol and acetoxypinoresinol, were less modified; in fact, they seem to be independent of the O_2 level in the malaxer.

The volatile composition of VOO, reported in **Table 3**, shows significant differences between the different malaxation conditions, particularly for C_5 and C_6 alcohols, and as deeply described in the literature (*6*) between the two cultivars. However, as previously shown in the pastes' headspace, no relationships were observed between the concentration of volatile compounds and the O_2 concentration in the covered malaxer during processing. The formation of volatile compounds observed also in the trials at 0 kPa of initial O_2 suggests that, for the LPO pathway activation and its development during all the malaxation time, the amount of the oxygen incorporated by the olive pastes during crushing is probably adeguate.

All the findings presented in this paper point out that the oxygen concentration in the headspace of olive pastes during malaxation selectively influences the phenolic composition of VOO. As a result, it is possible to assume that the oxygen concentration in the malaxer may be used as an indirect parameter to monitor the modifications of phenols in the pastes and in the oil during processing. At the same time, those results are particularly important because they show the feasibility of altering oxygen levels during processing to optimize the phenolic concentration in VOO. This is a fundamental aspect from a technological standpoint. In fact, due to the strong variability in phenol concentrations in the olive fruits, related to agronomic factors such as cultivar, fruit ripening, and agronomic practices, the malaxing conditions may be manipulated to obtain the optimal values of phenols in VOO without significant modifications to the aroma profile. That possibility is particularly relevant for the healthful and sensory properties involved in VOO quality. In fact, several papers (*1, 30, 31*) have reported on the role of secoiridoid derivatives in the reduction of cardiovascular diseases and in cancer prevention. At the same time, those compounds affect the sensory properties of VOO, and indeed, as shown previous papers (*1, 32–35*), the *p*-HPEA-EDA is responsible for the "pungency" while the other secoiridoid derivatives are related to the "bitter" taste in 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 with mass spectrometer; EFC, electronic flow controller; EI, electron ionization.

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