Air Exposure Time of Olive Pastes During the Extraction Process and Phenolic and Volatile Composition of Virgin Olive Oil

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ABSTRACT: The time of exposure of olive pastes to air contact (TEOPAC) during malaxation was studied as a processing parameter that could be used to control endogenous oxidoreductases, such as polyphenoloxidase, peroxidase, and lipoxygenase, which affect virgin olive oil quality. Phenolic and volatile compounds were analyzed in the oils obtained using progressive TEOPAC at three ripening stages of olives. Multivariate statistical analysis was applied to the raw data. The phenolic concentration of virgin olive oil progressively decreased with increasing TEOPAC. On the contrary, a positive relationship was found with the concentration of several volatile compounds responsible for virgin olive oil aroma. The effect of TEOPAC, however, was strictly related to fruit ripening.

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KEY WORDS: Oil mechanical extraction process, phenols, virgin olive oil quality, volatile compounds.

The mechanical oil extraction process affects a number of sensory and health parameters of virgin olive oil (1–3). The genesis of volatile compounds and the release of phenolic antioxidants in the oil, which greatly affect the quality of virgin olive oil, are directly related to the extraction process (4–9).

In this context, it is well known that the concentration of secoiridoids in virgin olive oil is affected by the activation of endogenous enzymes during the mechanical extraction process (2,6,9). Glycosidases of the olive fruit activate the formation of aglycon secoiridoids, the oxidation of which is promoted by oxidoreductases such as polyphenoloxidase (PPO) and peroxidase (POD) (2,3,7,9). Moreover, during crushing and malaxation lipoxygenase catalyzes the formation of C_5 and C_6 saturated and unsaturated aldehydes, alcohols, and esters, which are correlated with the "cut grass" and "floral" sensory notes of virgin olive oil (5,10,11). The time and temperature of malaxation also affect the concentrations of these compounds in the oil (8,12,13).

As shown in previous works performed in the laboratory and on a pilot-plant scale, the possibility of having selective control of endogenous oxidoreductases during processing might be a new approach to improving the quality of virgin olive oil (14–17). For this purpose, the time of exposure of olive pastes to air contact (TEOPAC) during malaxation could be used as a processing parameter to control O_2 concentration and, as a consequence, the above-mentioned enzymatic oxidative reactions. Therefore, the main aim of the present work was to determine the effects of TEOPAC on the presence and concentration of volatile and phenolic compounds in virgin olive oil.

MATERIALS

Olives. Drupes of Moraiolo cultivar, harvested during the year 2000, were used at three different ripening stages, evaluated by a pigmentation index according to Pannelli *et al.* (18). The period investigated covers about 40 d from the last week of October to the first week of December, which is the industrial harvesting period for the Moraiolo cultivar grown in central Italy.

Reference compounds. 3,4-(Dihydroxyphenyl)ethanol (3,4-DHPEA) was synthesized in the laboratory according to the procedure of Baraldi *et al.* (19). The dialdehydic form of elenolic acid linked to (*p*-hydroxyphenyl)ethanol (*p*-HPEA) or 3,4-DHPEA (*p*-HPEA-EDA or 3,4-DHPEA-EDA, respectively) and the isomer of oleuropein aglycon (3,4-DHPEA-EA) were extracted from virgin olive oil using a procedure reported previously (20,21). The purity of these substances was tested by HPLC and their chemical structures were verified by NMR. *p*-HPEA was obtained from Janssen Chemical Co. (Beerse, Belgium), and caffeic acid and vanillic acid were obtained from Fluka Co. (Buchs, Switzerland). Pure analytical standards of volatile compounds were purchased from Fluka (Milan, Italy) and Aldrich (Milan, Italy).

METHODS

The process was performed on a laboratory scale. Olives (10 kg) were crushed and placed in a malaxer with an internal volume of 12 L. Malaxation was allowed for 60 min at 30°C, after which the following TEOPAC were tested: 0, 10, 20, 30, 40, 50, and 60 min. During the residual times of malaxation, air was replaced with pure N_2. After malaxation, the oil was separated by centrifugation. Each experiment was performed in

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triplicate. During malaxation, oxygen in the pastes was measured using a Mettler Toledo Oxygen Transmitter, model 4100 (Greifensee, Switzerland). Results are expressed as ppm of O_2.

Phenolic compounds. Phenolic compounds were extracted, estimated colorimetrically at 765 nm using the Folin–Ciocalteau reagent, and expressed as 3,4-DHPEA equivalents as reported previously (20). The extraction and HPLC separation of phenolic compounds were carried out according to Montedoro *et al.* (20,21), the only exception being that an Inertsil ODS-3 column (150 × 4.6 mm i.d.) from Alltech Italia S.r.l. (Milan, Italy) was used.

Volatile compounds. (i) Solid-phase microextraction (SPME). Virgin olive oil (3 g) was placed into a 10-mL vial and thermostated at 35°C; a 65-μm CARBOWAX/divinyl-

TABLE 1
List of Variables Used in Model Building[a]

Factor no.	Variable	Factor no	Variable
	Alcohols		Ketones
7	1-Propanol	2	3-Pentanone
12	2-Methyl-1-propanol	6	1-Penten-3-one
14	3-Pentanol	26	2-Octanone
15	1-Butanol	28	3-Hydroxy-2-butanone
18	1-Penten-3-ol	36	6-Methyl-5-hepten-2-one
21	2-Methyl-1-butanol	53	3,5-Octadien-2-one
22	3-Methyl-1-butanol		
24	1-Pentanol		Esters
31	(*E*)-2-Penten-1-ol	1	Ethyl acetate
33	(*Z*)-2-Penten-1-ol	25	Hexyl acetate
37	1-Hexanol	30	3-Hexenyl acetate
38	(*E*)-3-Hexen-1-ol	32	(*Z*)-4-Hexenyl acetate
39	(*Z*)-3-Hexen-1-ol	35	(*E*)-2-Hexenyl acetate
41	(*Z*)-2-Hexen-1-ol	43	Ethyl caprylate
42	(*Z*)-2-Hexen-1-ol	59	Ethyl caprate
45	1-Hexen-3-ol	65	Methyl salicilate
46	1-Heptanol	71	Butyl caprate
49	2-Ethyl-hexanol		
52	2-Hepten-1-ol		Hydrocarbons
55	1-Octanol	4	3-Ethyl-1,5-octadiene
57	2-Butyl-1-octanol	5	3-Ethyl-1,5-octadiene (i)
61	2-Ethyl-1-decanol	8	1,1-Dimethyl-2-(1-methyl-2-propenyl)-cyclopropane
64	2-Hexyl-1-octanol		
67	Benzyl alcohol	9	1,1-Dimethyl-2-(2-methyl-2-propenyl)-cyclopropane
68	Phenylethyl alcohol		
70	3-Phenyl-2-propin-1-ol	10	1,1-Dimethyl-2-(2-methyl-2-propenyl)-cyclopropane (i)
	Aldehydes	19	Limonene
3	Pentanal		
11	Hexanal		Free acids
13	4-Pentenal	48	Acetic acid
16	Heptanal	56	Propionic acid
17	2-Pentenal	58	Formic acid
20	(*Z*)-2-Hexenal		
23	(*E*)-2-Hexenal		Nitrogen compound
27	Octanal	29	Geranyl nitrile
34	(*Z*)-2-Heptenal		
40	Nonanal		Phenolic compounds
44	(*E*)-2-Octenal	72	3,4-DHPEA
47	(*E,E*)-2,4-Heptadienal	73	*p*-HPEA
50	Decanal	74	Vanillic acid
51	2,4-Heptadienal (i)	75	Caffeic acid
54	Benzaldehyde	76	3,4-DHPEA-EDA
60	(*E*)-2-Nonenal	77	*p*-HPEA-EDA
62	Ethylbenzaldehyde	78	*p*-HPEA derivative
63	2,6 or 2,5 or 2,4-Dimethylbenzaldehyde	79	3,4-DHPEA-EA
66	3-Phenyl-2-propenal	80	Sum *ortho*-diphenols
		81	Total phenols
	Phenols		
69	Phenol		Dependent variable
		82	Air exposure time

[a]3,4-DHPEA, 3,4-(dihydroxyphenyl)ethanol; 3,4-DHPEA-EDA, the dialdehydic form of elenolic acid linked to 3,4-DHPEA; *p*-HPEA-EDA, the dialdehydic form of elenolic acid linked to *p*-HPEA; *p*-HPEA, (*p*-hydroxyphenyl)ethanol; 3,4-DHPEA-EA, the isomer of oleuropein aglycon.

benzene fiber (Supelco, Inc., Bellefonte, PA) was then exposed to the vapor phase for 30 min to sample the volatile compounds. The fiber was inserted into the gas chromatograph injector (set in splitless mode and using a splitless inlet liner of 0.75 mm i.d.) for thermal desorption, where it was left for 5 min. All SPME operations were automated using a Varian 8200 CX AutoSampler (Varian, Walnut Creek, CA).

(ii) GC–MS analysis. A Varian 3600 gas chromatograph equipped with a 1078 split/splitless injector coupled with a Varian Saturn 3 mass spectrometer (Varian, Walnut Creek, CA) was used. A fused-silica capillary column (DB-Wax, 50 m, 0.32 mm i.d., 1 mm film thickness; J&W Scientific, Folsom, CA) was used. The column was operated with helium at a pressure of 15 psi with a flow rate of 2.2 mL/min and a linear velocity of 30.7 cm/s at 35°C.

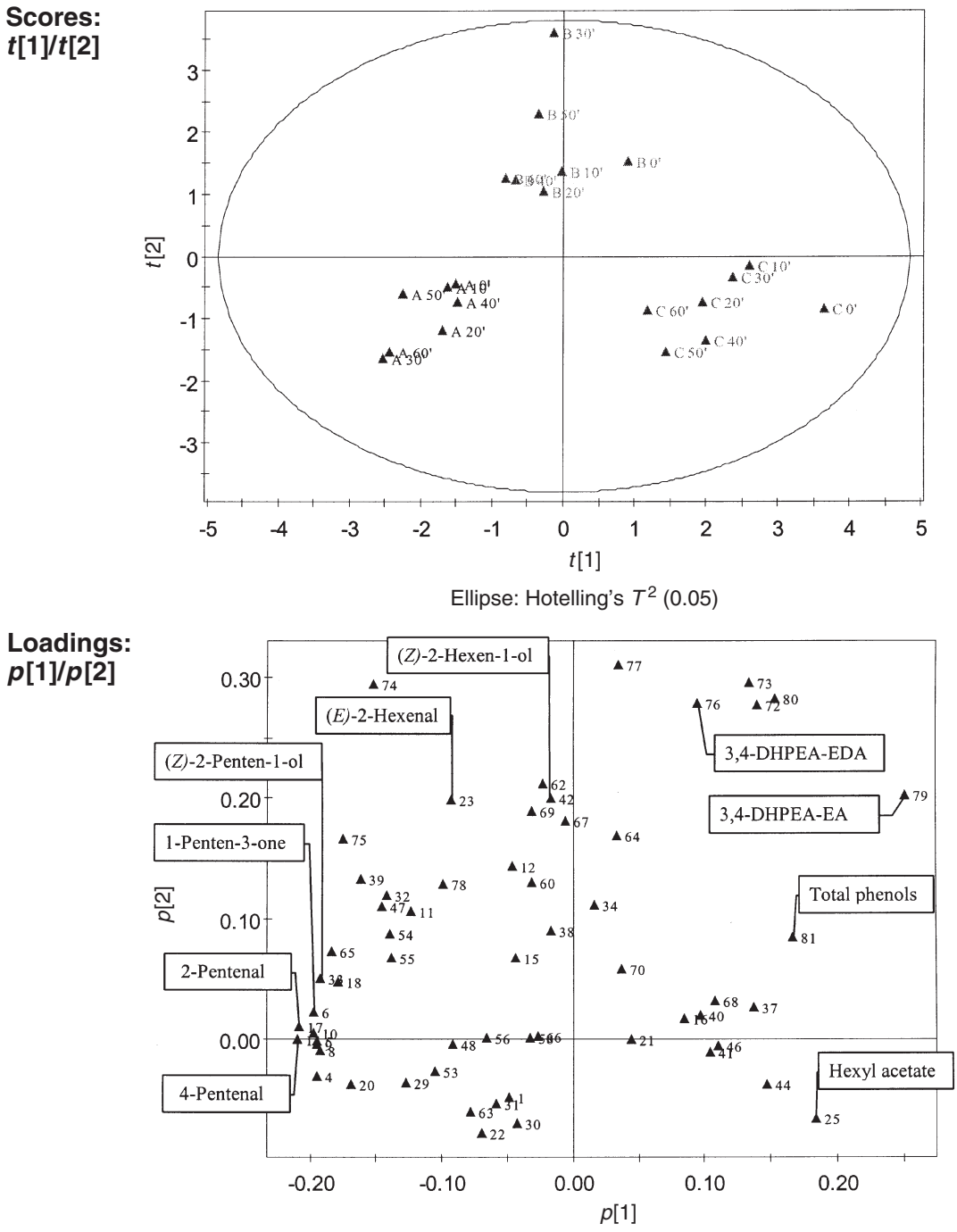

FIG. 1. Score and loading plots of the first two principal components of the model built with all virgin oil samples. Letters in the score plot represent the ripening stages (A = first; B = second; C = third), whereas the numbers represent the exposure times in minutes (′) of olive pastes to air contact. In the loading plot, the numbers correspond to the compounds reported in Table 1. 3,4-DHPEA, 3,4-(dihydroxyphenyl)ethanol; 3,4-DHPEA-EDA, the dialdehydic form of elenolic acid linked to 3,4-DHPEA, 3,4-DHPEA-EA, the isomer of oleuropein aglycon.

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 held for 14.51 min; the total time of analysis was 80 min. The injector temperature was maintained at 250°C. The temperature of the transfer line was fixed at 220°C.

The mass spectrometer was operated in the EI mode at an ionization voltage of 70 eV in the mass range of 10–350 a.m.u. at a scan rate of 1 s/scan and a manifold temperature of 180°C. The gas chromatograph–mass spectrometer was operated through Saturn GC–MS Version 5.2 software (Varian, Walnut Creek, CA). Volatile compounds were identified by comparing their mass spectra and retention times with those of authentic reference compounds. When standards were not available, volatile compounds were identified by comparing their mass spectral data with those of the NIST-92 library. Integration of all the chromatographic peaks was

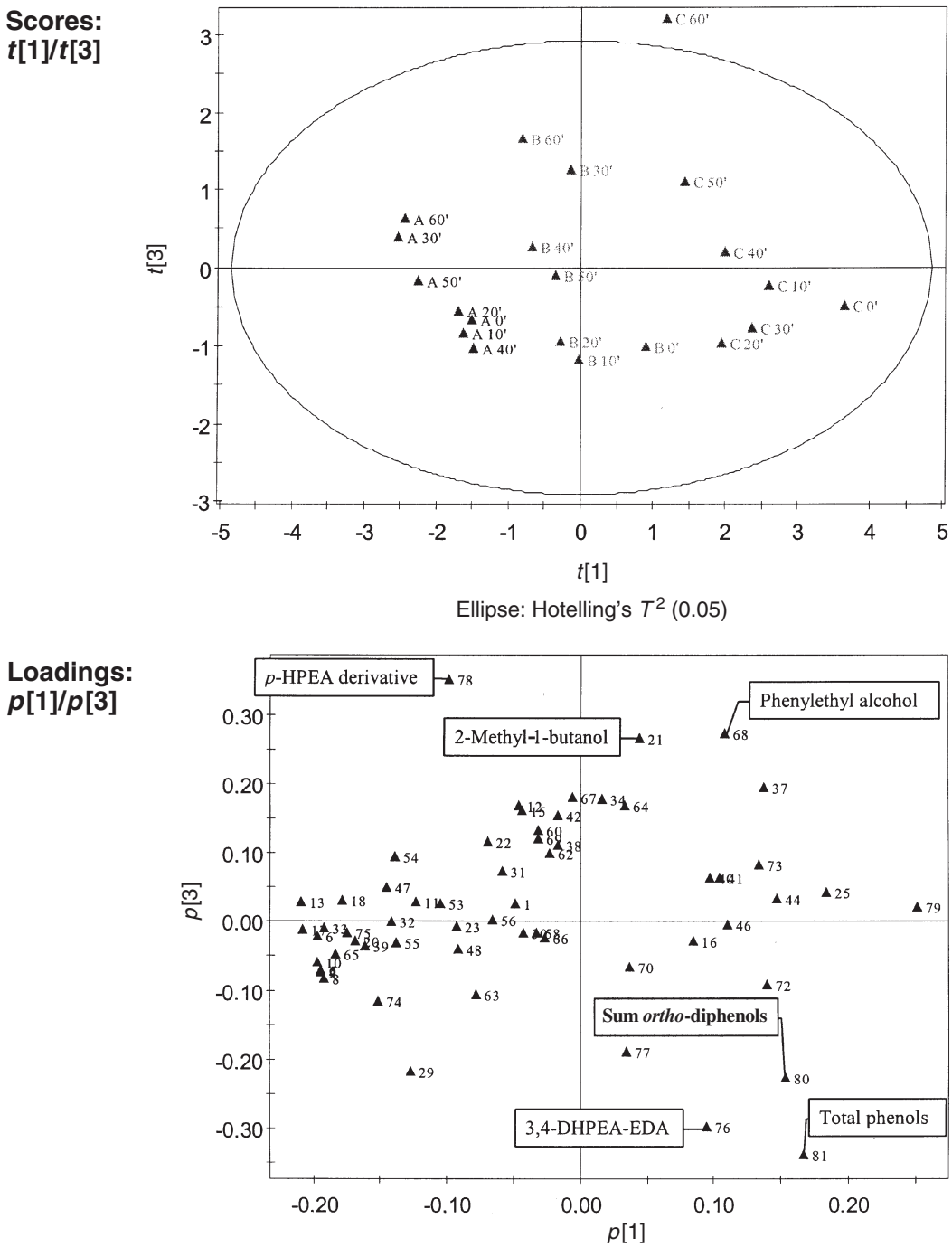

FIG. 2. Score and loading plots of the third vs. the first principal component of the model built with all samples. *p*-HPEA, (*p*-hydroxyphenyl) ethanol; see Figure 1 for all other abbreviations used.

performed by choosing the three masses, from among those specific to each compound, with the highest intensities so as to selectively discriminate them from their nearest neighbors. The results of the peak areas were expressed as area counts.

Quantitative determination of selected volatile compounds was performed by constructing calibration curves, using as the interpolation method quadratic equations passing through the origin. A stock solution at a fixed concentration was obtained by dissolving the substances in refined olive oil and then diluting this solution to obtain solutions at six concentration levels. These solutions were analyzed by headspace SPME under the same conditions as those used for the analyses of virgin olive oils. The analyses were carried out in triplicate, and the CV was less than 15% (15,22,23).

Statistical analysis. Principal components analysis (PCA), partial least squares projection to latent structures (PLS), and soft independent modeling of class analogy (SIMCA) were

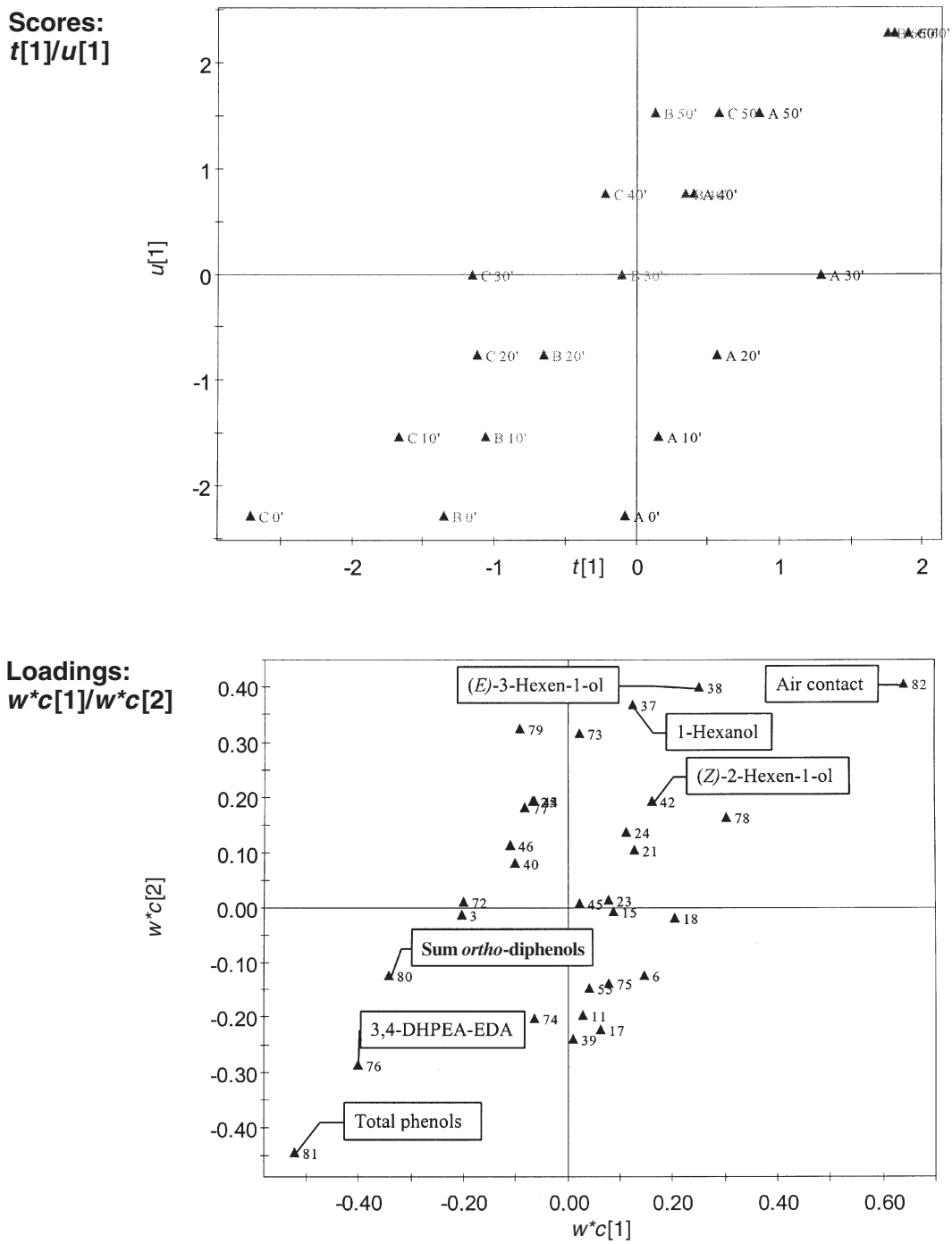

FIG. 3. Score plot of *u*[1] vs. *t*[1] and loading plot of *w*c*[2] vs. *w*c*[1] of the partial least squares projection to latent structures (PLS) model built with all samples. See Figure 1 for the abbreviations used. *w*c* represent both the *x*-weights (*w**) and *y*-weights (*c*) and show how the *x*-variables relate to the *y*-variables.

performed by using the chemometric package SIMCA-P, version 8.0 (Umetrics AB, Umeå, Sweden). The results of PCA and PLS modeling are presented in graphic form: (i) The score plots of samples show the similarity (clusters) or dissimilarity of the objects, and (ii) the loading plots of the variables evidence their relevance and correlation in the components.

RESULTS AND DISCUSSION

To evaluate the possible relationships between TEOPAC and the concentration of O_2 in the olive pastes, the latter was measured every 3 min during malaxation under both air and N_2 atmospheres. The results showed strong modifications of the O_2 levels as a consequence of atmospheric variations, the oxygen levels in the pastes being 5.7 ± 1.2 and 0.18 ± 0.07

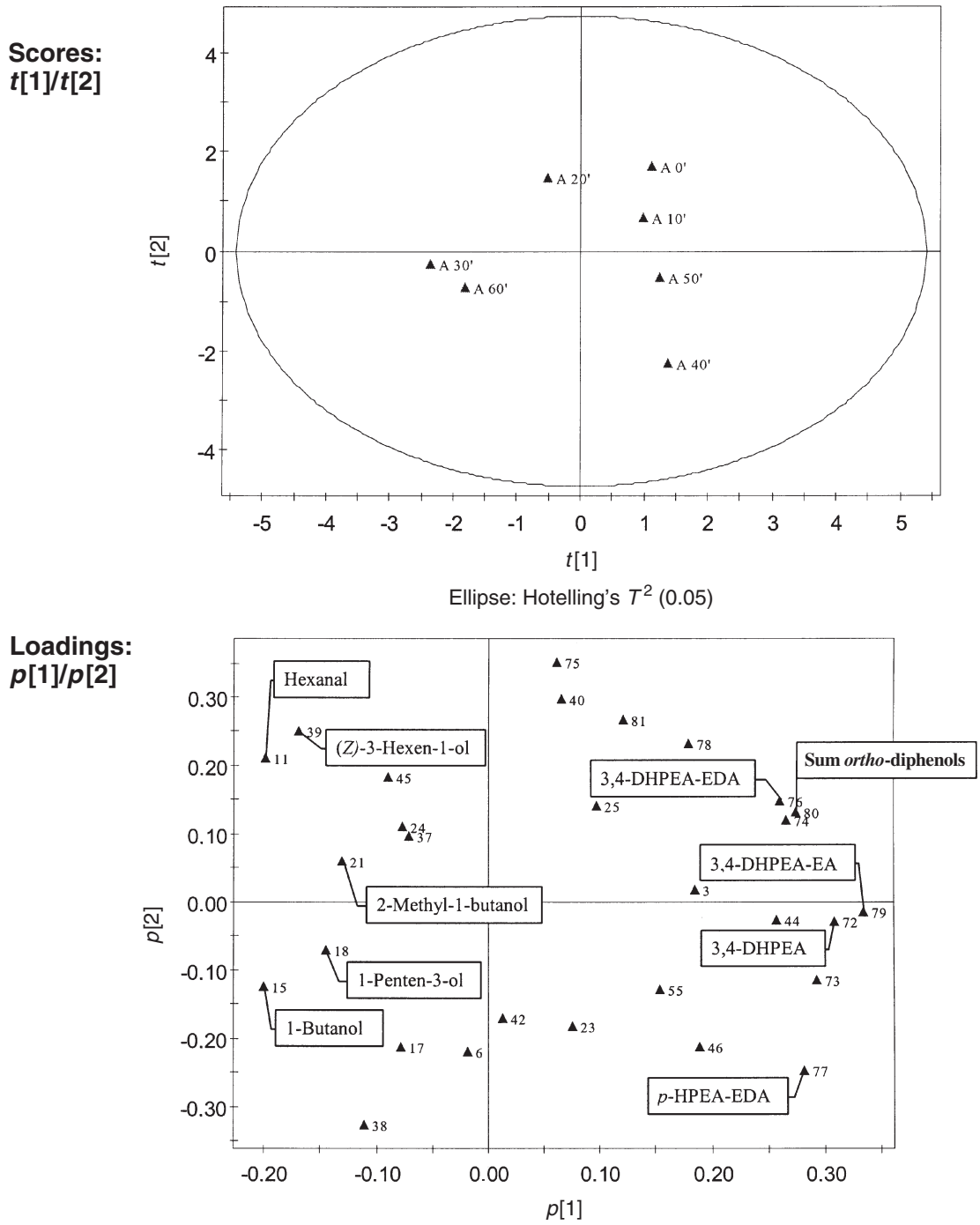

FIG. 4. Score and loading plots of the first two principal components of the model built with samples from the first ripening stage. *p*-HPEA-EDA, the dialdehydic form of elenolic acid linked to *p*-HPEA; See Figures 1 and 2 for other abbreviations used.

ppm under air and N_2, respectively. The relationships between TEOPAC and the volatile and phenolic compositions of virgin olive oil were determined using the multivariate statistical analysis methods PCA and PLS. Table 1 reports the variables used for model building.

The PCA model built using all virgin olive oil samples (21 objects) accounts for 64% of the total variance with three principal components, and the relative score plot of the first two components shows good discrimination among samples according to the ripening stage of the fruit: Along the first component, which accounts for 32% of the total variance, the distribution of objects is related to their increasing maturation; the variables with the higher absolute values in the loadings are 2-pentenal, 4-pentenal, 1-penten-3-one, (*Z*)-2-penten-1-ol, 3,4-DHPEA-EA, and hexyl acetate. The second, which accounts for 20% of the total variance, differentiates

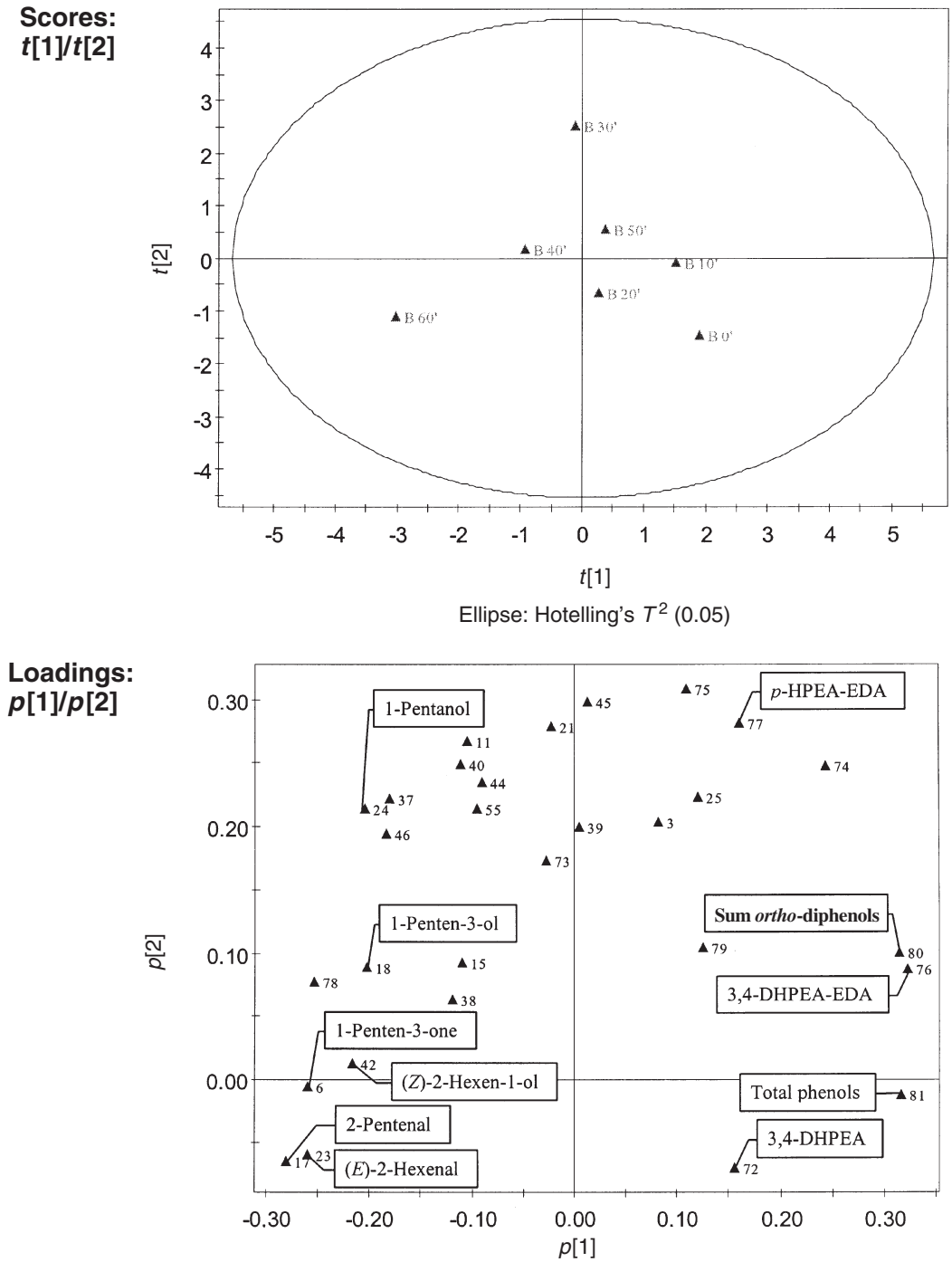

FIG. 5. Score and loading plots of the first two principal components of the model built with samples from the second ripening stage. See Figure 1 for the abbreviations used.

the samples at the second ripening stage; the most important variables are some phenols, the sum of *ortho*-diphenols, (*Z*)-2-hexen-1-ol, and (*E*)-2-hexenal (Fig. 1). The third component, which accounts for 12% of the total variance, discriminates among virgin olive oils according to the TEOPAC; the most important variables are the total phenols, 3,4-DHPEA-EDA, the *p*-HPEA derivative, 2-methyl-1-butanol, and phenylethyl alcohol (Fig. 2).

The PLS model, built considering as the dependent variable the time of exposure to air, which was correlated with the analytical data (independent variables), clearly shows the effect of TEOPAC on sample distribution and, at the same time, confirms the strong interaction with fruit ripening; in fact, as the TEOPAC increased, the discrimination of samples decreased as a function of ripening stage (Fig. 3). The relative loading plot reported in Figure 3 shows that secoiridoid derivatives such as 3,4-DHPEA-

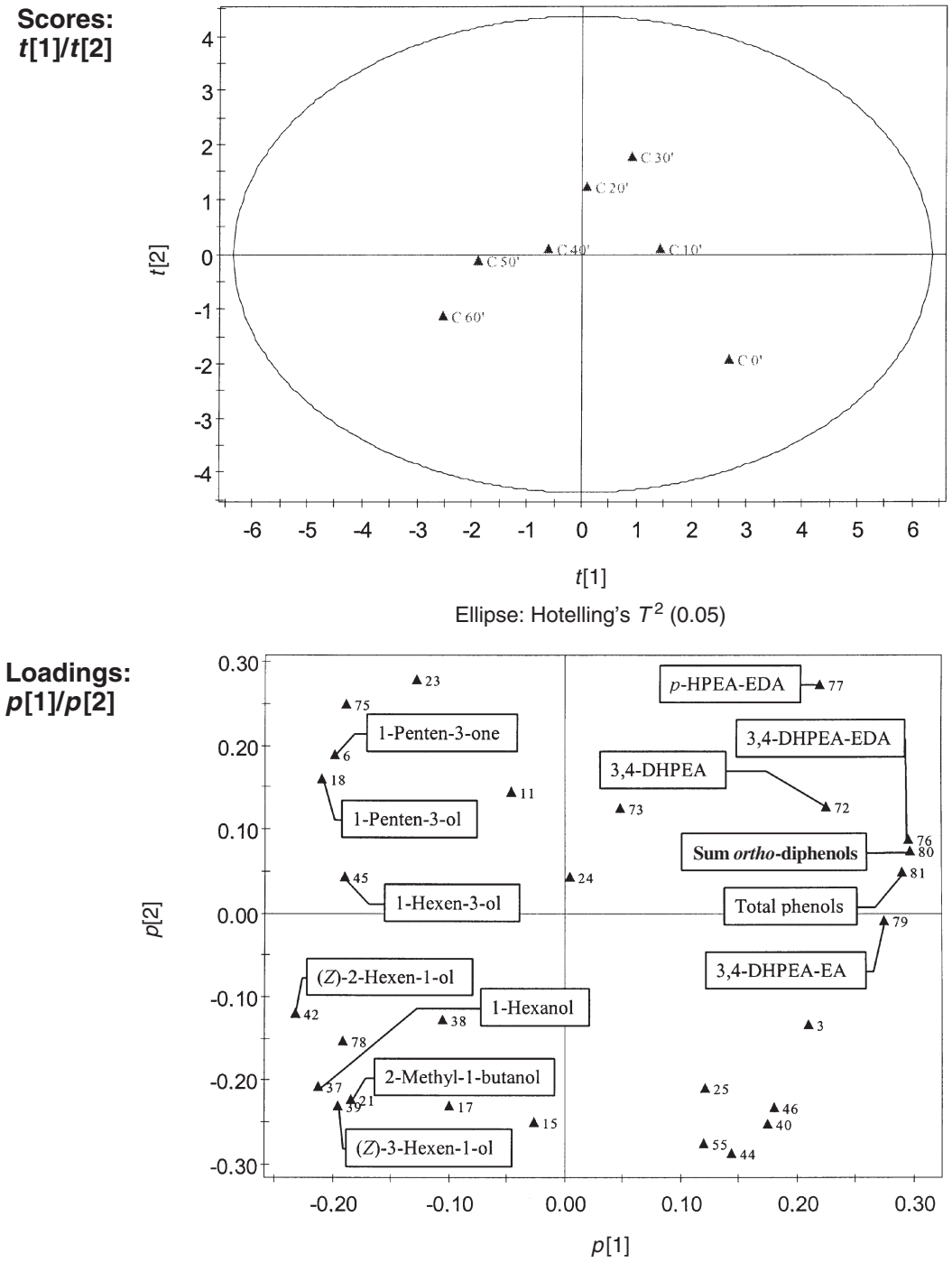

FIG. 6. Score and loading plots of the first two principal components of the model built with samples of the third ripening stage. See Figure 1 for the abbreviations used.

EDA, total phenols, and the sum of ortho-diphenols were negatively correlated with TEOPAC during malaxation, whereas volatile compounds such as (*E*)-3-hexen-1-ol, 1-hexanol, and (*Z*)-2-hexen-1-ol showed a positive correlation.

In addition, the effect of TEOPAC on the phenolic and volatile compounds of olive oil was evidenced by the results of the SIMCA analysis at each ripening stage of olive fruit. The relative score plots are reported in Figures 4–6. A similar distribution of samples along the first component, as a function of TEOPAC, was found at all ripening stages. The loading plots show that the phenolic compounds discriminated by the models were significantly affected by the process and were the same at all ripening stages. The highest loading values were observed for 3,4-DHPEA-EA, 3,4-DHPEA-EDA,

TABLE 2
Quantitative Values of Variables Selected from the SIMCA Models[a]

	Air exposure time						
	0′	10′	20′	30′	40′	50′	60′
	Pigmentation index: 2.2						
Phenolic compounds (mg/kg)							
3,4-DHPEA-EA	146.0 ± 16.6	137.0 ± 6.4	117.7 ± 28.8	111.9 ± 11.3	149.3 ± 7.5	143. ± 1.2	95.8 ± 6.0
3,4-DHPEA	1.35 ± 0.21	1.10 ± 0.08	0.82 ± 0.26	0 ± 0.00	1.33 ± 0.03	1.49 ± 0.05	0.95 ± 0.40
3,4-DHPEA-EDA	804.3 ± 26.0	848.6 ± 14.5	738.1 ± 11.4	681.6 ± 20.3	622.0 ± 15.2	581.4 ± 13.2	337.6 ± 8.6
p-HPEA-EDA	29.8 ± 1.3	33.7 ± 0.7	27.5 ± 0.2	26.2 ± 0.1	40.4 ± 1.3	37.9 ± 2.5	28.6 ± 4.1
Sum of *ortho*-diphenols	951.7 ± 30.9	986.7 ± 15.8	856.6 ± 30.9	793.5 ± 23.2	772.6 ± 17.0	726.2 ± 13.3	434.4 ± 10.5
Volatile compounds (μg/kg)							
Hexanal	512 ± 26	490 ± 13	469 ± 10	560 ± 27	348 ± 29	420 ± 35	494 ± 25
1-Penten-3-ol	640 ± 22	891 ± 51	782 ± 16	781 ± 43	711 ± 41	810 ± 21	1027 ± 95
(*E*)-2-Hexenal	12,754 ± 586	18,044 ± 856	12,254 ± 780	12,124 ± 541	17,146 ± 511	14,035 ± 750	17,465 ± 266
1-Hexanol	144 ± 18	252 ± 9	170 ± 19	117 ± 11	104 ± 12	108 ± 17	251 ± 10
(*Z*)-3-Hexen-1-ol	1454 ± 43	1448 ± 74	1469 ± 86	1429 ± 48	1199 ± 77	1337 ± 20	1505 ± 30.0
(*Z*)-2-Hexen-1-ol	321 ± 21	471 ± 14	333 ± 36	380 ± 11	406 ± 13	394 ± 22	424 ± 20
	Pigmentation index: 2.6						
Phenolic compounds (mg/kg)							
3,4-DHPEA-EA	354.9 ± 28.9	384.8 ± 12.6	327.7 ± 20.9	291.7 ± 21.1	290.8 ± 20.0	281.3 ± 27.6	245.9 ± 8.1
3,4-DHPEA	3.74 ± 1.11	2.69 ± 0.32	1.89 ± 0.08	2.84 ± 0.74	1.55 ± 0.08	2.41 ± 0.30	1.87 ± 0.11
3,4-DHPEA-EDA	1206.2 ± 37.8	1103.9 ± 23.8	1108.1 ± 14.1	1063.7 ± 10.0	1060.0 ± 23.1	834.3 ± 18.7	645.3 ± 6.5
p-HPEA-EDA	40.5 ± 1.4	41.4 ± 1.2	44.3 ± 1.7	49.8 ± 3.1	39.0 ± 0.3	32.5 ± 0.4	53.3 ± 0.2
Sum of *ortho*-diphenols	1564.8 ± 47.6	1491.4 ± 26.9	1437.7 ± 25.3	1358.2 ± 23.4	1352.4 ± 31.2	1118.0 ± 33.3	893.1 ± 10.4
Total phenols	575 ± 52	609 ± 48	581 ± 56	514 ± 45	456 ± 50	506 ± 61	389 ± 40
Volatile compounds (μg/kg)							
Hexanal	287 ± 9	552 ± 32	492 ± 24	611 ± 13	549 ± 67	482 ± 30	490 ± 32
1-Penten-3-ol	494 ± 52	786 ± 51	816 ± 21	769 ± 13	771 ± 46	780 ± 78	892 ± 75
(*E*)-2-Hexenal	22,918 ± 800	28,060 ± 469	27,977 ± 276	27,050 ± 329	29,663 ± 168	24,393 ± 756	40,994 ± 1288
1-Hexanol	289 ± 20	345 ± 28	386 ± 13	429 ± 13	437 ± 20	358 ± 15	385 ± 15
(*Z*)-3-Hexen-1-ol	1095 ± 39	1826 ± 113	1903 ± 63	1801 ± 44	1660 ± 60	1542 ± 134	1376 ± 70
(*Z*)-2-Hexen-1-ol	707 ± 48	519 ± 36	600 ± 20	770 ± 30	711 ± 27	635 ± 57	864 ± 39
1-Pentanol	21 ± 4	30 ± 4	47 ± 8	60 ± 10	42 ± 6	42 ± 7	52 ± 5
	Pigmentation index: 2.9						
Phenolic compounds (mg/kg)							
3,4-DHPEA-EA	368.5 ± 27.8	381.7 ± 24.5	337.5 ± 15.8	310.3 ± 16.6	314.5 ± 17.0	279.6 ± 11.1	246.0 ± 16.5
3,4-DHPEA	1.89 ± 0.13	2.71 ± 0.71	2.00 ± 0.34	1.96 ± 0.03	1.25 ± 0.03	1.31 ± 0.21	1.20 ± 0.26
3,4-DHPEA-EDA	1003.0 ± 54.5	956.5 ± 61.1	841.0 ± 45.3	882.7 ± 26.9	665.8 ± 50.4	492.2 ± 60.9	364.6 ± 36.0
p-HPEA-EDA	34.5 ± 1.1	36.8 ± 2.2	44.9 ± 1.6	37.1 ± 1.2	29.6 ± 2.2	24.9 ± 1.4	25.4 ± 1.4
Sum of *ortho*-diphenols	1373.4 ± 61.2	1340.9 ± 65.8	1180.5 ± 48.1	1195.0 ± 31.6	981.6 ± 53.2	773.1 ± 61.9	611.8 ± 39.6
Total phenols	666 ± 70	624 ± 61	589 ± 63	556 ± 45	494 ± 38	453 ± 42	345 ± 38
Volatile compounds (μg/kg)							
Hexanal	258 ± 15	467 ± 20	409 ± 18	314 ± 45	365 ± 39	354 ± 59	352 ± 28
1-Penten-3-ol	275 ± 37	496 ± 36	552 ± 47	452 ± 18	506 ± 21	606 ± 34	538 ± 35
(*E*)-2-Hexenal	4872 ± 163	8532 ± 230	12,194 ± 658	9309 ± 293	8771 ± 316	11,011 ± 332	8028 ± 418
1-Hexanol	441 ± 9	412 ± 12	417 ± 22	308 ± 24	507 ± 17	672 ± 31	699 ± 19
(*Z*)-3-Hexen-1-ol	412 ± 21	384 ± 9	381 ± 18	307 ± 23	402 ± 21	539 ± 21	575 ± 19
(*Z*)-2-Hexen-1-ol	334 ± 19	365 ± 21	406 ± 20	340 ± 20	389 ± 41	499 ± 25	615 ± 31
1-Penten-3-one	315 ± 12	458 ± 25	584 ± 16	504 ± 13	562 ± 17	667 ± 23	513 ± 20
1-Hexen-3-ol	13 ± 12	19 ± 2	24 ± 4	19 ± 3	18 ± 2	20 ± 4	29 ± 3

[a]Results are the mean values ± SD of three indenpendent determinations. SIMCA, soft independent modeling of class analogy.

3,4-DHPEA, *p*-HPEA-EDA, and the sum of *ortho*-diphenols. On the contrary, the volatile compounds that positively correlated with TEOPAC changed according to the stage of fruit ripening. The most important variables exhibiting such behavior were hexanal, 1-butanol, (*Z*)-3-hexen-1-ol, 1-penten-3-ol, and 2-methyl-1-butanol for the first stage; 2-pentenal, (*E*)-2-hexenal, 1-penten-3-one, (*Z*)-2-hexen-1-ol, 1-pentanol, and 1-penten-3-ol for the second; and (*Z*)-2-hexen-1-ol, 1-hexanol, 1-penten-3-ol, 1-penten-3-one, (*Z*)-3-hexen-1-ol, 1-hexen-3-ol, and 2-methyl-1-butanol for the third stage. The absolute values of phenolic and volatile compounds, characterized by high loadings (Table 2), confirmed a negative trend in the phenolic concentration according to the TEOPAC, however, the concentration of volatile compounds showed an opposite evolution in all the ripening stages studied, demonstrating that reduced TEOPAC limited the activity of PPO and POD, which in turn improved the phenolic concentration in the olive oil. However, reduced TEOPAC appeared to limit the lipoxygenase activity, as clearly indicated by the fact that several volatile compounds such as (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, hexanal, (*Z*)-2-hexen-1-ol, 1-hexanol, 1-penten-3-ol, and (*E*)-3-hexen-1-ol, produced during crushing and malaxation by LPO, decreased significantly.

The results obtained in the present study clearly indicate that TEOPAC can be used to control the oxidative reactions that affect virgin olive oil quality during malaxation: The concentration of phenolic and volatile compounds responsible for the flavor of virgin olive oil can be optimized by controlling this parameter. Furthermore, as reported in preliminary works, control of the oxygen level during malaxation can be easily transferred to an industrial scale (14–16). Moreover, since fruit ripening strongly interacts with TEOPAC to determine the composition of volatile and phenolic compounds, the optimal TEOPAC can be reached by changing its duration as a function of fruit ripening. Work is in progress to define the relationships between individual olive cultivars and TEOPAC in order to optimize, for each cultivar, the time of exposure to air contact for the most desirable concentration of volatile and phenolic compounds.

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REFERENCES

1. Servili, M., M. Baldioli, F. Mariotti, and G.F. Montedoro, Secoiridoids of Virgin Olive Oil: Modification During Mechanical Oil Extraction, in *Advances in Oils and Fats, Antioxidants, and Oilseed By-products, Proceedings of the World Conference on Oilseed and Edible Oils Processing*, edited by S.S. Koseoglu, K.C. Rhee, and R.F. Wilson, AOCS Press, Champaign, 1998, pp. 289–295.
2. Servili, M., M. Baldioli, F. Mariotti, and G.F. Montedoro, Phenolic Composition of Olive Fruit and Virgin Olive Oil: Distribution in the Constitutive Parts of Fruit and Evolution During the Oil Mechanical Extraction Process, *Acta Hortic. 474*: 609–619 (1999).
3. Servili, M., M. Baldioli, and G.F. Montedoro, Phenolic Composition of Virgin Olive Oil in Relationship to Some Chemical and Physical Aspects of Malaxation, *Ibid. 356*:331–336 (1994).
4. Servili, M., G. De Stefano, P. Piacquadio, L. Di Giovacchino, and V. Sciancalepore, Effect of Extraction Systems on the Phenolic Composition of Virgin Olive Oils, *Fett/Lipid 101*:328–332 (1999).
5. Olias, J.M., A.G. Perez, J.J. Rios, and L.C. Sanz, Aroma of Virgin Olive Oil: Biogenesis of the "Green" Odor Notes, *J. Agric. Food Chem. 41*:2368–2373 (1993).
6. Heredia, A., R. Guillén, A. Jiménez, and J.F Bolaños, Activity of Glycosidases During Development and Ripening of Olive Fruit, *Lebensm. Unters. Forsch. 196*:147–151 (1993).
7. Sciancalepore, V., Enzymatic Browning in Five Olive Varieties, *J. Food Sci. 50*:1194–1195 (1988).
8. Morales, M.T., F. Angerosa, and R. Aparicio, Effect of the Extraction Conditions of Virgin Olive Oil on the Lipoxygenase Cascade: Chemical and Sensory Implications, *Grasas Aceites 50*:114–121 (1999).
9. Servili, M., M. Baldioli, A.L. Begliomini, R. Selvaggini, and G.F. Montedoro, 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*, edited by V. Lanzotti and O. Taglialatela-Scafati, Kluwer Academic, Dordrecht, The Netherlands, 2000, pp. 163–173.
10. Servili, M., J.M. Conner, J.R. Piggott, S.J. Withers, and A. Paterson, Sensory Characterization of Virgin Olive Oil and Relationship with Head Space Composition, *J. Sci. Food Agric. 67*:61–70 (1995).
11. Servili, M., R. Selvaggini, A. Taticchi, and G.F. Montedoro, Headspace Composition of Virgin Olive Oil Evaluated by Solid Phase Microextraction: Relationships with the Oil Sensory Characteristics, in *Food Flavors and Chemistry—Advances of the New Millennium, Proceedings of the 10th International Flavor Conference*, edited by A.M. Spanier, F. Shahidi, T.H. Parliment, C. Mussinan, C.-T. Ho, and E. Tratras Contis, The Royal Society of Chemistry, Cambridge, United Kingdom, 2001, pp. 236–247.
12. Aparicio, R., M.T. Morales, G. Luna, and R. Aparicio-Ruiz, Biochemistry and Chemistry of Volatile Compounds Affecting Consumer's Attitudes Towards Virgin Olive Oil, in *Flavour and Fragrance Chemistry, Proceedings of the Phytochemical Society of Europe*, edited by V. Lanzotti and O. Taglialatela-Scafati, Kluwer Academic, Dordrecht, The Netherlands, 2000, pp. 3–14.
13. Angerosa, F., R. Mostallino, C. Basti, and R. Vito, Influence of Malaxation Temperature and Time on the Quality of Virgin Olive Oils, *Food Chem. 72*:19–28 (2001).
14. Servili, M., M. Baldioli, R. Selvaggini, F. Mariotti, E. Federici, and G.F. Montedoro, Effect of Malaxation Under N_2 Flush on Phenolic and Volatile Compounds of Virgin Olive Oil, in *Advances in Plant Lipid Research, Proceedings of the 13th International Symposium on Plant Lipids*, edited by J. Sánchez, E. Cerdá-Olmedo, and E. Martínez-Force, 1998, pp. 307–310.
15. Servili, M., R. Selvaggini, A. Taticchi, and G.F. Montedoro, Volatile Compounds of Virgin Olive Oil Evaluated by Solid Phase Microextraction. Application in the Discrimination of Virgin Olive Oil According to the Cultivar and Area, in *Flavour and Fragrance Chemistry, Proceedings of the Phytochemical Society of Europe*, edited by V. Lanzotti and O. Taglialatela-Scafati, Kluwer Academic, Dordrecht, The Netherlands, 2000, pp. 211–220.
16. Vierhuis, E., M. Servili, M. Baldioli, H.A. Schols, A.G.J. Voragen, and G.F. Montedoro, Study of the Effect of Enzyme Treatment During the Mechanical Extraction of Olive Oil on Phenolic Compounds and Polysaccharides, *J. Agric. Food Chem. 49*:1218–1223 (2001).

17. García, M.B., F. Martínez, J. Alba, P. García, and A. Garrido, High-Performance Liquid Chromatography Evaluation of Phenols in Virgin Olive Oil During Extraction at Laboratory and Industrial Scale, *J. Am. Oil Chem. Soc. 78*:625–629 (2001).
18. Pannelli, G., M. Servili, R. Selvaggini, M. Baldioli, and G.F. Montedoro, Effect of Agronomic and Seasonal Factors on Olive (*Olea europaea* L.) Production and the Qualitative Characterization of the Oil, *Acta Hortic. 356*:239–243 (1994).
19. Baraldi, P.G., D. Simoni, S. Manfredini, and E. Menziani, Preparation of 3,4-Dihydroxy-1-benzene Ethanol: A Reinvestigation, *Liebigs Ann. Chem.*:684–686 (1983).
20. Montedoro, G.F., M. Servili, M. Baldioli, and E. Miniati, Simple and Hydrolyzable Compounds in Virgin Olive Oil. 1. Their Extraction, Separation and Quantitative and Semiquantitative Evaluation by HPLC, *J. Agric. Food Chem. 40*:1571–1576 (1992).
21. Montedoro, G.F., M. Servili, M. Baldioli, R. Selvaggini, E. Miniati, and A. Macchioni, Simple and Hydrolyzable Compounds in Virgin Olive Oil. 3. Spectroscopic Characterization of the Secoiridoid Derivatives, *Ibid. 41*:2228– 2234 (1993).
22. Servili, M., R. Selvaggini, J. Fereidon, and G.F. Montedoro, Comparison Between Different Methods for the Qualitative and Quantitative Evaluation of Volatile Compounds in Virgin Olive Oil by "Head Space" Analysis, in *Rivista Italiana Eppos,* Special Issue 1997, edited by S. Porretta, Omina Arti Grafiche, Bergamo, Italy, pp. 311–322.
23. Servili, M., R. Selvaggini, A. Taticchi, A.L. Begliomini, and G.F. Montedoro, Relationships Between the Volatile Compounds Evaluated by Solid Phase Microextraction and the Thermal Treatment of Tomato Juice, *Food Chem. 71*:407–415 (2000).

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