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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

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The operative conditions of malaxation such as temperature and time of exposure of olive pastes to air contact (TEOPAC) affect volatile and phenolic composition of virgin olive oil (VOO) and, as a consequence, its sensory and healthy qualities. In this paper, optimal temperature and TEOPAC during malaxation were studied, in lab scale, in two Italian cultivars using phenolic compounds, volatile composition, and sensory analysis of VOO as markers. The optimal temperature and TEOPAC, selected by response surface modeling,were cultivar-dependent being 30 min of TEOPAC at the lowest temperature investigated (22 °C) and 0 min of TEOPAC at 26 °C for Frantoio and Moraiolo cultivars, respectively.

KEYWORDS: Virgin olive oil quality; oil mechanical extraction process; phenols; volatile compounds

INTRODUCTION

Sensory and healthy properties of VOO are highly related to its volatile and phenolic composition (*1*−*3*). The occurrence of these substances in the oil is the final result of various endogenous enzymatic activities of olive fruit that are activated during processing (*4*−*9*). The relationships between concentration of hydrophilic phenols in VOO and endogenous oxidoreductases, such as PPO and POD, which promote their oxidation during malaxation, are well-known (*10*−*12*). Moreover, the LPO, activated during crushing, catalyzes the genesis of C5 and C6 saturated and unsaturated aldehydes, alcohols, and esters that are correlated to the "cut grass" and "floral" sensory notes of VOO (*13*−*16*). 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 strictly related to the sensory and healthy quality of VOO. The processing temperature affects the PPO, POD, and LPO activities and, as a consequence, the volatile and phenolic composition of VOO (*14*, *17*−*19*), but for the moment, it is not the only parameter that can be well-studied to control these enzymes. In fact, as shown in previous works performed in lab and pilot plant scales, the selective control of PPO, POD, and LPO may be obtained regulating O_2 concentration in the pastes during processing (*20*−*24*). For this purpose, the TEOPAC, during malaxation, was recently proposed as a processing parameter to control O_2 concentration, and consequently, the above enzymatic oxidative reactions (*11*, *20*−*24*). For this reason, the malaxing temperature and TEOPAC are the operative processing conditions that can be well-associated to optimize the concentration of volatile and phenolic compounds in VOO.

The main aim of the present work was the optimization of the operative conditions of malaxation, in terms of temperature and TEOPAC, using volatile compounds, phenolic concentration, and quantitative−descriptive sensory analysis data as markers.

MATERIALS AND METHODS

Olives. Drupes of the Moraiolo and Frantoio cultivars, harvested during the year 2001, were used. The ripening stage evaluated as the pigmentation index, according to Pannelli et al. (*25*), was 1.05 and 0.95 for Moraiolo and Frantoio cultivars, respectively.

Reference Compounds. (3,4-Dihydroxyphenyl)ethanol (3,4-DHPEA) was synthesized in the laboratory according to the procedure of Baraldi et al. (*26*). The (*p*-hydroxyphenyl)ethanol (*p*-HPEA) was obtained from Janssen Chemical Co. (Beerse, Belgium) while caffeic acid and vanillic acid were obtained from Fluka (Milan, Italy). The dialdehydic form of elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively) and the isomer of oleuropein aglycon (3,4-DHPEA-EA) were extracted from VOO using a previously reported procedure (*27*). The purity of these substances was tested by high-performance liquid chromatography (HPLC), and their chemical structures were verified by NMR (**Figure 1**). Pure analytical standards of volatile compounds were purchased from Fluka and Aldrich (Milan, Italy).

Experimental Procedure. *Optimization of Temperature and TEOPAC during Malaxation.* The processing parameters were studied in a lab scale. Olives (10 kg) were crushed using a hammer crusher and malaxed for 60 min in a malaxer of 12 L of internal volume, and the oil

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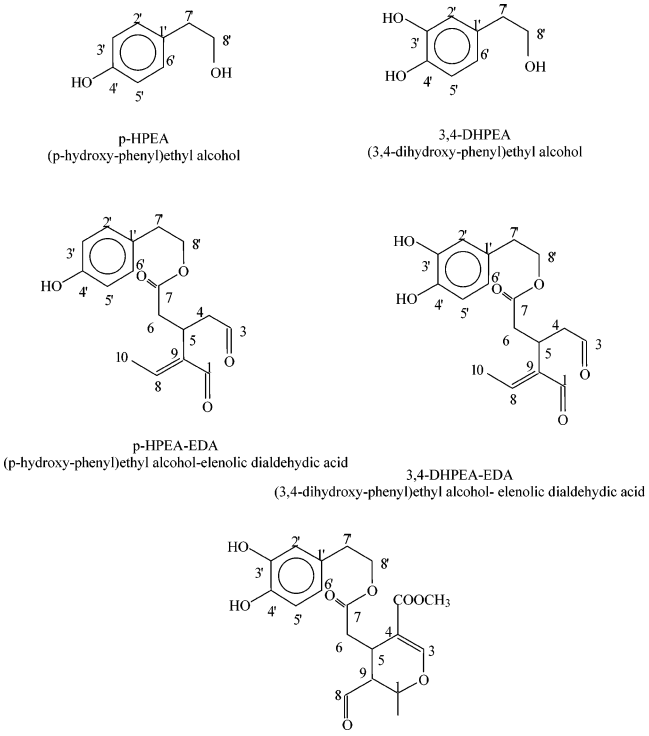


Figure 1. Secoiridoid compounds occurring in VOO (*27*, *29*).

Table 1. Combinations of Malaxation Parameters Selected by CCD

temp (°C)	TEOPAC (min)	temp (°C)	TEOPAC (min)	temp (°C)	TEOPAC (min)
24	–51	36	–9	30	–60
36	–51	22	–30	30	–0
24	–9	38	–30	30	–30[a]

[a] Central point with four repetitions.

separation was obtained by centrifugation. The data were collected according to a CCD with the central point replicated four times (*28*). As reported in **Table 1**, the temperatures of malaxation ranged, according to the common industrial conditions of processing, between 22 and 38 °C while the TEOPAC ranged between 0 and 60 min. During the residual times of malaxation, air was replaced with pure N_2. The O_2 concentration in the pastes was measured using a Mettler Toledo Oxygen Feeler model 4100 (Greifensee, Switzerland).

Phenolic Compounds. Phenolic compounds were extracted and estimated colorimetrically at 765 nm using the Folin−Ciocalteau reagent as previously reported (*29*) with the only exception that results are expressed as 3,4-DHPEA equivalents. The extraction and HPLC separation of phenolic compounds were carried out according to Montedoro et al. (*29*) except that an Inertsil ODS-3 column, 150 mm × 4.6 mm i.d., 5 μm, from Alltech Italia S.r.l. was used.

Volatile Compounds. SPME. VOO (3 g) was put into a 10 mL vial and thermostated at 35 °C, and then, the fiber (65 μm Carbowax/divinylbenzene) (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 chromatography (GC) injector and set in splitless mode using a splitless inlet liner of 0.75 mm i.d. for thermal desorption where it was left for 5 min (*30*). All of the SPME operations were automated using a Varian 8200 CX AutoSampler (Varian, Walnut Creek, CA).

GC-MS Analysis. A GC Varian 3600 equipped with a 1078 split/splitless injector coupled with a mass spectrometer Varian Saturn 3 (Varian) was used. A fused-silica capillary column DB-Wax, 50 m, 0.32 mm i.d., 1 μm film thickness (J&W Scientific, Folsom, CA) was employed. 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.

Table 2. List of the Variables Used in the Model Building

	Alcohols		
6	1-propanol	32	(*Z*)-3-hexen-1-ol
11	2-methyl-1-propanol	34	(*E*)-2-hexen-1-ol
12	3-pentanol	35	(*Z*)-2-hexen-1-ol
13	1-butanol	37	2-ethyl-hexanol
16	1-penten-3-ol	40	1-octanol
18	2-methyl-1-butanol	44	2-ethyl-1-decanol[a]
19	3-methyl-1-butanol	46	2-hexyl-1-octanol[a]
21	1-pentanol	49	benzyl alcohol
28	(*E*)-2-penten-1-ol	50	2-phenylethyl alcohol
30	(*Z*)-2-penten-1-ol	52	3-phenyl-2-propin-1-ol[a]
31	1-hexanol		
	Aldehydes		
2	pentanal	24	octanal
10	hexanal	33	nonanal
13	4-pentenal	39	benzaldehyde
14	heptanal	43	(*E*)-2-nonenal
15	(*E*)-2-pentenal	45	2,6-o-2,5-o-2,4-dimethyl-benzaldehyde[a]
17	(*Z*)-2-hexenal[a]	48	(*E*)-3-phenyl-2-propenal
20	(*E*)-2-hexenal		
	Phenols		
51	phenol	58	caffeic acid
53	total polyphenols	59	3,4-DHPEA-EDA
54	ortodiphenols	60	p-HPEA-EDA
55	3,4-DHPEA	61	p-HPEA derivative[b]
56	p-HPEA	62	3,4-DHPEA-EA
57	vanillic acid		
	Ketones		
2	3-pentanone	23	2-octanone
5	1-penten-3-one	25	3-hydroxy-2-butanone
	Esters		
1	ethyl acetate	29	(*Z*)-4-hexenyl acetate[a]
22	hexyl acetate	47	methyl salicilate
27	(*Z*)-3-hexenyl acetate		
	Hydrocarbons		
3	3-ethyl-1,5-octadiene[a]	8	1,1-dimethyl-2-(2-methyl-2-propenyl)cyclopropane[a]
4	3-ethyl-1,5-octadiene (i)[a]		
7	1,1-dimethyl-2-(1-methyl-2-propenyl)cyclopropane[a]	9	1,1-dimethyl-2-(2-methyl-2-propenyl)cyclopropane (i)[a]
	Free Acids		
36	acetic acid	42	formic acid
41	propionic acid		
	Nitrogen Compound		
26	geranyl nitrile*		
	Sensory Notes		
63	green	68	hay
64	yellow/green	69	floral
65	fruity	70	tomato
66	cut grass	71	bitter
67	artichoke	72	pungent

[a] Tentatively identified. [b] Tentatively identified according to Montedoro et al. (*27*).

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 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 electron ionization (EI) mode at an ionization voltage of 70 eV in the mass range of 10−350 amu at a scan rate of 1 s/scan and a manifold temperature of 180 °C. The GC-MS was operated through the software Saturn GC-MS version 5.2 (Varian). The volatile compounds were identified by comparison of their mass spectra and retention times with those of authentic reference compounds. When standards were not available, identification of the volatile compounds was obtained by comparing their mass spectral data with those of the NIST-92 library. Integration of all of the chromatographic peaks was performed choosing the three masses,

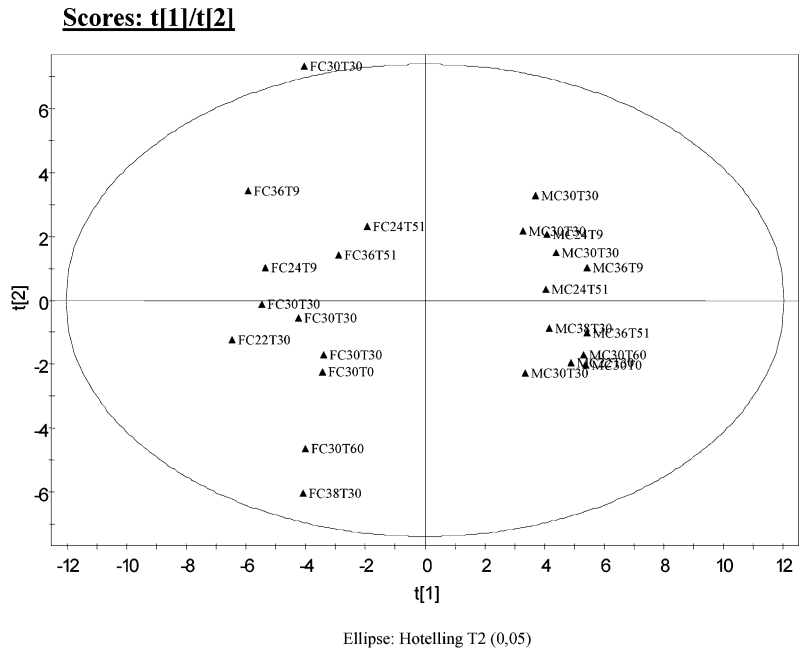


Figure 2. Score plot and loading plot of the first two principal components of PCA of VOO obtained from Moraiolo and Frantoio cultivars in different operative conditions. Abbreviations: F = Frantoio; M = Moraiolo; C = temperature (°C); and T = time (min) (see **Table 2** for variables).

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 expressed as area counts (*31, 32*).

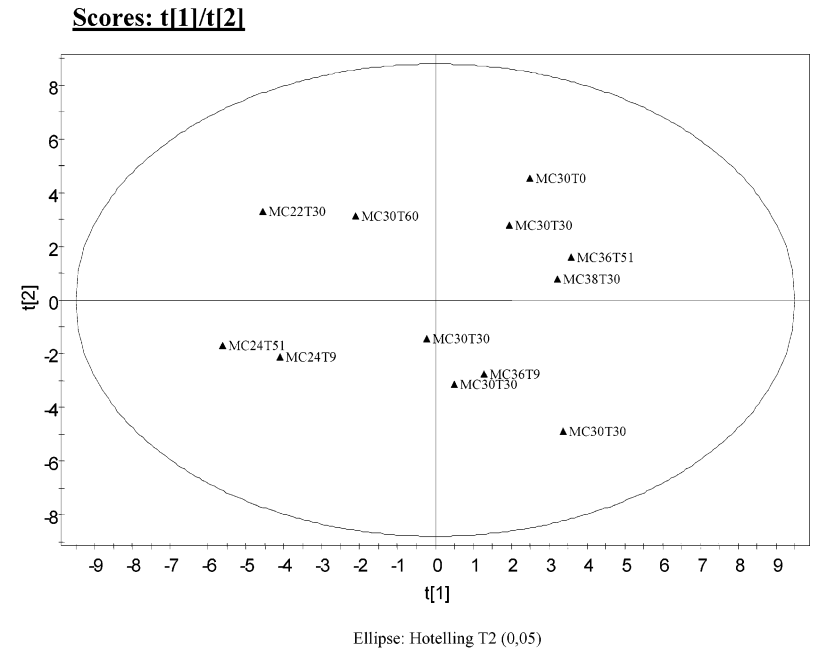
Sensory Analysis. Descriptive−quantitative analysis was carried out by a panel composed of eight assessors trained to perform VOO sensory analysis. Oil samples (15 g) were presented to the assessors in duplicate in amber-colored glasses at room temperature. The samples were presented in balanced order to each assessor. The following descriptors were proposed to the assessors: fruity, cut grass, artichoke, haylike, green apple, floral, tomato-like, almond, and fatty. The intensity of each property was graded using a line scale for each descriptor and thus converted in numerical score by measuring the position of the placed mark along the 10 cm line. Results were calculated as averages among assessor judgments (*33*).

Statistical Analysis. *ANOVA on Sensory Data.* A priori one way ANOVA, using the Tukey's honest significant differences test, was performed on sensory data to evaluate the response homogeneity of the panelists (*34*).

PCA. A PCA model was built to analyze the influence of the processing parameters on the instrumental and sensory data of VOO. The chemometric package "SIMCA-P v. 8.0" (Umetrics AB, Umeå, Sweden) was used.

The analytical data were put in a matrix with the rows corresponding to the samples (*n* objects) and the columns corresponding to the analytical parameters (*k* variables). The raw data were normalized, with the subtraction of the mean, and autoscaled, dividing these results by the standard deviation. The number of significant components has been found applying the cross-validation. The results of PCA modeling are presented in graphical form (*35*−*37*).

Optimization by RSM. RSM was performed with the chemometric package "MODDE v. 4.0" (Umetrics AB). Two preliminary PCA models were made, one for each cultivar, for selecting few variables



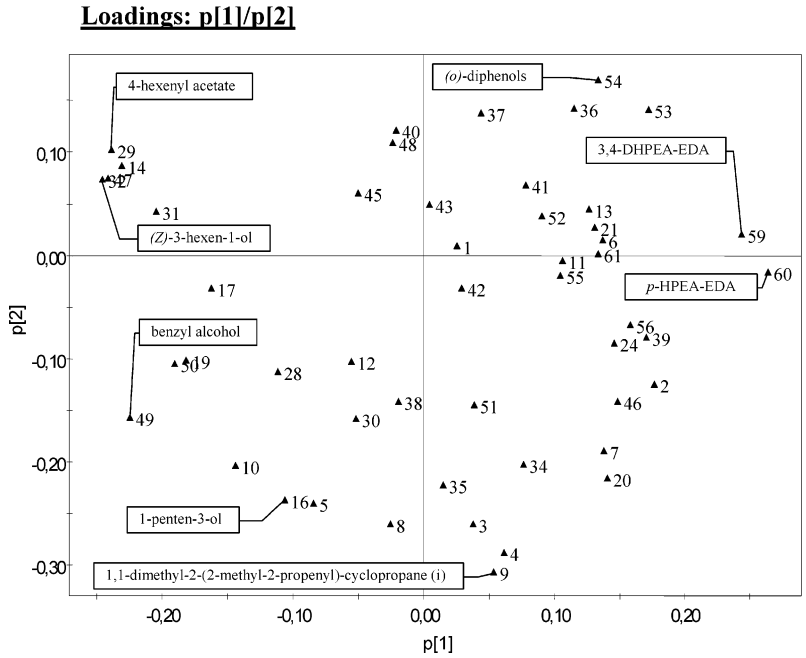


Figure 3. Score plot and loading plot of the first two principal components of PCA applied to the data set of phenolic and volatile compounds of VOO from the Moraiolo cultivar. Abbreviations: M = Moraiolo; C = temperature (°C); and T = time (min) (see **Table 2** for variables).

(volatile and phenolic compounds), among those with the highest absolute values of loadings that are relevant for sensory and healthy properties of VOO. Moreover, ANOVA was first applied to the sensory data to select the descriptors showing significant differences between the oil samples.

To optimize the malaxation parameters, the original data (Y), expressed as area counts, ppm, and average value for volatile compounds, hydrophilic phenols, and sensory analysis, respectively, were transformed in a desirability function (d_i) that is a dimensionless value, using a linear transformation, according to Derringer and Suich (*38*), with a little modification, so to obtain a range of desirability between 0.1 and 1:

$$d_i = \frac{0.9Y + 0.1Y_{max} - Y_{min}}{Y_{max} - Y_{min}}$$

(for volatile, phenolic compounds and sensory notes of VOO that must be maximized) and

$$d_i = \frac{-0.9Y + Y_{max} - 0.1Y_{min}}{Y_{max} - Y_{min}}$$

(for sensory notes of VOO that must be minimized). Y_{min} and Y_{max} corresponded to the minimum and the maximum value of the original data of the variables chosen, respectively. The overall desirability (D) was calculated as the geometric mean of the individual d_i values:

$$D = \sqrt[n]{d_1 * d_2 * ... * d_n}$$

The partial least-squares analysis (PLS) was employed for developing the model (*39*).

RESULTS

Preliminarily, the PCA was applied to the complete data set with included sensory and instrumental results; the list of volatile

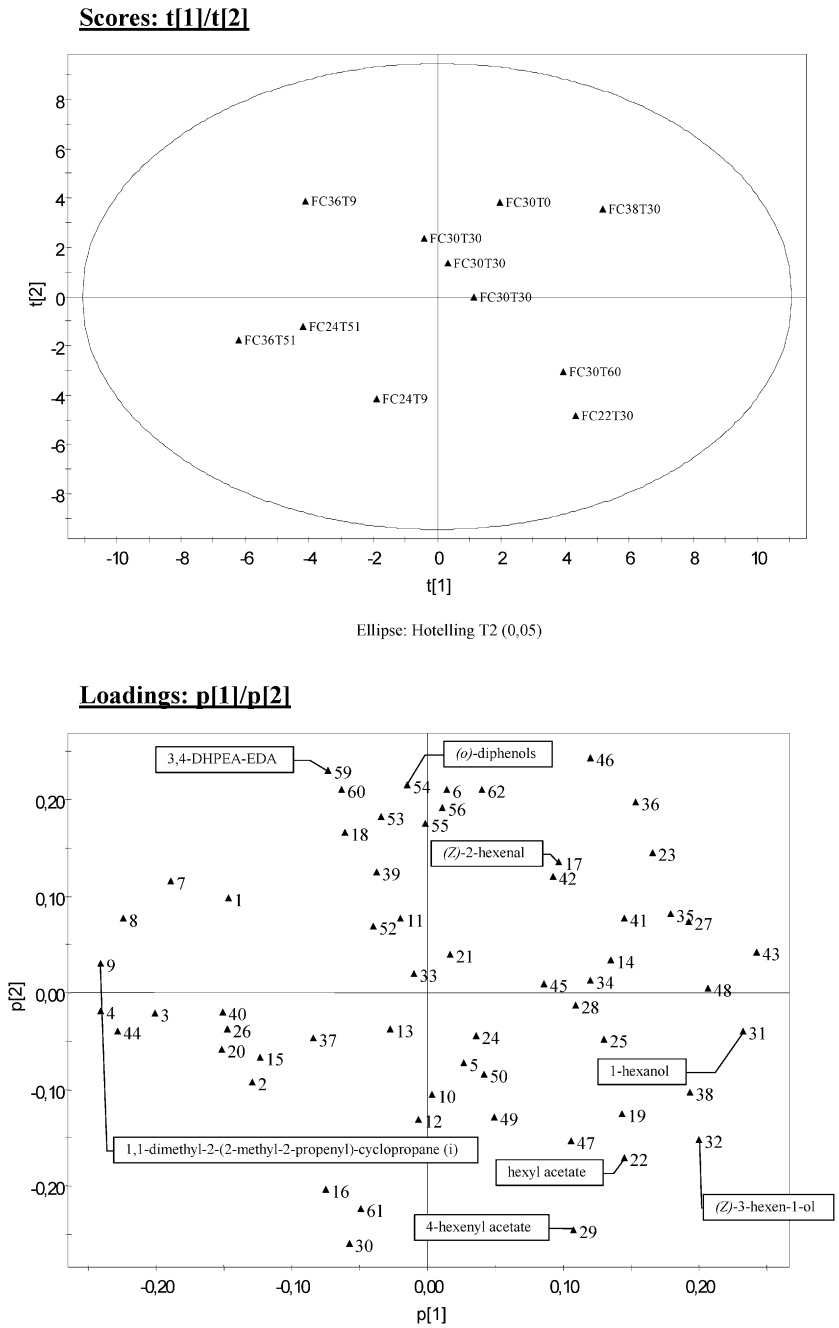


Figure 4. Score plot and loading plot of the first two principal components of PCA applied to the data set of phenolic and volatile compounds of VOO from the Frantoio cultivar. Abbreviations: F = Frantoio; C = temperature (°C); and T = time (min) (see **Table 2** for variables).

and phenolic compounds used to build the model is reported in **Table 2**. The model explains 55% of the total variance with three components and **Figure 2**, which reports the score plot and the loading plot of the first two components, shows a clear distribution of VOOs in two clusters, according to the olive cultivar, along the first component, while the second component differentiates the operative conditions of malaxation. As reported in the loading plot in **Figure 2**, a large quantity of volatile and phenolic compounds and sensory descriptors shows high loading values. These results appear to be very interesting since they prove that the genetic origin of the raw material has a great influence on the final composition of oil apart from the operative conditions applied during the oil mechanical extraction process.

To apply the RSM optimization model, few sensory and instrumental variables must be selected to define the desirability function; to this end, two different statistical approaches were chosen. Because of the low number of descriptors used by assessors in the descriptive sensory analysis of VOO, the ANOVA was applied to the sensory data set. The results showed significant differences between the samples for most descriptors including cut grass, haylike, floral, fruity, bitter, and pungent for the Frantoio cultivar, while for Moraiolo the descriptors chosen were cut grass, haylike, tomato-like, fruity, bitter, and pungent. As a consequence, these descriptors were used as sensory markers to build the optimization models. Moreover, in high quality VOO, several sensory notes such as cut grass, floral, tomato-like, and pungent could be stronger than others such as bitter and haylike; for this reason, in the RSM model, the first group of sensory data was maximized while the second was minimized.

Concerning the instrumental data of volatile and phenolic compounds, two PCA models were built for Frantoio and

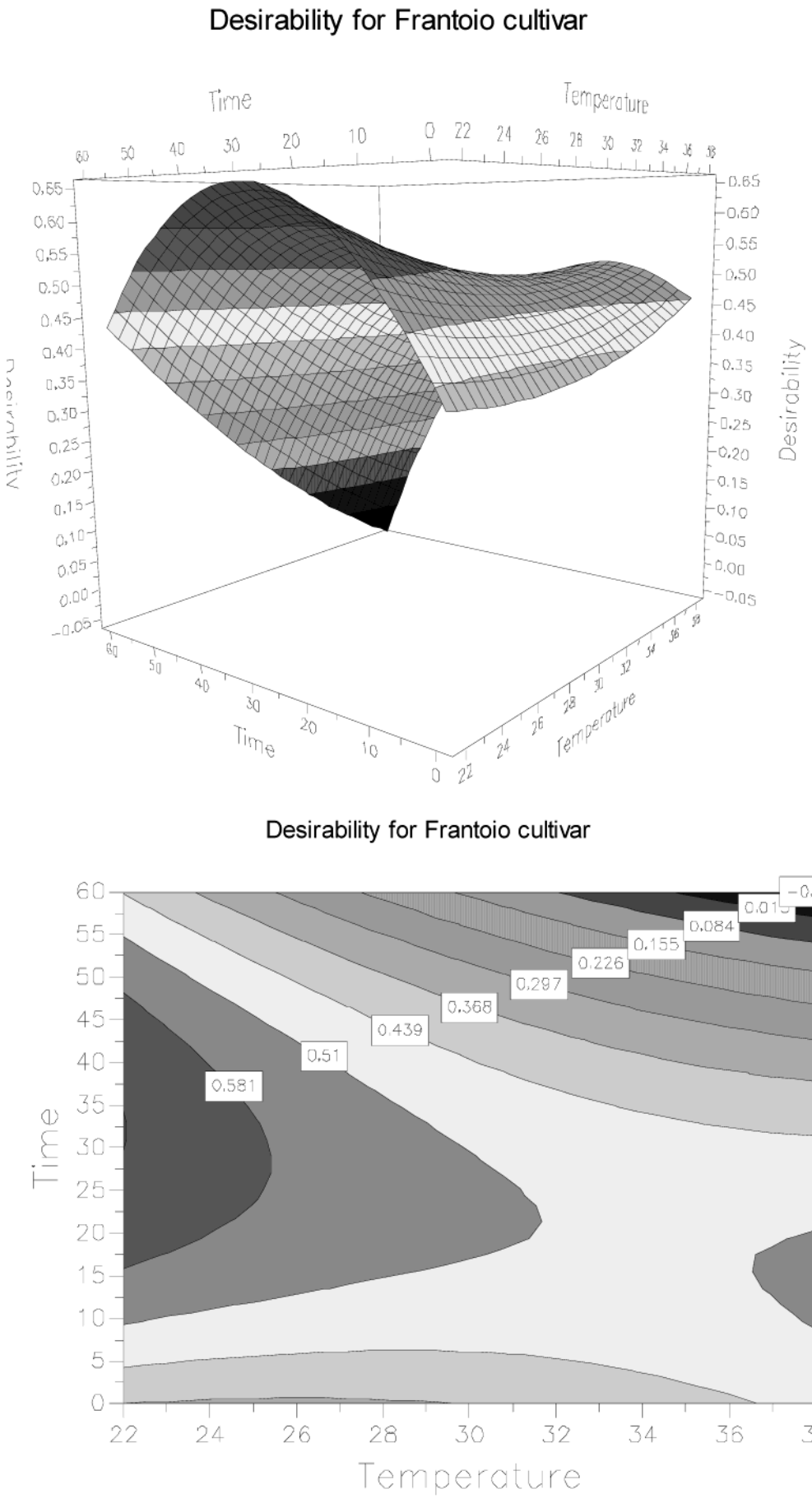


Figure 5. RSM and contour plots obtained using the PLS built to optimize the temperature and TEOPAC during malaxation in the Frantoio cultivar.

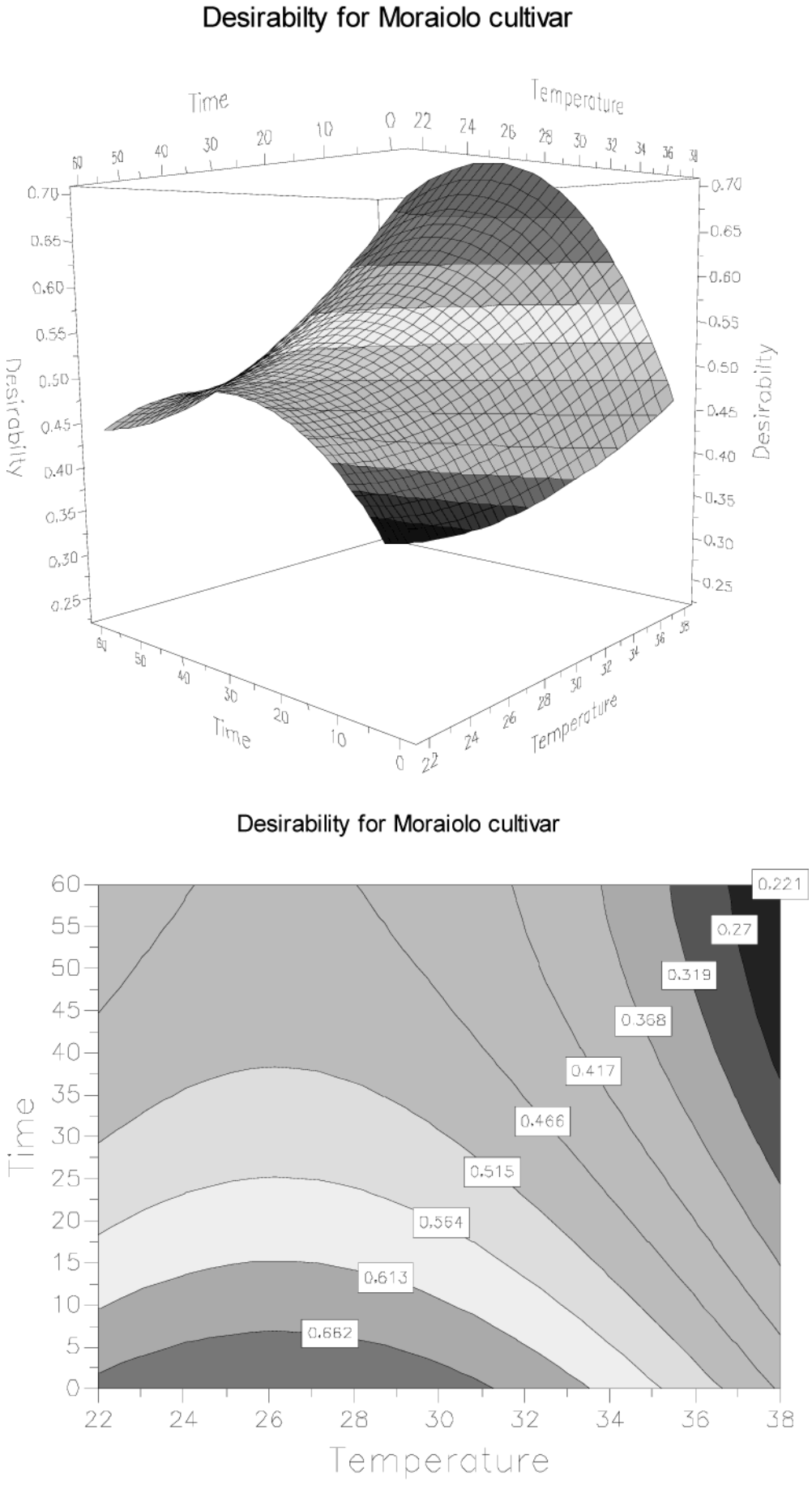


Figure 6. RSM and contour plots obtained using the PLS built to optimize the temperature and TEOPAC during malaxation in the Moraiolo cultivar.

Moraiolo cultivars, respectively (**Figures 3** and **4**). The former explains with three components 56% of the total variance while the latter explains 55% of the total variance with the same number of principal components. According to these results, eight compounds with the highest loadings such as 1,1-dimethyl-2-(2-methyl-2-propenyl)cyclopropane (i), (*Z*)-2-hexenal, hexyl acetate, (*Z*)-4-hexyl acetate, 1-hexanol, (*Z*)-3-hexen-1-ol, *o*-diphenols, and 3,4-DHPEA-EDA were selected for the Frantoio cultivar (**Figure 4**). The following RSM model, which explains 89% of the total variance with two PLS components, has a saddle shape, and the better operative conditions can be found at the lowest temperature investigated (22 °C) for 30 min of TEOPAC (**Figure 5**). For the Moraiolo cultivar, temperature and TEOPAC during malaxation were optimized using, in addition to the sensory descriptors above-discussed, the following phenolic and volatiles compounds: 1,1-dimethyl-2-(2-methyl-2-propenyl)cyclopropane (i), 1-penten-3-ol, benzyl alcohol, (*Z*)-4-hexenyl acetate, (*Z*)-3-hexen-1-ol, *o*-diphenols, *p*-HPEA-EDA, and 3,4-DHPEA-EDA (**Figure 2**). Furthermore, the resulting RSM model, which explains 95% of the total variance with two PLS components, also this time showed a saddle and the better operative conditions of malaxation were 26 °C and 0 min of TEOPAC (**Figure 6**).

DISCUSSION

As reported in previous papers (*12*, *18*−*24*), the selective control of oxidoreductases such as PPO, POD, and LPO during processing is a new aspect of oil processing, strictly related to the healthy and sensory quality of VOO. The results reported in this work confirm that the TEOPAC can be used to perform a selective control of deleterious enzymes such as PPO and POD preserving the activity of LPO. Moreover, the space distribution of the RSM surface showed a strong incidence of the temperature in the optimization model. In fact, the desirability's value highly decreased when the temperature increased in both cultivars studied. On the contrary, the modification of TEOPAC showed a variation of the desirability's values lower in comparison to temperature. These results are in agreement with previous papers, which reported the deleterious effect of the high temperature on several sensory and healthy markers of VOO quality, such as volatile compounds and hydrophilic phenols (*14*, *17*, *18*, *40*, *41*).

Two different biochemical mechanisms explain these results. Concerning the phenolic antioxidants, the increased temperature improves the oxidation of these compounds due to the PPO and POD activities (*12*, *17*, *18*). The reduced concentration of oxygen in the pastes, obtained replacing air with N_2 in the headspace of malaxer during processing, can inhibit these enzymes minimizing the oxidative degradation of phenolic compounds during processing. Nevertheless, the most negative effects of high malaxation temperatures were related to the genesis of volatile compounds due to the LPO pathway. As reported by Salas and Sànchez (*41*), temperatures higher than 25 °C reduce the activity of two basic enzymes involved in the LPO pathway, such as LPO and hydroperoxide lyase. The partial inhibition of these enzymes reduces the formation of C6 saturated and unsaturated aldehydes, alcohols, and esters responsible for cut grass and floral sensory notes of VOO and, as a consequence, justifies the negative modifications of VOO flavor associated to processing temperatures higher than 25 °C (*14*, *18*, *40*).

Even so, the significant differences observed in terms of optimal temperatures and TEOPAC during malaxation for Moraiolo and Frantoio confirm that the cultivar has a fundamental importance for the final composition of volatile and phenolic compounds of VOO (*12*, *16*, *40*) but, at the same time, prove that to produce high quality VOO, the processing parameters must be differentiated according to the olive cultivar. In fact, the strong variations in olive composition, obtained by different cultivars, in terms of phenolic precursors of VOO, such as oleuropein and demethyloleuropein, and PPO, POD, and LPO activities are well-known (*6*, *10*−*12*, *14*, *16*).

ABBREVIATIONS USED

TEOPAC, time of exposure of olive pastes to air contact; VOO, virgin olive oil; RSM, response surface modeling; PPO, polyphenolidoxidase; POD, peroxidase; LPO, lipoxygenase; CCD, central composite design; SPME, solid phase microextraction; ANOVA, analysis of variance; PCA, principal components analysis.

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