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Changes in Volatile and Phenolic Compounds with Malaxation Time and Temperature during Virgin Olive Oil Production

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Virgin olive oils produced at wide ranges of malaxation temperatures (15, 30, 45, and 60 °C) and times (30, 60, 90, and 120 min) in a complete factorial experimental design were discriminated with stepwise linear discriminant analysis (SLDA) revealing differences with processing conditions. Virgin olive oils produced at 15 and 60 °C for 30 min showed the most significant ($p < 0.01$) differences. Discrimination was based upon volatile and phenolic compounds detected in olive oils, peroxide value (PV), free fatty acids (FFA), ultraviolet (UV) absorbances, and oil yield. There were different discriminating variables for processing conditions illustrating the dependence of virgin olive oil quality on malaxation time and temperature. Volatile compounds were the dominant discriminating variables. Common oxidation indicators of olive oil (PV, K_{232}, and K_{270}) were not among the variables that significantly ($p < 0.01$) changed with malaxation time and temperature. Variables that discriminated both malaxation time and temperature were hexanal, 3,4-dihydroxyphenyl ethyl alcohol−decarboxymethyl elenolic acid dialdehyde (3,4-DHPEA-DEDA) and FFA, whereas 1-penten-3-ol, *E*-2-hexenal, octane, tyrosol, and vanillic acid significantly ($p < 0.01$) changed with temperature only and *Z*-2-penten-1-ol, (+)-acetoxypinoresinol, and oil yield changed with time only. Virgin olive oil quality was significantly influenced by malaxation temperature, whereas oil yield discriminated malaxation time. This study demonstrates the two modes of hexanal formation: enzymatic and nonenzymatic during virgin olive oil extraction.

KEYWORDS: Virgin olive oil; volatile and phenolic compounds; processing conditions; hexanal formation; stepwise linear discriminant analysis (SLDA); time−temperature response surfaces; contour plots

INTRODUCTION

Mechanical oil extraction affects formation of volatile compounds and the release of phenolic antioxidants, which greatly influence the quality of virgin olive oil (*1*, *2*). During mechanical extraction of virgin olive oil, the olive paste, formed after the fruit has been crushed, is mixed in a process called malaxation (*3*, *4*). Malaxation induces the coalescence of minute oil droplets into large droplets and the subsequent formation of a continuous lipid phase, which is then separated from the paste (*5*). To assist in the coalescence process, the temperature of the paste is raised to decrease the viscosity of the mix. Higher oil yields are obtained by malaxing at higher temperatures (*6*), but oil quality may deteriorate if the temperature is too high. Thus, a balance between oil yield and quality must be achieved. Furthermore, higher malaxation temperatures and shorter malaxation times may be advantageous in increasing oil yield and daily production capacity, respectively, provided that such a combination retains the extra virgin status of the oil.

Several studies have shown that malaxation time and temperature are important factors strongly influencing the quality and yield of virgin olive oil (*4*, *5*, *7*−*9*). These studies have investigated the effect of malaxation time and temperature either as sole variables or in combination with other variables, for example, cultivar. For instance, Ranalli's group have studied the effect of malaxation temperature (*7*) and time (*5*) on oil quality and yield from Caroleo, Leccino, and Dritta cultivars. Several studies (*4*, *9*, *10*) have investigated the effect of both malaxation time and temperature on olive oil quality during mechanical extraction. Across all of these studies, limited ranges of malaxation temperature (20−38 °C) and time (15−90 min) have been reported (*4*, *5*, *7*, *9*−*11*).

Malaxing olive paste at 30 °C for at least 45 min (*5*) produced both pleasant green extra virgin olive oil and satisfactory oil extraction outputs, but malaxing at 35 °C introduced numerous defects in the oil without substantially increasing oil yield (*7*). Conversely, it has been reported (*12*) that malaxing at 35 °C for 60 min produces the best quality olive oil and yield. Anecdotal evidence suggests that some processors are malaxing at temperatures significantly lower than 25 °C mainly due to low ambient temperatures early in the processing day, without

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guidance as to how this may affect oil yield and quality. Little is known about the changes extreme processing conditions can introduce into virgin olive oil. Morales and co-workers (*9*) suggested an alternative way of obtaining pleasant green olive oils through processing at higher temperatures (>35 °C) with minimum malaxation times (<30 min). A recent paper by Garcia et al. (*13*) on the treatment of olives with hot water (60 and 72 °C) prior to oil extraction raises the question of how high a temperature is possible before oil quality deteriorates below the virgin classification. In the case of oil extraction from Coratina olive fruit (*4*), investigations of extremely high malaxation temperatures (35 and 45 °C) were not carried out due to technical troubles.

In the evaluation of the various malaxation time and temperature combinations for processing, different parameters have been used to describe the effect of these variables on virgin olive oil quality. For example, some studies have focused on volatile compounds only (*9*), some on volatile compounds and sensory analysis (*4*), and some on phenolic and volatile compounds combined with sensory analysis (*10*); in contrast, Ranalli's group (*5*, *7*) considered a diverse array of measures including quality indices, oil yield, and volatile and phenolic compounds as well as sensory analysis.

Because of wide variations in experimental designs, different ranges of malaxation times and temperatures, and the different parameters used to define the changes of virgin olive oil quality, it is difficult to ascertain the key oil quality parameters that are indicative of, or predictors for, quality changes in the oil due to a combination of malaxation time and temperature. For instance, studies that considered a wide spectrum of quality attributes (*5*, *7*) did not consider the simultaneous effect of malaxation time and temperature. On the other hand, a study (*9*) with a sound statistical experimental design and data analysis that simultaneously investigated the effect of malaxation time and temperature considered only volatile compounds and no other quality parameters.

The objective of this study was to systematically identify volatile and phenolic compounds that significantly ($p < 0.01$) change with simultaneous changes in malaxation time and temperature during virgin olive oil production. This study is unique as it applies complete four-level factorial combinations of malaxation time and temperature over a wide range to explore changes in volatile and phenolic compounds, quality indices, and oil yield in a single study. The systematic approach of using response surfaces, contour plots, and multivariate analysis applied in this study is rare in studies of olive oil processing conditions. The extremes of malaxation temperatures and times can reveal some of the changes in virgin olive oil quality that can be explored for the benefit of possible future advancement in olive-processing technology.

MATERIALS AND METHODS

Materials. Reagents and phenolic and volatile standards from the indicated sources were used without further purification: acetic acid (Biolab, Sydney, Australia); hexane and methanol (Mallinckrodt Chemicals, Paris, France); acetonitrile (J. T. Baker, Phillipsburg, NJ); formic acid, caffeic acid, *p*-coumaric acid, and gallic acid (Sigma, St. Louis, MO); tyrosol (Aldrich, Milwaukee, WI); hydroxytyrosol (Sapphire Bioscience, Sydney, Australia); oleuropein (Extrasynthese, Genay, France). Verbascoside was kindly donated by Prof. Okuyama of Chiba University, Japan. Standards were prepared in methanol + water (50 + 50 v/v) and filtered through 0.45 μm plastic nonsterile filters prior to chromatographic analysis. Grade 1 water (ISO3696) purified through a Milli-Q water system was used for chromatographic preparations.

The volatile standards used were as follows: pentanal, *E*-2-hexenal, and nonanol (Merck, Hohenbrunn, Germany); hexanal, heptanal, *E*-2-octenal, *E*-2-nonenal, 1-penten-3-ol, 2-penten-1-ol, heptanol, octanol, hexyl acetate, methyl isobutyl ketone (MIBK), and 2-nonanone (Aldrich, Milwaukee, WI); octanal, octane, nonane, decane, undecane and dodecane (Sigma, St. Louis, MO); benzaldehyde (Ajax Chemicals, Auburn, Australia); ethanol (Biolab, Sydney, Australia); ethyl acetate (Mallinckrodt Chemicals, Paris, France); and hexanol (Riedel de Haen, Seelze, Germany).

Table 1. Independent Variables for the Characterization of Malaxation Time and Temperature in Virgin Olive Oil from Frantoio Fruit

volatile compound	phenolic compound	other variables
acetic acid	tyrosol	free fatty acid
1-penten-3-one	vanillic acid	peroxide value
1-penten-3-ol	3,4-DHPEA-DEDA[a]	K_{232}
Z-2-penten-1-ol	(+)-acetoxypinoresinol	K_{270}
octane	oleuropein aglycone	ΔK
hexanal	hemiacetal of oleuropein	oil yield
E-2-hexenal		
E-2-hexen-1-ol		
hexanol		
6-methyl-5-hepten-2-one		
2-pentyl furan		
octanal		
hexyl acetate		
octanol		

[a] 3,4-Dihydroxyphenyl ethyl alcohol–decarboxymethyl elenolic acid dialdehyde.

Reagents were used in the determination of peroxide values (PV), ultraviolet (UV) absorbances (K_{232}, K_{270}, and ΔK) and free fatty acid (FFA) were as follows: chloroform, acetic acid, and potassium iodide (Biolab, Sydney, Australia), sodium thiosulphate (Asia Pacific Speciality Chemicals Ltd., Seven Hills, Australia), and starch (Scharlau Chemie S.A., Barcelona, Spain) for PV; cyclohexane spectrophotometric grade (Sigma, St. Louis, MO) for UV absorbances; and propan-2-ol (Mallinckrodt Chemicals, Paris, France), sodium hydroxide (Ajax Chemicals, Auburn, Australia), and phenolphthalein indicator (Sigma, St. Louis, MO) for FFA determination.

Fruit and Oil Samples. Frantoio olive fruit (50 kg) was hand-picked from Cookathama farm, near Darlington Point in southwestern New South Wales, Australia, during the 2004-harvest season. The fruit was harvested at 34 weeks after flowering when the skin color was red to black (maturity index = 3.7 ± 0.1). The maturity index (MI) was assessed using the method of the Instituto Nacional de Investigaciones Agronomicas, Estacion de Jaen (Spain), and described by IOOC (*14*). The oil extracted from the olive fruit was stored (<1 week) in the dark at room temperature prior to analysis of quality indices (PV, FFA, *K* values) and volatile and phenolic compounds.

Methods. *Olive Oil Extraction.* Forty-eight samples (16 treatments × 3 replicates × 1 kg of olive fruit/extraction) were extracted using a cold-press Abencor extraction unit (Abencor, Spain). Olive oil was extracted according to the time−temperature processing conditions based on a four-level (4^2) complete factorial experimental design with malaxation time (30, 60, 90, and 120 min) and malaxation temperature (15, 30, 45, and 60 °C) as factors. Water (100 mL/kg of fruit) was added at processing temperature to improve the rheology of the paste. The oil and paste mixture was separated into two phases after centrifugation, and the top oil layer was decanted into foil-covered pharmaceutical bottles (200 mL) prior to analysis.

Determination of Quality Parameters. Determination of PV (milliequivalents of O_2 per kilogram of oil), FFA (percent oleic acid), and UV absorbances (*K* values) was performed according the standard EC and IOOC methods (*15*, *16*). The UV absorbances were measured at four wavelengths (232, 266, 270, and 274 nm) in spectrophotometric grade cyclohexane (Sigma, St. Louis, MO). The parameters K_{232} and K_{270} were calculated from UV absorbance at 232 and 270 nm, respectively, whereas ΔK was calculated from the absorbances at 266, 270, and 274 nm. These parameters (PV, FFA K_{232}, K_{270}, and ΔK) are commonly used to assess the quality of olive oil (*16*) and were used as independent variables in the characterization of malaxation time and temperature (**Table 1**).

Qualitative and Quantitative Analysis of Volatile Compounds. Qualitative and quantitative analysis of volatile compounds (micrograms per gram) was performed using solid-phase microextraction−gas chromatography−mass spectrometry (SPME-GC-MS) and solid-phase microextraction−gas chromatography−flame ionization detection (SPME-GC-FID), respectively, as described in earlier papers (*17*, *18*). Volatile compounds identified in virgin olive oil are listed in **Table 1**.

Qualitative and Quantitative Analysis of Phenolic Compounds. Qualitative and quantitative analysis of phenolic compounds (micrograms per gram) in **Table 1** was performed using liquid chromatography−electrospray ionization−mass spectrometry (LC-ESI-MS) and high-performance liquid chromatography−diode array detection (HPLC-DAD), respectively, as described earlier (*17*).

Statistical Data Analysis. Stepwise linear discriminant analysis (SLDA) was used to identify malaxation time and temperature patterns with quality indices and concentrations of volatile and phenolic compounds (**Table 1**) as independent variables. Unlike the other multivariate exploratory procedures, standardizing the variables in linear discriminant analysis has no effect on the outcome but merely rescales the axes (*19*); hence, no transformation was performed on the independent variables in our data set.

SLDA involves variable selection, testing significance of discriminant functions, and pattern recognition (*20*). Variable selection was based on a stringent criterion ($p = 0.01$) for entry of an independent variable (**Table 1**) into a discriminant function to select the most likely discriminating variables of malaxation times and temperatures. The Wilks' lambda statistic tested the significance of the discriminant functions; in this test, values close to 0 indicate different group means, whereas values close to 1 indicate similar group means (*20*). The group differences explained by the canonical discriminant functions should be significant ($p < 0.05$) to necessitate discrimination in the underlying dimension (*20*). The outcome of discriminant analysis in recognizing malaxation time and temperature patterns was visualized in two dimensions by combined-group scatter plots, where the *x*-axis plots the values of discriminant function 1 and the *y*-axis plots the values of discriminant function 2.

Quantitative data generated from a complete factorial design are explained with respect to changes within the variables and in relation to other virgin olive oil components. Notable and interesting changes within a discriminating variable at different combinations of malaxation times and temperatures during virgin olive oil production were illustrated through response surfaces and contour plots generated using S-PLUS 6.1 (Insightful Corp., Reinach, Switzerland). Significant ($p < 0.01$) differences of variables in relation to other virgin olive oil components were examined using one-way ANOVA post hoc multiple-comparison tests using Duncan's test with SPSS 11.5 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Virgin olive oils extracted from Frantoio fruit were used to identify volatile and phenolic compounds that significantly ($p < 0.01$) change with malaxation time and temperature. To identify significant ($p < 0.01$) changes in virgin olive oil, multivariate statistical methods were applied. The global indicators of changes in virgin olive oil quality (FFA, PV, K_{232}, K_{270}, and ΔK), olive oil phenolic compounds, volatile compounds, and oil yield (**Table 1**) with processing have been identified, and the changes for selected individual phenolic and volatile compounds with malaxation time and temperature have also been explored.

Malaxation Time and Temperature Discrimination. Discrimination of malaxation time and temperature with SLDA was undertaken to recognize patterns and identify discriminating variables. The highest cumulative percent variance explained (99.5%) was observed for malaxation time discrimination (**Table 2**), indicating an overall success in the discrimination. However, the strong influence of discriminant function 1, explaining 98.5% of the variance (**Table 2),** limited malaxation time discrimination along the *y*-axis (**Figure 1**). The Wilks' lambda statistic for discriminant function 2 was close to 1, and the means of the scores were not significantly different ($p > 0.05$), consistent with earlier reports (*5*) on the limited influence of malaxation time during virgin olive oil production. Malaxing for 30 min produced oil that was separated from the other malaxation times (60, 90, and 120 min), whereas malaxing for 90 and 120 min formed a cluster that was not mutually exclusive (**Figure 1**).

Table 2. Discriminating Variables for Malaxation Times and Temperatures during Virgin Olive Oil Production

discriminated group	% variance explained (function 1, V_1)	% variance explained (function 2, V_2)	% variance explained (cumulative)	discriminating variable
time	98.5[a]	1.0	99.5	*Z*-2-penten-1-ol hexanal 3,4-DHPEA-DEDA[b] acetoxypinoresinol FFA[c] yield
temperature	69.0[a]	21.8[a]	90.8	1-penten-3-ol hexanal *E*-2-hexenal octane tyrosol vanillic acid 3,4-DHPEA-DEDA[b] FFA[c]

[a] Wilks' lambda statistic significantly ($p < 0.05$) different. [b] 3,4-Dihydroxyphenyl ethyl alcohol–decarboxymethyl elenolic acid dialdehyde. [c] Free fatty acid as percent oleic acid.

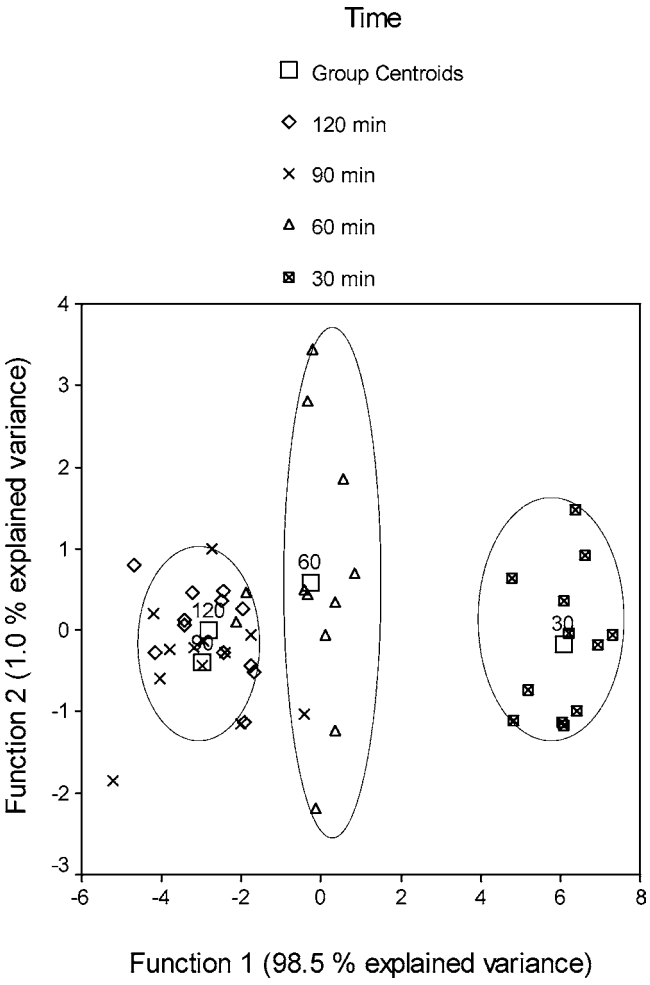

Figure 1. Scatter plot for the first two canonical discriminant functions separating malaxation time.

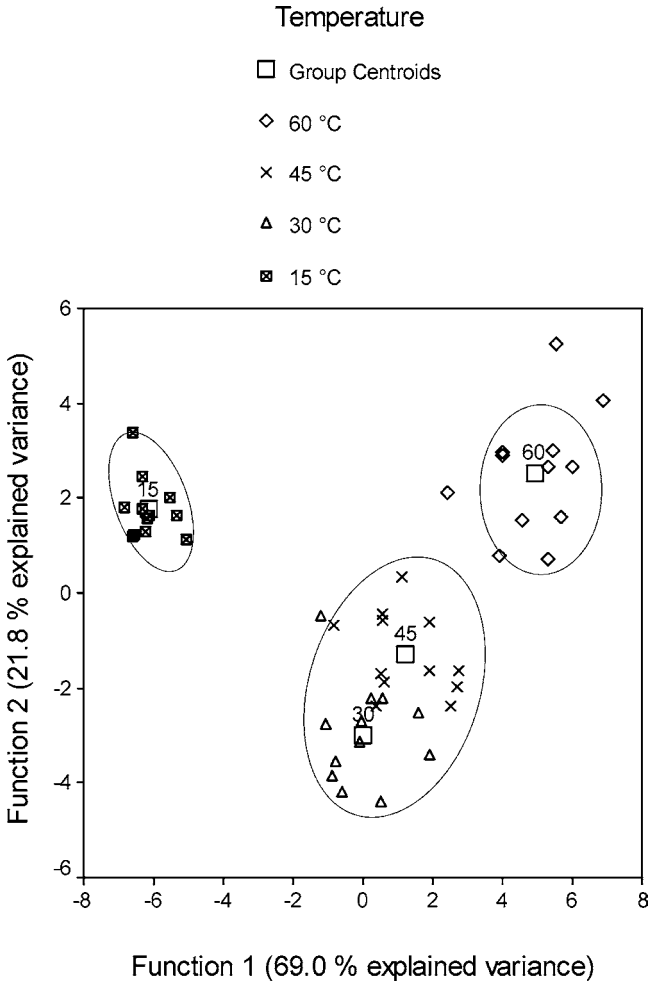

Figure 2. Scatter plot for the first two canonical discriminant functions separating malaxation temperature.

The formation of a cluster indicates that there were no significant ($p < 0.01$) differences in the oils produced at 90 and 120 min, whereas malaxing for 30 min produced a significantly ($p < 0.01$) different virgin olive oil.

Discrimination based on malaxation temperature separated the group centroids, apart from 30 and 45 °C, which formed a cluster (**Figure 2**). Malaxing at 15 and 60 °C displayed a distinct difference in the *x*-axis (**Figure 2**), indicating differences in quality and composition of minor components with processing at the respective malaxation temperatures. The extreme temperatures (15 and 60 °C) were also separated from the intermediate malaxation temperatures (30 and 45 °C) on the *y*-axis (**Figure 2**).

Malaxation temperature explained a lower cumulative variance (90.8%) than malaxation time (99.5%) but had a higher percent of variance explained for function 2 (**Table 2**). Malaxation temperature separated the group centroids better than malaxation time (**Figures 1** and **2**). Other studies (*4*, *7*, *10*, *11*) have shown that malaxation temperature is important in the production of premium quality virgin olive oil.

Parameters That Discriminate Malaxation Times. Discrimination of malaxation times along the *x*-axis of **Figure 1** is given in the linear discriminant equation (V_1, eq i) below.

$$V_1 = 1.29[\text{hexanal}] + 1.44[\text{DHPEA-DEDA}] + 0.83 \times \text{FFA} - 1.12[Z\text{-2-penten-1-ol}] - 0.73[\text{acetoxypinoresinol}] - 1.65 \times \text{yield} \quad \text{(i)}$$

Table 3. Variables Separating Individual Malaxation Times and Temperatures

	discriminating variables
time	
30 min	hexanal, 3,4-DHPEA-DEDA,[a] and FFA[b]
60 min	*Z*-2-penten-1-ol, hexanal, acetoxypinoresinol, and FFA[b]
90 min	*Z*-2-penten-1-ol, acetoxypinoresinol, and yield
120 min	*Z*-2-penten-1-ol, acetoxypinoresinol ,and yield
temperature	
15 °C	1-penten-3-ol, *E*-2-hexenal, and vanillic acid
30 °C	*E*-2-hexenal
45 °C	tyrosol and FFA[b]
60 °C	hexanal, octane, and 3,4-DHPEA-DEDA[a]

[a] 3,4-Dihydroxyphenyl ethyl alcohol–decarboxymethyl elenolic acid dialdehyde. [b] Free fatty acid as percent oleic acid.

A malaxation time of 30 min, which is on the positive side of the scatter plot (**Figure 1**), was discriminated by hexanal, 3,4-DHPEA-DEDA, and FFA. Long malaxation times (90 and 120 min), which lie on the negative side of the scatter plot, were discriminated by *Z*-2-penten-1-ol, acetoxypinoresinol, and oil yield. Discrimination on the *y*-axis was not distinct as almost all of the malaxation times lie along the axis at function 2 equal to 0 (**Figure 1**). Nevertheless, there was a slight separation between the group centroids for malaxing at 30 and 60 min, which lie on the negative and positive sides, respectively, of function 2. Discrimination of malaxation times along function 2 of **Figure 1** is given in the linear discriminant equation (V_2, eq ii) below.

$$V_2 = 0.58[\text{hexanal}] + 0.52[Z\text{-2-penten-1-ol}] + 0.10 \times \text{FFA} + 0.16[\text{acetoxypinoresinol}] - 0.34 \times \text{yield} - 0.44[\text{DHPEA-DEDA}] \quad \text{(ii)}$$

With the separation of the group centroids for malaxing at 30 and 60 min alluded to above, oil yield and 3,4-DHPEA-DEDA, variables with negative coefficients in eq ii, discriminated virgin olive oil with 30 min of malaxation time, whereas hexanal, *Z*-2-penten-1-ol, FFA, and acetoxypinoresinol discriminated virgin olive oil with 60 min of malaxation time, which lies on the positive side of function 2 (**Figure 1**).

The validity of discrimination was checked by examining the variance explained and the significance of separating the malaxation time group centroids. The discrimination by function 1, V_1 explained more variance (98.5%) than function 2, V_2 (1.0%), with a nonsignificant ($p > 0.05$) Wilks' lambda statistic (**Table 2**). The nonsignificant Wilks' lambda statistic confirmed the poor separation of malaxation times on the *y*-axis (**Figure 1**), which is explained by discriminant function 2, V_2 (**Table 2**). Compilation of discriminating variables that separated individual malaxation times (**Table 3**) was accomplished by considering the significance of the discriminant function and the percentage variance explained by the function in the scatter plot (**Figure 1**).

A malaxation time of 30 min produced significantly different virgin olive oils from those malaxed for 60, 90, and 120 min (**Figure 1**). Quantitative data in **Table 4** are consistent with this observation for 3,4-DHPEA-DEDA, which discriminated 30 min of malaxation (**Table 3**) when the concentrations at 30 min were significantly ($p < 0.01$) higher than those at the other malaxation times. Apart from FFA, malaxation time discriminating variables (**Table 2**) are not directly associated with virgin olive oil quality. Results of this study are consistent with earlier reports (*5*, *10*) of observed minimal influence of malaxation time on the quality of virgin olive oil.

It can be observed in **Table 2** that concentrations of hexanal, 3,4-DHPEA-DEDA, and FFA changed with both malaxation

Table 4. Quantitative Data for Different Malaxation Time–Temperature Combinations in the Production of Virgin Olive Oil[a]

processing conditions	hexanal (μg/g)	octane (μg/g)	3,4-DHPEA-DEDA[b]	tyrosol (μg/g)	FFA[c]	PV[d]	oil yield (% m/m)
30 min/15 °C	27 ± 3cd	0.30 ± 0.01ab	<0.1	2.6 ± 0.6a	0.35 ± 0.02ab	15.3 ± 0.8ab	34.6 ± 0.6a
60 min/15 °C	29 ± 8d	0.37 ± 0.04ab	<0.1	3.4 ± 0.4a	0.37 ± 0.01abc	14 ± 2ab	40.0 ± 0.1ef
90 min/15 °C	25 ± 2abcd	0.31 ± 0.02ab	<0.1	3.2 ± 0.4a	0.37 ± 0.01abc	14 ± 2ab	42.0 ± 0.1gh
120 min/15 °C	26 ± 4bcd	0.36 ± 0.04ab	<0.1	3.5 ± 0.2a	0.37 ± 0.02abc	14 ± 1ab	43.0 ± 0.1hi
30 min/30 °C	21.8 ± 0.3abcd	0.16 ± 0.01a	2.3 ± 0.6b	5.5 ± 0.5b	0.38 ± 0.01bc	13.7 ± 0.4ab	38.0 ± 0.1cd
60 min/30 °C	19 ± 2abc	0.16 ± 0.01a	0.5 ± 0.9a	6.1 ± 1.2bc	0.37 ± 0.02bc	16 ± 2b	40.3 ± 0.6ef
90 min/30 °C	20 ± 3abc	0.25 ± 0.04a	0.6 ± 0.9a	6.7 ± 0.6bc	0.42 ± 0.02de	16 ± 2ab	44.3 ± 0.6j
120 min/30 °C	24 ± 1abcd	0.30 ± 0.03ab	<0.1	5.8 ± 1.0bc	0.43 ± 0.01e	15 ± 1ab	44.0 ± 0.1ij
30 min/45 °C	18 ± 2abc	0.26 ± 0.03ab	2.8 ± 0.6b	5.2 ± 0.4b	0.34 ± 0.01a	12.4 ± 0.9a	33.8 ± 0.2a
60 min/45 °C	21 ± 4abcd	0.51 ± 0.08abc	0.5 ± 0.9a	6.1 ± 0.4bc	0.38 ± 0.01bc	14.5 ± 0.9ab	37.0 ± 1.0bc
90 min/45 °C	16 ± 2a	0.52 ± 0.07abc	0.5 ± 0.9a	5.5 ± 0.8b	0.37 ± 0.01abc	13 ± 1ab	38.4 ± 0.5d
120 min/45 °C	18 ± 3ab	0.8 ± 0.2cd	<0.1	5.7 ± 0.4b	0.39 ± 0.01cd	13.5 0.4ab	39.6 ± 0.6e
30 min/60 °C	23.6 ± 1.0abcd	0.6 ± 0.1bc	2.8 ± 0.3b	6.0 ± 1.6bc	0.39 ± 0.01bc	13.0 ± 0.8ab	36.3 ± 0.6b
60 min/60 °C	25 ± 5bcd	1.0 ± 0.2cd	0.6 ± 1.1a	7.7 ± 0.6c	0.43 ± 0.02e	12.7 ± 0.7ab	40.3 ± 0.6ef
90 min/60 °C	18 ± 2ab	1.4 ± 0.2e	<0.1	6.7 ± 0.4bc	0.43 ± 0.01e	13 ± 2ab	42.0 ± 0.1gh
120 min/60 °C	18 ± 2ab	1.7 ± 0.4e	<0.1	6.9 ± 0.6bc	0.45 0.01e	14 ± 1ab	41.0 ± 0.1fg

[a] Different letters in a column indicate significantly ($p < 0.01$) different mean ± standard deviation of triplicate determinations. [b] Concentration of 3,4-dihydroxyphenyl ethyl alcohol–decarboxymethyl elenolic acid dialdehyde in micrograms per gram. [c] Free fatty acid as percent oleic acid. [d] Peroxide value expressed as milliequivalents of oxygen per kilogram of oil.

time and temperature, whereas concentrations of *Z*-2-penten-1-ol, (+)-acetoxypinoresinol, and oil yield significantly ($p < 0.01$) changed with time only; concentrations of 1-penten-3-ol, *E*-2-hexenal, octane, tyrosol, and vanillic acid changed with temperature only. The different discriminating variables of processing conditions illustrate the dependence of virgin olive oil quality on malaxation time and temperature.

Parameters That Discriminate Malaxation Temperatures. Virgin olive oil produced at different malaxation temperatures was separated by selected variables (**Table 2**) and not all parameters measured in olive oil from Frantoio fruit (**Table 1**). Discrimination of malaxation temperatures along the *x*-axis of **Figure 2** is given in the linear discriminant equation (V_1, eq iii) below.

$$V_1 = 0.32[\text{octane}] + 0.14[\text{hexanal}] + 0.49 \times \text{FFA} + 0.68[\text{tyrosol}] + 1.26[\text{DHPEA-DEDA}] - 0.37[\text{1-penten-3-ol}] - 0.13[E\text{-2-hexenal}] - 0.96[\text{vanillic acid}] \quad \text{(iii)}$$

Discriminating variables with positive coefficients (octane, hexanal, FFA, tyrosol, and 3,4-DHPEA-DEDA) discriminated high malaxation temperatures (45 and 60 °C), which are on the positive side of **Figure 2**. Low-temperature (15 °C) malaxation, which lies on the negative side of **Figure 2**, is discriminated by compounds with negative coefficients (1-penten-3-ol, *E*-2-hexenal, and vanillic acid). Discrimination by V_1 explained more variance (69.0%) than V_2 (21.8%). Discriminant analysis on the *y*-axis (V_2, eq iv) separates the extremes of malaxation temperatures (15 and 60 °C), which lie on the positive side of the *y*-axis (**Figure 2**), from the intermediate temperatures (30 and 45 °C).

$$V_2 = 1.08[\text{octane}] + 0.19[\text{1-penten-3-ol}] + 1.91[\text{hexanal}] + 0.09[\text{vanillic acid}] + 0.50[\text{DHPEA-DEDA}] - 1.87[E\text{-2-hexenal}] - 0.44 \times \text{FFA} - 0.38[\text{tyrosol}] \quad \text{(iv)}$$

Extreme malaxation temperatures (15 and 60 °C) on the positive side of the *y*-axis (**Figure 2**) are discriminated by octane, 1-penten-3-ol, hexanal, vanillic acid, and 3,4-DHPEA-DEDA. *E*-2-Hexenal, FFA, and tyrosol discriminate intermediate malaxation temperatures (30 and 45 °C) on the negative side of the *y*-axis (**Figure 2**). Discriminating variables that significantly ($p < 0.01$) discriminated individual malaxation temperatures were deduced (**Table 3**) from discriminant functions V_1 and V_2, above.

As shown in **Table 3,** virgin olive oils produced by malaxation at lower temperatures (15 and 30 °C) were discriminated by compounds (1-penten-3-ol, *E*-2-hexenal, and vanillic acid) associated with the freshness of olive oil (*4*, *7*, *9*, *21*). Similarly, high malaxation temperatures (45 and 60 °C) produced oils that were discriminated by variables (hexanal, octane, tyrosol, and FFA) that are often associated with low-quality olive oil (*22*, *23*). Interestingly, 3,4-DHPEA-DEDA is also among the high-temperature-discriminating variables. Increasing levels of this phenolic compound may protect the oil from oxidation, consistent with the nonsignificant changes in PV and *K* values at higher temperatures. The identification of high malaxation temperature discriminating variables is consistent with quantitative data (**Table 4**), which show that some of these variables (octane, 3,4-DHPEA-DEDA, tyrosol, and FFA) have significantly ($p < 0.01$) higher values at high temperatures (45 and 60 °C) than at low temperatures (15 and 30 °C).

Effect of Malaxation Time–Temperature Combination on Virgin Olive Oil Quality and Yield. Malaxation time and temperature significantly changed the volatile and phenolic profile of virgin olive oil, which are important in the sensory quality of virgin olive oil (*2*, *23*, *24*) in addition to changing FFA content, which is used to grade olive oil into different commercial classes (*16*). Hence, it is not surprising that FFA and volatile and phenolic compounds appear as discriminating variables in **Table 2**. Common oxidation indicators of olive oil, PV, K_{232}, and K_{270} were not among the variables that significantly ($p < 0.01$) discriminated malaxation times and temperatures. This observation is supported by quantitative data (**Table 4**) that show PV having minimum significant ($p < 0.01$) changes with both malaxation time and temperature. Previous studies (*7*) have shown an acceleration of lipolysis and oxidation processes with an increase in malaxation temperatures. The nonsignificant ($p > 0.01$) influence of oxidation indicators compared to the significant ($p < 0.01$) influence of FFA indicates that lipolysis is more important than oxidation during virgin olive oil production.

The processing parameters also affected oil yield. Malaxation time showed a highly significant effect on oil yield (**Table 2**) with a 30 min malaxation time producing significantly ($p < 0.01$) less oil (**Table 4**) than the other malaxation times (60, 90, and 120 min) at all malaxation temperatures (15, 30, 45, and 60 °C). Malaxing at 45 °C had significantly ($p < 0.01$) lower yields compared to other temperatures (15, 30, and 60

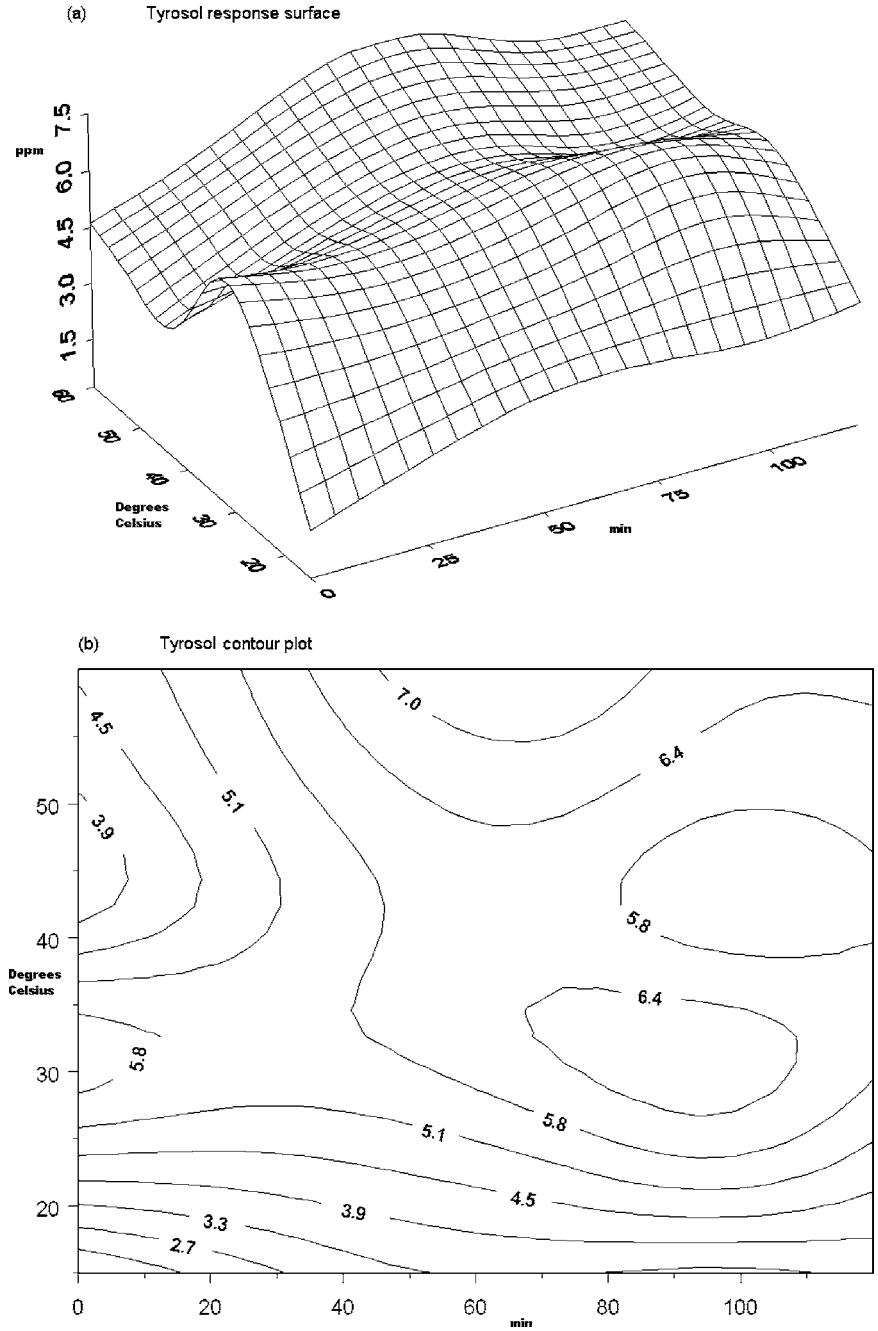

Figure 3. (**a**) Malaxation time–temperature response surface for tyrosol (ppm is μg/g of oil). (**b**) Malaxation time–temperature contour plot for tyrosol with numbers in the plot representing concentration in μg/g of oil.

°C), which might be due to change in the rheology of the paste and increased interactions between lipids, proteins, and carbohydrates, culminating in the entrapment of oil in the olive paste. Amirante et al. (*6*) observed that raising the temperature of the olive paste reduces the viscosity, leading to better separation and higher oil yields; this was not the case in our study as malaxation time had a more significant ($p < 0.01$) effect on oil yield. Earlier studies (*5*) reported a small decrease in oil yields with time from 60 to 75 min, which was attributed to a reformation of oil−water or oil−solid emulsions. In our study, decrease in yield with time was not observed (**Table 4**). These variations may possibly be attributed to the different paste rheologies probably arising from different cultivars and maturity stages.

Changes in Phenolic Compounds with Processing. In addition to affecting the volatile and phenolic compounds of virgin olive oil in relation to other virgin olive oil components, malaxation time and temperature affected concentrations of individual compounds in different ways. In the case of tyrosol, it is observed from the response surface (**Figure 3a**) that concentration predominantly increases with malaxation temperature.

A closer look at the contour plot reveals that the concentration predominantly increases along the temperature axis (**Figure 3b**). The low density of contour lines at temperatures above 30 °C (**Figure 3b**) indicates less sensitivity of tyrosol formation toward high malaxation temperatures. The formation of tyrosol is sensitive at low temperatures, and quantitative data (**Table 4**) show a significant ($p < 0.01$) difference in tyrosol concentration at 15 °C with minimal significant differences at higher malaxation temperatures. No significant ($p > 0.01$) differences of tyrosol concentration with malaxation time (**Table 4**) were

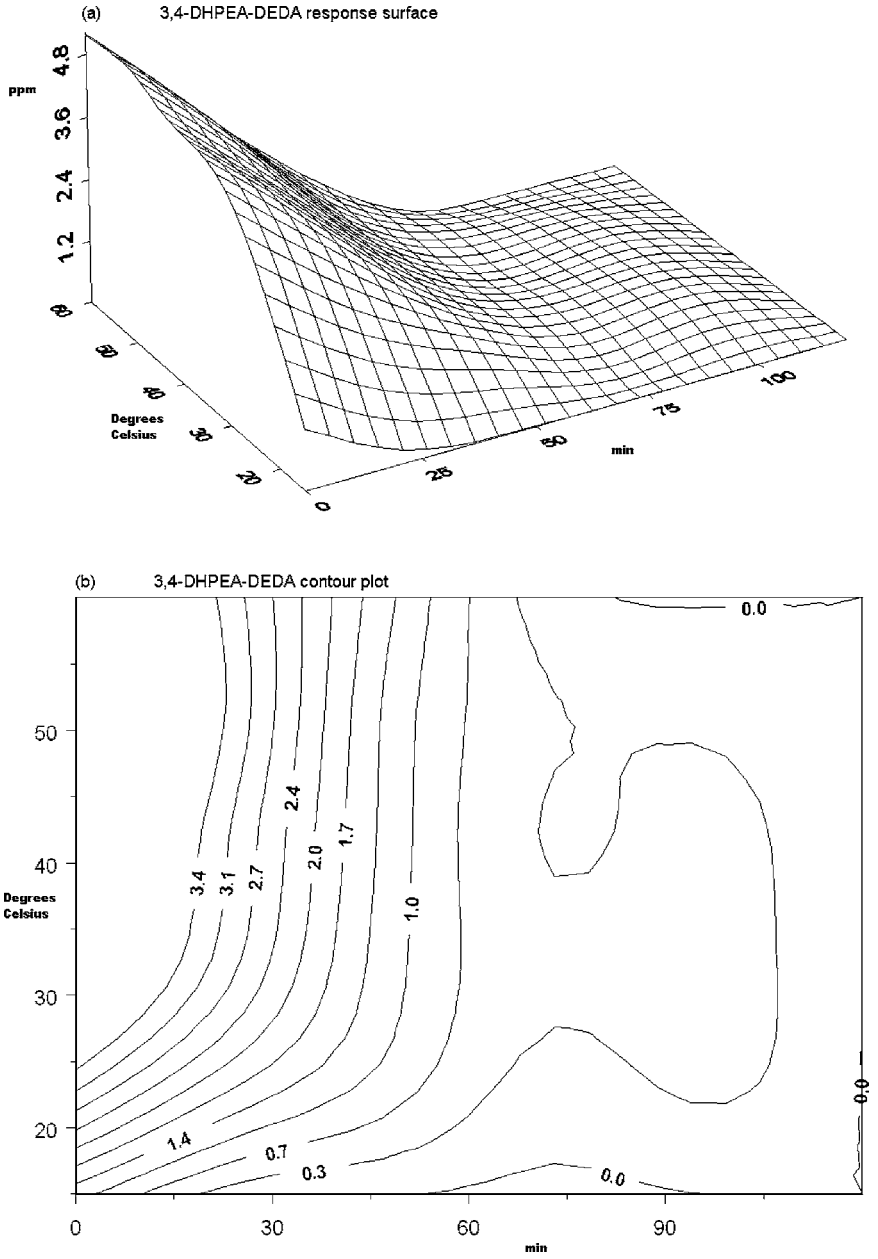

Figure 4. (**a**) Malaxation time–temperature response surface for 3,4-DHPEA-DEDA formation (ppm is μg/g of oil). (**b**) Malaxation time–temperature contour plot for 3,4-DHPEA-DEDA with numbers in the plot representing concentration in μg/g of oil.

found, consistent with an earlier observation that tyrosol is significantly affected by temperature only (**Table 2**).

Looking at an example of a discriminating variable that predominantly changed with malaxation time, 3,4-DHPEA-DEDA, the response surface (**Figure 4a**) clearly shows high concentrations at short malaxation times, in agreement with earlier observations in this study (**Table 3**). Complementary observations are made on the contour plot (**Figure 4b**), where contour lines span along the time axis and concentration increases toward shorter times with a high density below 60 min, indicating a high sensitivity in 3,4-DHPEA-DEDA formation at short malaxation times. Quantitative data (**Table 4**) show low concentrations of 3,4-DHPEA-DEDA at 15 °C, and a significant ($p < 0.01$) difference was found at 30 min of malaxation time for higher temperatures (30, 45, and 60 °C).

The observed low concentrations of the phenolic compounds (tyrosol and 3,4-DHPEA-DEDA) at low temperatures (15 °C) suggest their levels in virgin olive oil are strongly influenced by processing temperature. Malaxation temperature plays a crucial role in the formation and degradation of such phenolic compounds. Degradation may be accelerated at elevated temperatures, whereas formation may involve bond cleavages to release phenolic compounds that are bound to other molecules in the olive fruit. This is in line with earlier observations (*25*−*27*) on the formation of phenolic compounds, such as 3,4-DHPEA-DEDA and tyrosol from high molecular weight phenolic compounds. Reaction kinetics will determine the concentration of all compounds in the oil. In the cases when malaxation time emerges as a critical variable, the importance of reaction kinetics also emerges. For instance, in the case of 3,4-DHPEA-DEDA, it is apparent from the density of contour lines (**Figure 4b**) that short processing times (<60 min) favor higher rates of 3,4-DHPEA-DEDA formation relative to degradation, whereas at higher processing temperatures/longer times the degradation rate of 3,4-DHPEA-DEDA increases.

Changes in Volatile Compounds with Processing. Volatile compounds were the most common discriminating variables (**Table 2**) of malaxation times and temperatures. The discrimi-

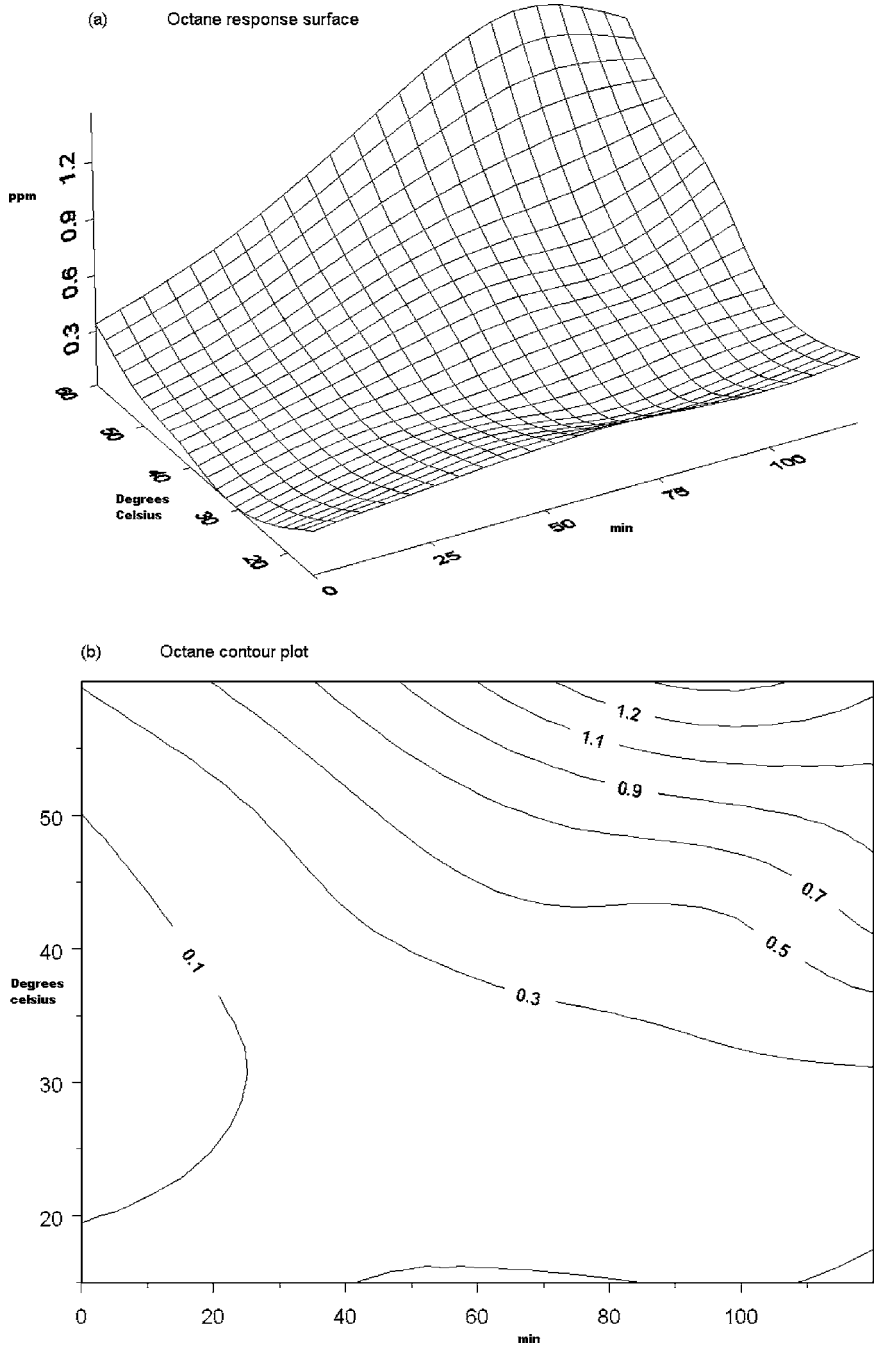

Figure 5. (**a**) Malaxation time–temperature response surface for octane formation (ppm is μg/g of oil). (**b**) Malaxation time–temperature contour plot for octane with numbers in the plot representing concentration in μg/g of oil.

nating volatile compounds were found to increase in chain length with malaxation temperature; 1-penten-3-ol, *E*-2-hexenal, and octane were discriminating variables at 15, 30, and 60 °C, respectively (**Table 3**). The increase toward long-chain volatile compounds with processing temperature is consistent with earlier observations (*11*) of virgin olive oil C6 volatile compounds decreasing with high malaxation temperature. Unlike malaxation temperature, malaxation time showed a decrease with chain length; the C6 volatile compound (hexanal) significantly ($p <$ 0.01) discriminated shorter times (30 and 60 min), whereas longer times (90 and 120 min) were discriminated by the C5 volatile compound, *Z*-2-penten-1-ol (**Table 3**).

It should be recognized that volatile compounds in virgin olive oil do not originate from the fruit, per se; they are formed during processing (*2*, *11*), which points us toward the importance of thermodynamic conditions. This idea is supported by data in **Table 2**, where four volatile compounds (1-penten-3-ol, octane, hexanal, and *E*-2-hexenal) significantly ($p < 0.01$) discriminated malaxation temperatures compared to only *Z*-2-penten-1-ol that discriminated malaxation time. Response surfaces and contour plots for octane and hexanal (**Figures 5** and **6**) illustrate the dominant influence of temperature in volatile formation.

Octane is produced from the decomposition of 10-hydroxy-peroxide of oleic acid and correlated with "fusty" defect in olive oil (*28*). High malaxation temperatures favored the formation of octane as illustrated by the response surface (**Figure 5a**). The increase of octane concentration with malaxation temperature is also apparent on the contour plots (**Figure 5b**). The formation of octane becomes more sensitive at elevated malaxation temperatures (>40 °C), indicated by a high density of contour lines (**Figure 5b**). Octane concentrations (**Table 4**) were significantly ($p < 0.01$) different at elevated malaxation

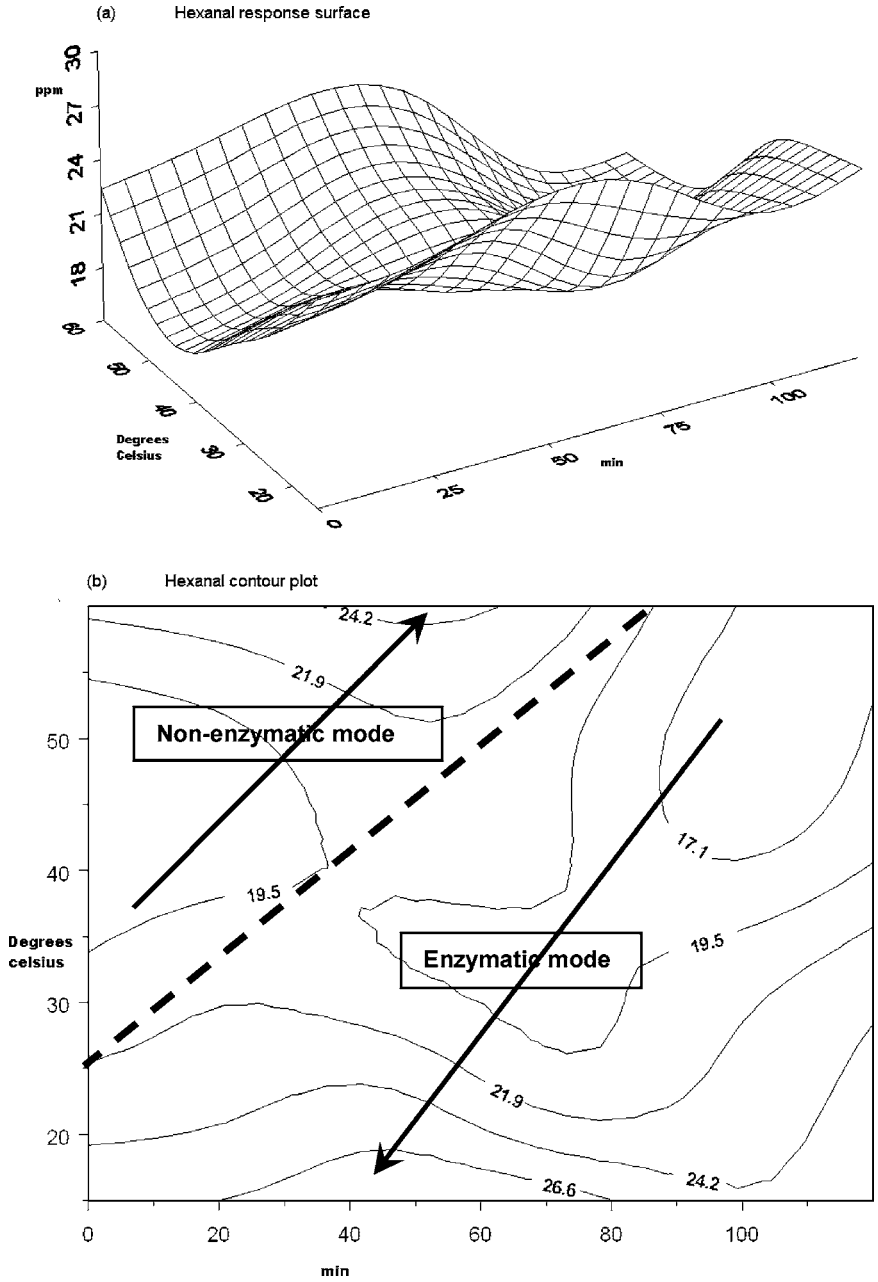

Figure 6. (**a**) Malaxation time–temperature response surface for hexanal formation (ppm is μg/g of oil). (**b**) Malaxation time–temperature contour plot for hexanal with numbers in the plot representing concentration in μg/g of oil.

temperatures (45 and 60 °C) but were not significantly ($p > 0.01$) different at low temperatures (15 and 30 °C).

The hexanal time−temperature response surface (**Figure 6a**) shows that as processing temperature is increased, the concentration of hexanal initially decreases, reaches a minimum at about 50 °C, and then increases as temperature is further increased. The 50 °C minimum forms a valley that runs virtually parallel with the time axis. An earlier study (*9*) reported that hexanal levels were more influenced by malaxation time than temperature. This is not consistent with our observations, probably due to the inclusion of higher malaxation temperatures (45 and 60 °C) in our study compared to the malaxation temperature range in the work by Morales and Aparicio (*9*). The contour lines in **Figure 6b** span diagonally along the time and temperature axis, showing that neither malaxation time nor temperature exerts a dominant influence on the formation of hexanal.

It is intriguing that hexanal concentration increases along opposite directions of the temperature axis (**Figure 6b**). This behavior suggests that there may be two different modes of hexanal formation during virgin olive oil extraction. Previous studies have reported that hexanal may be formed through both enzymatic and nonenzymatic pathways (*29*, *30*). The contour plot (**Figure 6b**) can be interpreted as displaying the two modes of hexanal formation; nonenzymatic mode (hexanal concentration increasing toward high temperature/long time) and enzymatic mode (low temperature/short time), illustrated by the direction of the arrows pointing toward increasing hexanal concentration (**Figure 6b**). The enzymes responsible for hexanal formation have a high activity at low temperatures (*11*) and lose their activity at high temperatures, consistent with the increase in the enzymatic hexanal formation toward low temperatures in this work. At high temperatures (>50 °C) and shorter malaxation times (<75 min), an increase in hexanal

concentration is observed (**Figure 6b**). Quantitative data (**Table 4**) show the lowest concentration at 90 min and 45 °C, which is along the 50 °C valley of the response surface (**Figure 6a**). Significantly ($p < 0.01$) higher hexanal concentrations (**Table 4**) were observed at short malaxation times (30 and 60 min) and low temperature (15 °C), consistent with an earlier study (*11*) in which maximum hexanal production was observed at 15 °C.

It can be hypothesized that the generation of hexanal at high temperatures is nonenzymatic because at such high temperatures enzyme activity is lost. The nonenzymatic nature of hexanal formation during oil extraction has been rarely reported, probably because most of the studies (*4*, *7*, *9*, *10*) on the effects of malaxation temperature were conducted at low temperatures (<40 °C). The nonenzymatic formation of hexanal at elevated temperatures is in agreement with the formation of other volatile compounds, such as octane shown above, and other C8 and C9 volatile compounds known to be formed through nonenzymatic oxidation during olive oil storage (*22*, *29*).

The above results suggest that alternative processing time−temperature combinations may be suitable for the production of virgin olive oil with pleasant sensory characteristics. It has been proposed (*9*) that elevated temperatures (>35 °C) and short malaxation times (<30 min) can produce pleasant olive oils with green aroma characteristics. These conditions represent a departure from the typically recommended processing temperature of 30 °C and malaxing for at least 45 min, according to olive paste rheology (*5*). The higher malaxation temperatures (>35 °C) and shorter times (<30 min) proposed by Morales and Aparicio (*9*) fall in the region of nonenzymatic hexanal formation (**Figure 6b**), where hexanal concentration increases with malaxation temperature. In a similar high-temperature (>35 °C) and short-time (<30 min) region (**Figure 5b)**, octane formation is minimized. This result of maximizing hexanal, associated with green aroma characteristic olive oils (*31*−*33*), and minimizing octane, associated with low-quality olive oils (*28*), supports the alternative approach of shorter time and higher malaxation temperature for the production of premium virgin olive oil.

ABBREVIATIONS USED

PV, peroxide value; FFA, free fatty acid; UV, ultraviolet; 3,4-DHPEA-DEDA, 3,4-dihydroxyphenyl ethyl alcohol−decarboxymethyl elenolic acid dialdehyde; IOOC, International Olive Oil Council; SLDA, stepwise linear discriminant analysis; SPME-GC-MS, solid phase microextraction−gas chromatography−mass spectrometry; SPME-GC-FID, solid phase microextraction−gas chromatography−flame ionization detection; LC-ESI-MS, liquid chromatography−electrospray ionization−mass spectrometry; HPLC-DAD, high-performance liquid chromatography−diode array detection.

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