Chromatic Evolution of Virgin Olive Oils Submitted to an Accelerated Oxidation Test

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ABSTRACT: Six samples of virgin olive oil obtained from several varieties of olive fruits (Picual, Manzanilla, Lechín, and Arbequina) were submitted to an accelerated oxidation process during a 63-h period under the conditions of the oil stability index (OSI), as measured by a Rancimat (100ºC) apparatus. Spectra were measured every 3 h, and chlorophyll and carotenoid indexes and CIELAB color ordinates were calculated. As oxidation time increased, remarkable changes in the spectral characteristics and color ordinates were observed. Oxidation provoked less vivid colors (lower values for chroma, C^*_{ab}) in all the samples; however, only some varieties became darker (lower values for lightness, L*). The pigment loss calculated for oxidized oils was 67% for the carotenoid index and 58% for the chlorophyll index. Mathematical models are offered to predict color changes with time of storage at 20ºC.

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KEY WORDS: CIELAB, oxidation, tristimulus colorimetry, virgin olive oil.

Color is one of the first sensory attributes of virgin olive oil evaluated by consumers, and it can be considered a quality parameter that highly influences its acceptance and preference. Virgin olive oil is a natural product obtained by simple pressing of olive fruit (*Olea europea*) and shows colors ranking from dark green to pale yellow. Among other components, olive oil color is directly related to the chlorophyll and carotene content (1) and can be influenced by several factors, i.e., the olive (olive variety, cultivar conditions, maturation index, or production zone), the extraction procedure, or the conservation conditions (humidity, temperature, light, oxygen exposure, container type, and material) (2–4). Olive oil color has been proposed as a characterizing factor, i.e., as a quality index related to the oil extraction method and to the olive variety (5,6).

No objective standardized method for measuring virgin olive oil color has yet been established. In Spain a color index based on a modification of the bromthymol blue method (UNE 55021) (7,8) is used to evaluate olive and seed oil colors varying from yellow to green but excluding red tonalities. This is a long and tedious procedure that lacks accuracy and shows some deficiencies when characterizing olive oil colors because of metamerism (9) and visual variability among observers. The accuracy, reliability, and reproducibility of this method has already been discussed (10–12). Similar problems arise when oil color is measured by means of visual or automatic colorimeters, mainly when green tonalities are present (13), which indicates that any method based on comparisons with standards shows an inherent error owing to the subjectivity of visual impressions.

Tristimulus colorimetry, based on transmittance values of the whole spectra, is an analytical method that widely improves the objective analysis of color in foods. The Commission Internationale de l'Eclairage (CIE) (14) has recommended since 1976 the use of the uniform color systems CIE 1976-(L*u*v*) or CIELUV color space, and CIE 1976-(L*a*b*), or CIELAB color space. In particular, the CIELAB system has been accepted worldwide in most industries. Its application to virgin olive oil samples provides better results than those obtained by visual methods (10).

The stability of virgin olive oils has been widely studied and related to major and minor compounds (15,16) as well as to technological procedures (17,18). However, few studies have shown the color evolution of virgin olive oils under storage conditions (19).

The aim of this work was to assess the chromatic evolution of virgin olive oils. Four olive varieties (Manzanilla, Picual, Arbequina, and Lechín) with similar maturation indexes were submitted to an accelerated oxidation test, which imitates changes occurring during storage. Tristimulus colorimetry, particularly the uniform color space colorimetric system CIELAB, was used to follow color changes during the accelerated oxidation test. The relationship between color changes and pigment content was also explored.

EXPERIMENTAL PROCEDURES

Samples. Olive oil samples were selected among the olive fruit varieties most frequently used by olive oil makers in Andalucía (South of Spain): one sample of Manzanilla (M) and Lechín (L), and two samples of Arbequina (A1 and A2) and Picual (P1 and P2) (Table 1).

Monovarietal oils were extracted in the laboratory by the Abencor® method (20). This is a laboratory-scale system that simulates a standard olive oil mill. Olive fruits are milled into a paste in an electric mill, and the resulting paste is mixed in a malaxator and spun at 3500 rpm to obtain the oil, which retains all its flavor and taste. To assess the effects of extraction conditions on oxidative stability, a sample of the Picual vari-

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TABLE 1
Sample Description and Oil Stability Indexes (OSI) Obtained with the Rancimat® Apparatus

Variety	Sample code	Extraction method[a]	Maturation index	$OSI_{100°C}$ (h)	$OSI_{20°C}$ (mon)
Lechín	L	A	2	54.6 ± 2.7	19.6
Arbequina	A1	A	2	44.3 ± 5.3	15.8
Arbequina	A2	A	3	46.2 ± 3.0	16.4
Manzanilla	M	A	2	99.3 ± 3.0	35.3
Picual	P1	I	3	111.8 ± 5.0	39.8
Picual	P2	A	3	103.5 ± 1.3	37.0

[a]A, Abencor; I, industrial.

ety was also extracted by an industrial procedure (P2). The influence of the maturation stage on color evolution was assessed on an Arbequina variety that was sampled at two different maturation indexes (A1 and A2).

Oil stability index (OSI). To evaluate the oil stability, the induction time for oxidation was measured by the Rancimat® apparatus (Metrohm CH 9100; Herisau, Switzerland). A flow of air (10 L/h) was bubbled through the oil heated at 100°C and cold water, successively. In this process, the volatile oxidation products were stripped from the oil and dissolved in the water, increasing the water conductivity. The time taken to reach a fixed level of conductivity was measured (21).

Oxidation study. Oxidation conditions for the chromatic study were as follows: 20.0 ± 0.1 g of the extracted and filtered oil was submitted to thermal oxidation in a Rancimat apparatus at a temperature of 100 ± 0.1ºC, with an air flow of 10 L/h and a surface-to-oil ratio of 6 mm. Sample oxidation was interrupted at night and on weekends, during which times oil was kept at 20ºC in the dark. The heating cycles at the conditions described above were of about 9 h/d, and seven cycles were completed. The total time that the six different olive oil samples were submitted to these accelerated oxidation conditions was 63 h. Complete duplicate oxidation procedures were done in a different batch (not simultaneously).

Chromatic measurements. Oil spectra (380–770 nm) were recorded using an UV-vis diode array spectrophotometer (HP8452; Hewlett-Packard, Palo Alto, CA) equipped with quartz cells with path lengths of 5 and 10 mm. Tristimulus values were obtained following the weighted-ordinate method at constant intervals ($\Delta\lambda$ = 2 nm). Recommendations by CIE were considered and the CIELAB system for color specification was applied. The CIE-1976 color difference formula (14) was applied to obtain an adequate expression of visually perceived color differences. A specific software package, PCROM®, was used for all the colorimetric calculations (22).

Colorimetric measurements were taken at the beginning of the working day and every 3 h thereafter (4 measurements/d). The first measurement taken daily was used to ascertain whether the night and weekend interruptions affected the color. No significant differences in color between the end-day and starting-day measurements were found for any of the samples studied (data not shown).

Extrapolation of results to storage conditions (20ºC). To roughly estimate the color stability of the oils during storage at ambient temperature (20ºC), the heating times at the conditions selected in the study (100ºC) were extrapolated to the time under storage conditions (20ºC). This extrapolation was done under the assumption that, during storage, autoxidation is the only relevant decomposition process and that temperature dependency is constant. The heating times were extrapolated to times under storage conditions as follows:

$$\text{Time}_{20°C} = \text{Time}_{100°C} \times 2^{(100-20)/2} \quad [1]$$

Time was expressed in months.

Pigment indexes. The chlorophyll and carotenoid indexes were calculated from the absorption spectra of the oils. The absorption maximum at 670 nm is usually considered to be related to the chlorophyll fraction, pheophytin *a* being its major component. The dominant pigment in the carotenoid fraction is lutein, and the absorption maximum at 470 nm is considered the zone without interference from pheophytin *a,* which can be used for estimating the yellow pigments. The values of the coefficients of specific extinction applied were those referenced in the bibliography (5): E_0 = 613 for pheophytin and E_0 = 2000 for lutein. Thus, the pigment indexes were calculated as follows:

$$[\text{chlorophylls}] = \left(A_{670} \times 10^6\right) \Big/ (613 \times 100 \times \text{d}) \text{ mg/kg} \quad [2]$$

$$[\text{carotenoids}] = \left(A_{470} \times 10^6\right) \Big/ (2000 \times 100 \times \text{d}) \text{ mg/kg} \quad [3]$$

where *A* is the absorption.

Statistical analysis. The statistical analyses were carried out using Statistica for Windows, v. 5.5, by Statsoft, Inc. (23).

RESULTS AND DISCUSSION

Oxidative stability. The oxidative stability determined by the Rancimat technique showed wide variation among the different varieties tested (Table 1). The oil stabilities ranged from a minimum of 44 h for Arbequina to 111 h for Picual under the conditions of the accelerated oxidation test. If we extrapolate to storage under ambient temperature (20ºC), this should mean about 16 mon of stability for Arbequina and more than 3 yr for Picual. According to these results and previously published data (18), the olive varieties used in this study could be considered representative examples of low- and high-stability olive oils.

In accordance with the overall mechanism proposed for the autoxidation process (24) and the OSI calculated for the virgin olive oil samples, we must differentiate two groups of oils. The more stable olive oils (Manzanilla and Picual) following the treatment period (63 h of accelerated oxidation) were those still in the induction period of oxidation (OSI > 63 h), whereas the less stable oils (Lechín and Arbequina) following the treatment period (63 h) were those that had reached the final stages of oxidation (OSI < 63 h) (Table 1).

Spectrophotometric study and pigment index evolution. The four olive oil varieties showed similar UV-vis absorption spectra profiles with a broad absorption band in the blue area, between 375 and 525 nm, corresponding to the yellow color

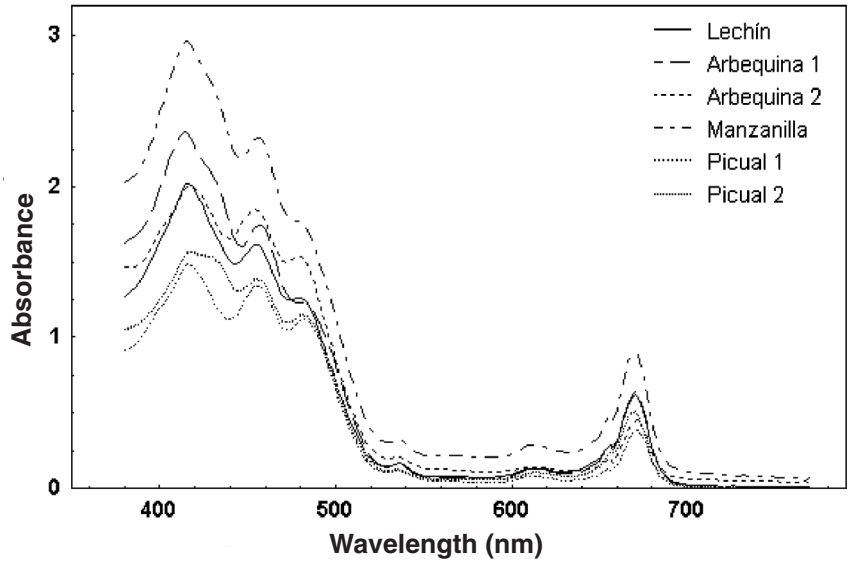

FIG. 1. Absorption spectra of the six varieties of virgin olive oil at the initial stage (fresh oils).

transmission region (Fig. 1). In this zone, three maxima were clearly defined: ones over 410 and 450 nm, which could be assigned to chlorophylls and carotenoids in general, and one over 470 nm, corresponding only to the carotenoid fraction, usually expressed as lutein, the dominant pigment in this fraction. At 670 nm a less intense maximum, corresponding to the red color absorption zone/green color transmission area was found, which could be assigned to chlorophyll and mainly to pheophytin *a,* its major component (5). The pigment concentration is known to be higher in oils extracted by the Abencor method than in commercial oils (16). However, the oil from the Picual variety extracted by the Abencor method was not significantly different, in relation to pigment contents, from that extracted in the industry. This could be inferred from the spectra of these oils (Fig. 1) and from the pigment indexes calculated for this variety (Table 2). On the other hand, the stage of maturity is known to be correlated with the pigment concentration and with chlorophyll in particular, as is clearly shown in the spectra obtained for the samples of the Arbequina variety. The A1 sample (maturation index 2) and the A2 sample (maturation index 3) showed significant differences in the chlorophyll absorption zone, and the A1 sample almost duplicated the chlorophyll index obtained for the A2 sample (Table 2).

TABLE 2
Chorophyll and Carotenoid Indexes for Different Virgin Olive Oils at the Initial Stage (fresh virgin olive oils) and at the Final Stage (after 63 h of accelerated oxidation)[a]

Sample code	Initial stage (t = 0)		Final stage (t = 63 h)		Pigment lost (%)	
	I_{Ca}	I_{Ch}	I_{Ca}	I_{Ch}	Ca	Ch
L	3.71	6.15	0.74	1.85	80.05	69.92
A1	4.67	7.20	0.72	1.63	84.58	77.36
A2	3.96	3.83	1.02	1.95	74.24	49.09
M	4.89	7.80	2.24	3.75	54.19	51.92
P1	2.94	4.36	1.28	1.91	56.46	56.19
P2	3.08	3.74	1.69	2.82	45.12	24.60

[a]I_{Ca}, carotenoid index; I_{Ch}, chlorophyll index; Ca, carotenoid fraction; Ch, chlorophyll fraction.

As oxidation time progressed, important changes in the UV-vis spectra were observed (Figs. 2,3). The chlorophyll peak (670 nm) underwent a hypochromic and bathochromic shift (toward a higher wavelength), and a broad band at 702 nm increased progressively as oxidation advanced. Carotenoids were also modified by the oxidation process, and differences were clearly shown among samples, depending on the stability of the oil. The olive oil varieties Manzanilla and Picual (Table 1) still showed absorption peaks in the regions 400–500 nm after the treatment period (Fig. 2) because these oils were still in the induction stage of the oxidation process, when oxidation increases more slowly. On the contrary, the olive oil varieties Lechín and Arbequina showed no absorption maximum in the same region (Fig. 3). These oils were at the end of the induction period, in the accelerated stage of oxidation, where a great number of secondary oxidation compounds are formed and absorb at <300 nm.

The carotenoid index is quantitatively more affected by oxidation than is the chlorophyll index: for Lechín and Arbequina, which were at the end stage of the induction period (OSI < 63 h), about 80% was lost, whereas for Picual and Manzanilla, which were still in the induction period (OSI > 63 h), the pigment loss was about 55% (Table 2). The antioxidative behavior of carotenoids is closely related to its own degradation; however, the oxidative degradation products formed may react with lipids, finally resulting in an acceleration of lipid oxidation (25). On the other hand, the presence of other antioxidants, such as α-tocopherol, has a synergistic effect. Other minor compounds, such as polyphenols, also have proved to have antioxidant properties and to exert a significant contribution to the stability of virgin olive oils (15). This could explain why olive oil samples with similar carotenoid indexes showed quite different OSI.

Chromatic study and color evolution with oxidation. As regards the chromatic study, the uniformity associated with the CIELAB color space, which allows small differences to be detected, offered a good chromatic characterization of the virgin

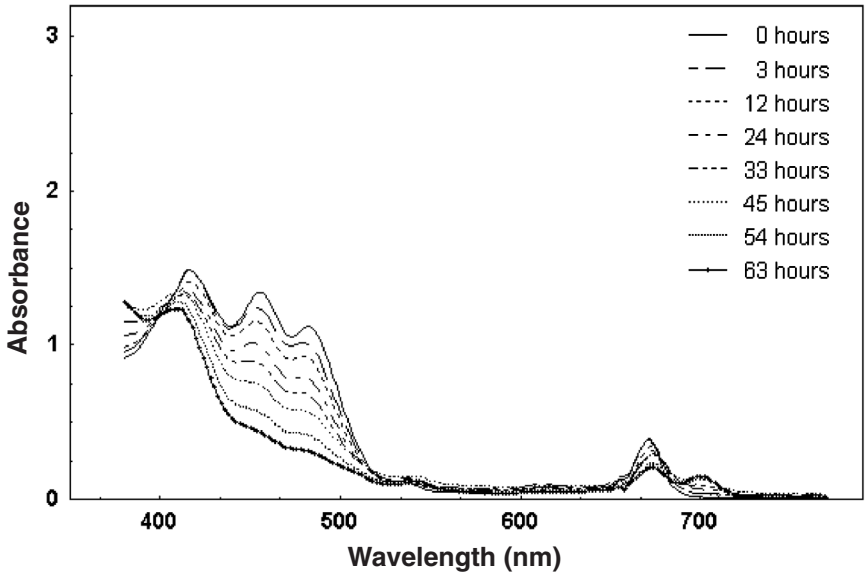

FIG. 2. Absorption spectra of the Picual variety extracted by the Abencor method (Picual 1) at different oxidation stages (from initial stage to 63 h of accelerated oxidation).

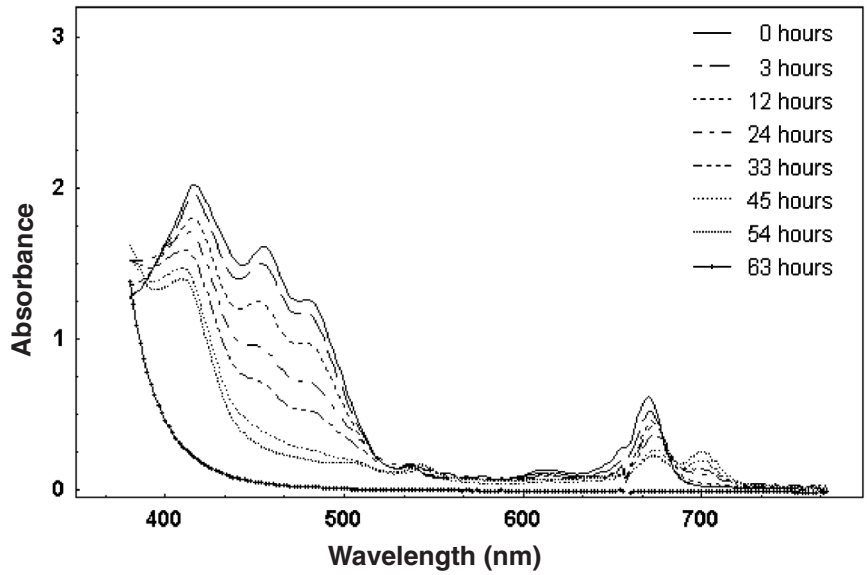

FIG. 3. Absorption spectra of the Lechín variety at different oxidation stages (from initial stage to 63 h of accelerated oxidation).

oil varieties studied. According to their location in the a*b* chromatic diagram (Fig. 4), the color of the samples showed a tendency toward greener hues for the paler oils, with lower values for chroma and toward yellowish colors as chroma values were higher. The initial and final values for the rectangular chromatic coordinates are shown in Table 3. The main differences among varieties were related to the proportion of yellow (carotenoids), represented by the coordinate b* (positive axis b*), and not to the proportion of green (pheophytins), represented by the negative axis a*. The tendency in the coordinates with oxidation time was toward less negative values for a* and lower values for b*, which indicates a browning effect. The oil sample from the variety Manzanilla visually seems the greenest one, but according to CIELAB parameters, the attribute that distinguishes it from other varieties is lightness (L*). L* is a parameter related to the transmission of light, and the Manzanilla oil sample showed the lowest values, with a slight increase during the oxidation assay. In contrast, b* showed a tendency toward lower values.

The visual sensation of colorfulness is correlated with the 2-D parameter (a*b*), an expression related to chromaticity. The qualitative component of this attribute is the hue angle (h_{ab}), a parameter that showed similar values for all the varieties studied (between 89 and 93º). However, as the oxidation advanced a higher dispersion of h_{ab} values (88–95º) was observed. The quantitative component of chromaticity, chroma (C^*_{ab}), decreased steadily, indicating less vivid colors with a loss of color intensity.

To find the significance of these changes, correlations between the chromatic parameters and pigment indexes were

TABLE 3
Chromatic Parameters of Fresh Virgin Olive Oil Samples (initial stage) and After Accelerated Oxidization (final stage) in the Rancimat Apparatus for 63 h[a]

Sample code	L^*_{t0}	L^*_{t63}	a^*_{t0}	a^*_{t63}	b^*_{t0}	b^*_{t63}	$C^*_{ab\ t0}$	$C^*_{ab\ t63}$	$h_{ab\ t0}$	$h_{ab\ t63}$
L	82.171	88.296	–2.709	–2.679	104.54	33.446	104.389	33.553	91.487	94.580
A1	76.695	88.145	–2.746	–2.854	107.811	31.487	107.846	31.616	91.459	95.179
A2	81.263	88.798	2.470	–3.778	104.760	46.084	104.789	46.239	88.649	94.687
M	73.275	73.153	0.762	3.114	104.918	62.698	104.921	62.776	89.584	87.157
P1	86.264	83.635	–2.167	–2.837	97.163	52.955	97.187	53.031	91.278	93.066
P2	83.049	79.854	0.361	–0.955	93.087	54.503	93.088	54.512	89.778	91.004

[a]L^*_{t0}, lightness of fresh virgin olive oil samples; L^*_{t63}, lightness at 63 h; a^*_{t0}, color coordinate a* of fresh virgin olive oil samples; a^*_{t63}, color coordinate a* at 63 h; b^*_{t0}, color coordinate b* of fresh virgin olive oil samples; b^*_{t63}, color coordinate b* at 63 h; $C^*_{ab\ t0}$, chroma of fresh virgin olive oil samples; $C^*_{ab\ t63}$, chroma at 63 h; $h_{ab\ t0}$, hue angle of fresh virgin olive oil samples; $h_{ab\ t63}$, hue angle at 63 h. For sample codes see Table 1.

explored. These results are shown in Table 4. At the initial and final stages, strong and significant correlations were found for both carotenoid and chlorophyll indexes and the coordinate L*, lightness. The correlation was negative in both cases, indicating an inverse relationship between light transmission and pigment content. In the fresh oil, very strong and significant correlations were also found between b* and C^*_{ab} coordinates and the carotenoid index, indicating the relevance of these compounds in the color of oils obtained from mature olives. For the oxidized oils, significant correlations were found for both indexes. Furthermore, the negative correlation with h_{ab} indicated that oxidation provokes a tendency toward more yellow colors.

CIELAB color differences. The color differences between fresh oils and the oxidized oils for the different varieties are represented in Figure 5. On average, the main contribution to the calculated color differences was the change in color coordinate b* (yellow), as mentioned above. In the varieties Arbequina (A1 and A2) and Lechín, the color differences became more marked after 40 h of accelerated oxidation, which is in accordance with their advanced oxidation stage (OSI about 40–50 h). The behavior of the other varieties included in this study was quite similar, increasing steadily with time. As oxidation advanced, the oil samples became less saturated (C^*_{ab} decreased) and L* dispersion increased. Only Picual and Manzanilla became darker with oxidation time, although they were still in the induction period of oxidation. An even more intense change could be expected when the induction time was over.

TABLE 4
Correlations Between Chromatic Parameters and Pigment Indexes at the Initial Stage (fresh virgin olive oils) and After Oxidizing in the Rancimat Apparatus for 63 h (final stage)[a]

	Initial stage (t = 0)		Final stage (t = 63 h)	
Parameter	I_{Ca}	I_{Ch}	I_{Ca}	I_{Ch}
L*	–0.96*	–0.83*	–0.94*	–0.99*
a*	0.11	–0.36	0.89*	0.95*
b*	0.85*	0.69	0.94*	0.82*
C^*_{ab}	0.84*	0.69	0.94*	0.82*
h_{ab}	–0.13	0.34	–0.98*	–0.82*

[a]Correlations marked with an asterisk are significant at $P < 0.05$. For abbreviations see Tables 2 and 3.

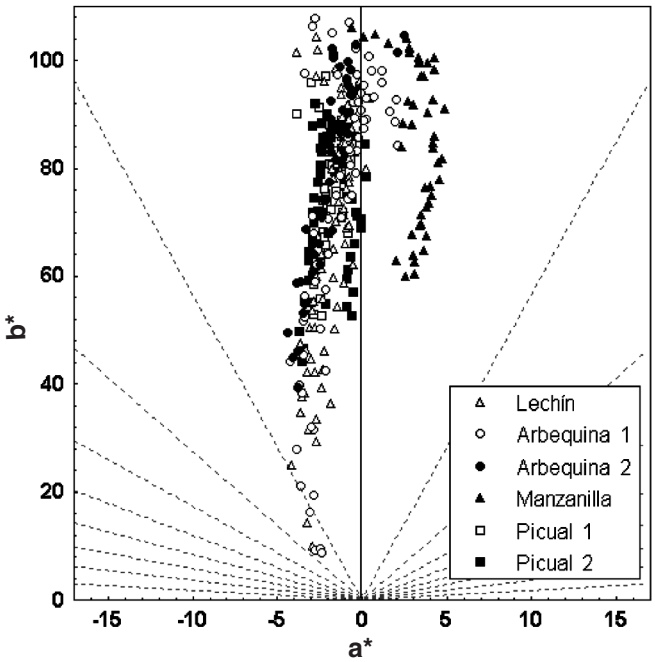

FIG. 4. Color evolution of olive oil varieties measured in CIELAB [CIE 1976-(L*a*b*)] color space at different oxidation times: (a*b*) diagram.

Although there is no general agreement about the visual threshold for a normal observer to appreciate the CIELAB color difference, it is usually considered to be between 1 and 3 units. Most recent papers usually consider the threshold to be over 3 CIELAB units. According to this view, the changes in olive oil color could become noticeable ($\Delta E > 3$) after about 1 mon of storage at ambient temperature. The color change would be more clear for the varieties Arbequina and Lechín ($\Delta E_{1mon} > 8$). Only for the variety Manzanilla would the color change be noticeable after 2 mon of storage at 20ºC. It is possible to obtain multiple regression models to predict color changes as a function of storage times. Mathematical equations for the different varieties are proposed in Table 5. Despite the small number of samples used in this study, the regression models proposed could be used for rough estimates of when consumers could notice the changes in olive oil color during storage (at a controlled temperature).

TABLE 5
Regression Linear Models Predict Color Changes as a Function of Time of Storage at 20ºC[a]

Sample code	Models	R^2	SEE
L	$\Delta E^*_{ab} = 4.111 + 2.644t - 0.008t^2 + 0.001t^3$	0.9938	1.7920
A1	$\Delta E^*_{ab} = 0.175 + 2.915t - 0.246t^2 + 0.012t^3$	0.9984	0.9253
A2	$\Delta E^*_{ab} = 3.074 + 3.266t - 0.298t^2 + 0.012t^3$	0.9878	1.8461
M	$\Delta E^*_{ab} = 0.146 + 1.563t + 6.092\cdot10^{-4}t^2 + 6.336\cdot10^{-4}t^3$	0.9941	1.0828
P1	$\Delta E^*_{ab} = 2.653 + 2.200t - 0.084t^2 + 0.003t^3$	0.9905	1.2918
P2	$\Delta E^*_{ab} = 1.022 + 1.724t - 0.038t^2 + 0.002t^3$	0.9913	1.1332

[a]R^2, coefficient of determination adjusted for DF; SEE, standard error of estimate. For all samples, $P \leq 0.00000$. See Table 1 for sample code.

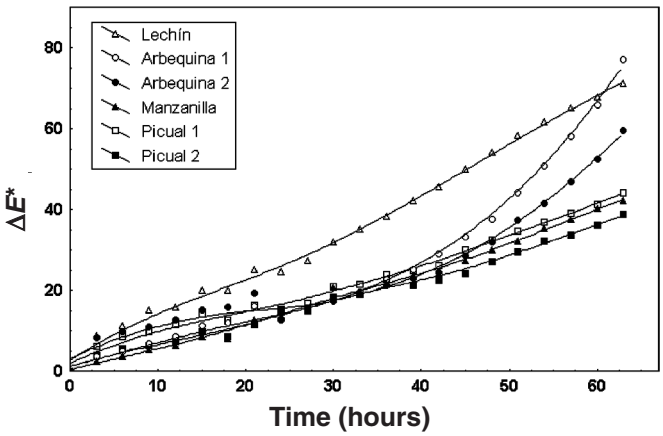

FIG. 5. Evolution of CIELAB color differences of the olive oil varieties under storage conditions at 20ºC. ΔE = difference in specific extinction coefficient.

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