Color-Pigment Correlation in Virgin Olive Oil

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The chlorophyll and carotenoid content of virgin olive oils from five varieties harvested at varying degrees of ripeness were determined. Colors were evaluated from the chromatic ordinates L*, a*, b* of the absorption spectrum.

Oil color changes for different varieties or stages of ripeness are directly related to pigment content and a* and b* values. The statistical study made on both series of parameters proves that there is a good correlation between them. The carotenoid content and b* have one of the best correlation coefficients (r) and is easily measured. This methodology evaluates chlorophyll and carotenoid content, an additional attribute for evaluation of virgin olive oil quality.

KEY WORDS: Carotenoid, chlorophyll, chromatic ordinates, lutein, oil color, Olea europaea, olive oil, pheophytin "a", pigments.

In a previous study (1) it was shown that virgin olive oil obtained from olive fruit (Olea europaea) by mechanical pressing had a color ranging from green-yellow to gold depending on the degree of the fruit's ripeness. Pheophytin "a" and lutein were the major components of the chlorophyll and carotenoid fractions, respectively. In oils from olives picked at the beginning of the harvest season, pheophytin "a" can comprise up to 42% of the total pigment composition, while in oils obtained from over-ripe olives picked at the end of the season, lutein is the dominant component, reaching an individual contribution of some 61% of the total color.

Although color is an important quality attribute, at present there is no properly standardized evaluation method for virgin olive oil. Up to now, color is measured using an adaptation of methods developed for palm oil (2-5) and seed oils in general (6,7). Recently, a specific method for virgin olive oil has been perfected based on the visual comparison of color with standard solutions (8). Nevertheless, a method is still needed for the objective evaluation of this qualitative attribute.

As virgin oil is a natural product whose color depends exclusively on biological compounds such as the chlorophyll and carotenoid pigments, their identification and individual evaluation make it possible to relate oil color with the content and type of these compounds present.

The color of a body is due to the selective absorption of the light falling on it, and is characterized by its hue, chroma, and value. Hue is related to the dominant wavelength, and permits classification of the color into reds, greens, etc. The chroma is related with the amount of whiteness and gives an idea of the vividness of the color. The value shows similarity with a scale going from white to black, and is due to the different quantity of complete absorption throughout the spectrum.

For the measurement of "color", different color spaces have been established (9). The CIELAB space was defined in 1974 by the Commission Internationale de l'Eclairage (CIE) and is the most widely used at present. It uses the chromatic ordinates L*, a*, b*, determined either directly by means of a colorimeter or deduced from the tristimulus values X, Y, Z, obtained from the experimental absorption curve (10). The cylindrical ordinates L*, C*, H*, calculated from the values L*, a*, b*, are also used to express color space.

In the present work the chromatic ordinates are calculated in order to check which have the closest relation to the pigment composition of the oils, and to establish how they vary with visual estimation. This study will be the basis for a color index permitting objective classification. This index, besides classifying the oil by its color, which is related with pigment content, may give information about the oil quality and stability.

The provitamin, anticarcinogenic, and anti-ulcer character recently attributed to the carotenoid pigments (11-13), and their controversial intervention (together with chlorophylls and derived products) in oxidative processes, important in the stability of virgin olive oil, make their measurement of great interest (14-16).

MATERIALS AND METHODS

Materials. Samples were analyzed throughout the harvest season from six varieties of olive: Lechín, Hojiblanca, Verdial, Picuda, Pajarero and Subbética. In the first two, oil was available from olives in eight degrees of ripeness; in Verdial, oil from green and ripe fruits; and in the last three, only one sample could be obtained from each, from fruits in the optimum ripeness stage for oil extraction.

Pigment quantification. The detailed evaluation technique is described in a previous work (17). The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of the pigment extract in cyclohexane (Merck for spectroscopy, art, 2822, D-1600, Darmstadt, F.R. Germany).

In outline, the procedure is based on the study of the absorption spectra of the two groups making up the pigmentation of virgin olive oil. The pigments are separated from the fat-free extract (18) by thin-layer chromatography (TLC) on Kieselgel GF$_{254}$ plates, using petroleum ether (65-95°C) acetone/diethylamine (10:4:1) as developer. The carotenoid fraction was

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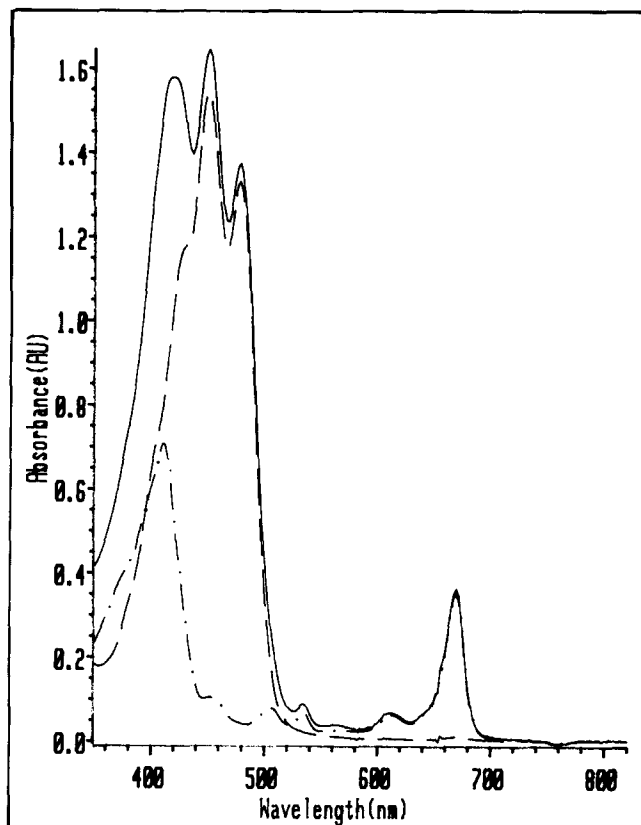*To whom correspondence should be addressed.

FIG. 1. Absorption spectra obtained from the total pigment extract (———), carotenoid fraction (-------------) and chlorophyll fraction (-•-•-•).

scraped and eluted from the plate with ethanol, and the chlorophylls with ethyl ether. The resulting absorption spectra and that obtained from the total pigment extract are shown in Figure 1.

The absorption maximum at 670 nm in the spectrum of the total extract is due exclusively to the presence of the chlorophyll fraction. As pheophytin "a" is the major component of this fraction, the group of chlorophyll derivatives can be evaluated as if all were pheophytin "a", after calculating the coefficient of specific extinction in cyclohexane. The value obtained from that given in the bibliography in ethyl ether is $E_o = 613$.

The spectrum of the carotenoid fraction takes the form of the dominant pigment, lutein. Thus, it is possible to evaluate the yellow pigments in the total spectrum as if all were lutein. The maximum at 470 nm is chosen, being a zone without interference from pheophytin "a", after obtaining the corresponding coefficient of extinction. This value, calculated from that given in the bibliography for lutein in ethanol, is $E_o = 2000$.

For the procedure, 7.5 g oil was weighed exactly, dissolved in cyclohexane and taken to a final volume of 25 mL. Once the absorption spectrum was obtained, the chlorophyll and carotenoid fractions were deduced at 670 nm and 472 nm, respectively.

Chromatic ordinate calculation. The tristimulus values X, Y, and Z were calculated for illuminant C from the absorption spectrum, using the weighted ordinate method, by means of a computer program, and the ordinates L*, a*, b*, and C* calculated in turn from them.

Statistical study. The statistical analyses were carried out on an Olivetti M-240 P.C. with the SPSS computer program (19).

Apparatus used. UV/Vis spectrophotometer Hewlett-Packard 8452 A, connected to a HP 89500 A computer, and provided with a HP 2225 printer and HP 7550 A graphic plotter.

RESULTS AND DISCUSSION

Table 1 shows the values corresponding to the concentration of chlorophyll and carotenoid pigments, expressed both as absolute value (mg/kg) and percentage (%). The pigment concentration in the oils from the variety Hojiblanca decreased gradually with increasing lateness of the date of harvest. This decrease was much more pronounced for the chlorophyll fraction than for the carotenoid. For the latest dates, the yellow fraction comprised some 70% while the green fluctuated around 30%.

From the results obtained, the behavior of the Lechín variety is totally different—ripening does not involve drastic variations in pigment content, and during the whole period studied, the chlorophyll and carotenoid fractions seemed to remain in equilibrium.

In the variety Verdial, as the data correspond to extreme stages of ripening, there are great differences.

Lastly, as the remaining varieties (Picuda, Subbética and Pajarero) supply only single data, it can only be stated that the chlorophyll and carotenoid content is quite balanced with reference to the percentage composition, but that in absolute values, Pajarero shows least pigmentation.

Figure 2 shows the absorption spectra of five oils from the variety Hojiblanca in different degrees of ripeness.

All the curves show a broad zone of absorption between 375 and 525 nm, with three well-defined maxima at 410, 450, and 470 nm. This zone, corresponding to blue absorption, transmits the complementary color, yellow. In the majority of cases, a rather narrow, more or less intense maximum is also observed at 670 nm, the red zone, which thus transmits green.

As harvesting time progresses, the concentration of pigments in the olives decreases, and consequently the height of the peaks of the respective absorption spectra. Because this decrease is much more pronounced for the chlorophyll fraction than for the carotenoid, the maximum at 670 nm is hardly detectable at the end of the season.

Table 2 shows the values of the chromatic ordinates a*, b*, C* and L*, obtained from the absorption spectra of the respective oils. The values of b*, all positive, correspond to the yellow zone, and the values of a*, all negative, are found in the green zone. If the values of absorption at 450 and 670 nm are expressed, a distribution is obtained similar to that of a* and b* (values not shown).

With increased ripening, the values of these ordinates decrease similarly to those of pigment concentra-

TABLE 1

Chlorophyll and Carotenoid Composition of Virgin Olive Oils from Fruits of Different Varieties and Degree of Ripeness

| Variety	Picking date	Sample code	Pigment content (mg/kg)		Percentage of the total pigment	
			Phys.[a]	Carot.[a]	Phys.	Carot.
Hojiblanca	12-20-88	H-1	23.7	16.5	59	41
	1-9-89	H-2	30.4	17.6	63	37
	1-19-89	H-3	18.9	16.1	54	46
	1-21-89	H-4	5.7	10.4	35	65
	1-30-89	H-5	9.1	13.1	41	59
	2-3-89	H-6	2.6	8.7	23	77
	2-20-89	H-7	3.1	7.6	29	71
	2-25-89	H-8	1.0	2.6	28	72
Lechín	1-10-89	L-1	3.8	4.7	45	55
	1-20-89	L-2	2.7	3.3	45	55
	1-30-89	L-3	6.6	5.0	57	43
	2-10-89	L-4	5.2	5.0	51	49
	2-18-89	L-5	2.4	3.4	41	59
Verdial	green color	V-1	54.4	22.5	71	29
	white color	V-2	2.2	3.3	40	60
Picudo	2-26-90	PC	16.6	14.0	54	46
Subbético	2-26-90	SB	15.7	13.5	54	46
Pajarero	2-26-90	PJ	7.3	6.9	51	49

[a]Carot., carotenoid fraction; Phys., derivatives chlorophyll fraction.
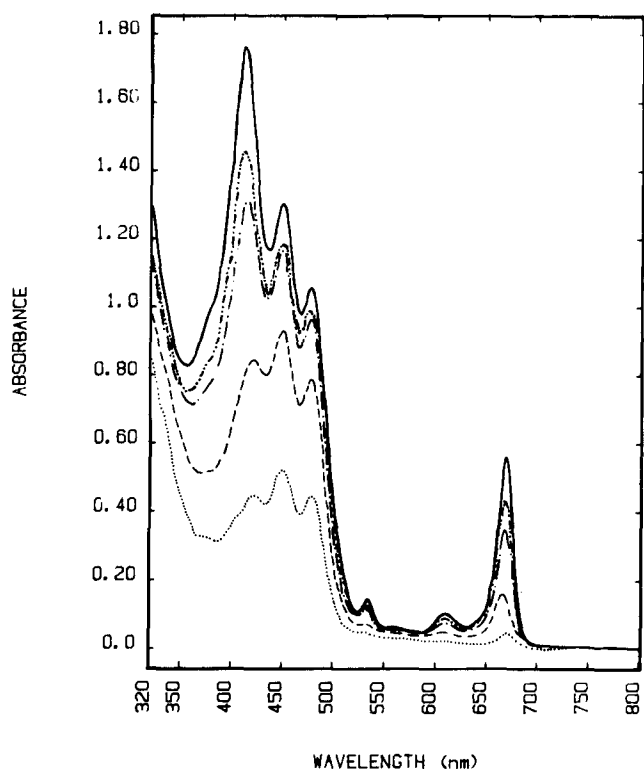
FIG. 2. Absorption spectra of five oils from the variety Hojiblanca in different degrees of ripeness.

tion, which is in agreement with the loss of color intensity in the corresponding oils.

TABLE 2

Color (expressed as chromatic ordinates a*, b*, C* and L*) of Virgin Olive Oils from Fruits of Different Varieties and Degree of Ripeness

Sample code	Chromatic ordinates			
	a*	b*	C*	L*
H-1	−15.6	86.6	88.0	91.2
H-2	−14.9	90.1	90.1	89.5
H-3	−15.2	84.6	86.0	91.2
H-4	−15.1	61.7	63.6	94.6
H-5	−14.6	72.9	74.4	92.7
H-6	−12.6	53.2	54.6	95.7
H-7	−10.9	46.3	47.6	94.7
H-8	− 3.6	15.9	16.3	96.4
L-1	− 9.8	34.2	35.5	97.5
L-2	− 5.8	21.8	22.6	95.6
L-3	− 8.2	32.9	33.9	94.2
L-4	− 9.7	32.9	34.3	95.9
L-5	− 7.0	21.9	22.9	96.1
V-1	−26.9	99.5	103.1	88.3
V-2	− 6.6	21.5	22.5	95.7
PC	− 9.5	64.0	65.1	77.9
SB	− 9.2	67.0	67.6	83.4
PJ	− 7.3	39.4	40.1	86.9

Table 3 shows the results in decreasing order of carotenoid content. The values of pigment concentration, chromatic ordinates, and relationships found between the main parameters are shown for all the oils studied. There is a clear relationship between the val-

TABLE 3

Comparison Among Pigment Content (mg/kg), Chromatic Ordinates and Rates Between the Main Parameters

Sample[a] code	Carot.[b]	Phys.[b]	Phys./Carot.	L*	a*	b*	C*	a*/b*	b*/L*	C*/L*
V-1	22.5	54.4	2.42	88.3	−26.9	99.5	103.1	−0.27	1.13	1.17
H-2	17.6	30.4	4.73	89.5	−14.9	90.1	90.1	−0.17	1.01	1.01
H-1	16.5	23.7	1.44	91.2	−15.6	86.6	88.0	−0.18	0.95	0.96
H-3	16.1	18.9	1.17	91.2	−15.2	84.6	86.0	−0.18	0.93	0.94
H-5	13.1	9.1	0.69	92.7	−14.6	72.9	74.4	−0.20	0.79	0.80
SB	13.5	15.7	1.17	83.4	− 9.2	67.0	67.6	−0.13	0.80	0.81
PC	14.0	16.6	1.19	77.9	− 9.5	64.0	65.1	−0.15	0.82	0.83
H-4	10.4	5.7	0.55	94.6	−15.1	61.7	63.6	−0.24	0.65	0.67
H-6	8.7	2.6	0.30	95.7	−12.6	53.2	54.6	−0.24	0.56	0.57
H-7	7.6	3.1	0.41	94.7	−10.9	46.3	47.6	−0.24	0.49	0.50
PJ	6.9	7.3	1.06	86.9	− 7.3	39.4	40.1	−0.18	0.45	0.46
L-1	4.7	3.8	0.81	97.5	− 9.8	34.2	35.1	−0.29	0.35	0.36
L-4	5.0	5.2	1.04	95.9	− 9.7	32.9	34.3	−0.29	0.34	0.36
L-3	5.0	6.6	1.32	94.2	− 8.2	32.9	33.9	−0.25	0.35	0.36
L-5	3.4	2.4	0.71	96.1	− 7.0	21.9	22.9	−0.32	0.23	0.24
L-2	3.3	2.7	0.80	95.6	− 5.8	21.8	22.6	−0.26	0.23	0.24
V-2	3.3	2.2	0.65	95.7	− 6.6	21.5	22.5	−0.31	0.22	0.23
H-8	2.6	1.0	0.40	96.4	− 3.6	15.9	16.3	−0.22	0.16	0.17

[a]Arranged in decreasing order of carotenoid content.
[b]Carot., carotenoid fraction; Phys., derivatives chlorophyll fraction.

TABLE 4

Correlation Between Chromatic Ordinates and Pigment Content for Those Variables Which Were Considered Best Correlated

Dependent variable	Independent variable (mg/kg)	Regression slope and intercept				
		m_1	m_2	b	r	p
a*	P[a]	−0.32868		−7.38686	0.83425	0.0000
a*	P + C	−0.24122		−6.07957	0.86192	0.0000
b*	C[a]	4.39430		10.04831	0.98508	0.0000
C*	C	4.45070		10.71571	0.98576	0.0000
L*	C	−0.52554		97.16972	0.60473	0.0078
C*/L*	C	0.05194		0.09169	0.99388	0.0000
C*/L*	P	0.01945		0.36580	0.84207	0.0000
C*/L*	C,P	0.06131	-4.65681×10^{-3}	0.05570	0.99813	0.0000
b*/L*	C	0.05124		0.08522	0.99255	0.0000
b*/L*	P	0.01909		0.35680	0.83665	0.0000
b*/L*	C,P	0.06144	-5.06313×10^{-3}	0.04609	0.99771	0.0000

[a]P, chlorophyll fraction; C, carotenoid fraction.

ues of b* and the carotenoid concentration, and between those of a* and the evolution of pheophytins.

As the values of b* and a* vary within rather wide ranges (15.9–99.5 and 3.6–26.9, respectively), the expression of each pair of `ues in the quadrant of color yellow-green gives very separated points. Thus, as even small differences in color will result in different positions, the different oils can be more easily classified.

The ratios a*/b* and pheophytins/carotenoids indicate different tonalities, the latter being greener as the absolute value of the ratio is higher. The chroma C* is very similar to b*, being slightly higher. The value L* generally presents very similar values; however, the differences are marked in determinate cases, making it possible to distinguish between oils having similar levels of a* and b*. The ratios C*/L* and b*/L* are

also included as being of possible interest in the establishment of a color index.

In order to know the significance of these relationships, a study of correlation between one parameter and another was made. Table 4 shows the relationships obtained for those variables which were considered best correlated. The coefficient of correlation is higher in those cases when carotenoid concentration participated than when pheophytins did so. The best correlations are obtained in the function of both variables together. However, the relationship between b* and carotenoid content is proposed as the most appropriate to correlate both parameters as they are easiest to evaluate.

In respect of the apparent color of the oils, a close relationship is confirmed between some of the chro-

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matic ordinates and the concentration of major pigments. Thus a color index can be established which, when applied to a sufficiently representative number of oils of different sources and varieties, allows their classification and correlation with the subjective classification. This relationship could be simplified to the determination of two absorption values, at 400 and 670 nm, and the color index obtained for a commercial oil.

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