An Efficient Method for a Numerical Description of Virgin Olive Oil Color with Only Two Absorbance Measurements

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ABSTRACT: Four polynomial expressions are obtained that provide a good approximation and an easy, rapid calculation of the chromatic coordinates and the chroma—L^*, a^*, b^*, and C—for the illuminant C and the standard observer, for a virgin or extra virgin olive oil; absorbance is measured at only 480 and 670 nm. These are as follows: $L^* = 0.556458(A_{480})^2 - 2.51145A_{480} + 0.55504(A_{670})^2 - 8.53016A_{670} + 98.4089$; $a^* = 0.177372(A_{480})^2 + 2.1363A_{480} + 1.43254(A_{670})^2 - 0.789231A_{670} - 13.9246$; $b^* = -16.0277(A_{480})^2 + 79.8932A_{480} - 5.06558(A_{670})^2 + 3.36169A_{670} + 31.9405$; $C = -15.8439(A_{480})^2 + 78.9312A_{480} - 5.26784(A_{670})^2 + 3.56917A_{670} + 33.3927$. These give acceptable results, making the method a practical alternative to the extremely laborious Commission Internationale d'Eclairage (CIE) $L^*a^*b^*$ system, by which 391 absorbance values must be measured individually, nanometer by nanometer, before applying more complex equations. The validity of the proposed method has been confirmed by comparison, using a set of 20 sample oils different from the set of 25 oils used to generate the order of the equations. The variations between the values provided by the proposed and standard methods, respectively, had a mean of 0.00 for each of the chromatic variables—L^*, a^*, b^*, and C; SD were moderate (0.71, 0.52, 1.22, and 1.22, respectively); the root mean square and the R^2 terms also confirmed the validity of the method.

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KEY WORDS: Chromatic coordinates, CIE$L^*a^*b^*$, color determination, virgin olive oil.

Food color stability is an indication of quality. For this reason, color is used increasingly as a key physicochemical parameter to describe a foodstuff. This description is expressed numerically according to the chromatic coordinates a^* and b^*, the lightness L^*, and the chroma C of the CIE$L^*a^*b^*$ standardized system of the International Commission on Illumination (1,2). These values describe an objective color measurement using a spectrophotometer, but this requires absorbance measurements at each nanometer from 380 to 770 nm, together with the spectral composition of the illuminant and the coefficients of the trichromatic color-matching function values at 1-nm intervals, and a computer program to apply the CIE$L^*a^*b^*$ system procedure to transform these data into a few representative numerical color parameters (3).

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UV-vis spectrophotometers are routinely used to measure some of the standard analytical parameters describing an olive oil; some of these parameters—such as the indices of oxidation—do not require the whole spectrum, but only the absorbance values at various wavelengths (4). The objectives of our study are: (i) to find a procedure for the numerical characterization of the color of an oil that does not require measurement across the entire visible spectrum, but only a few—two or three—absorbance values; (ii) to use for this procedure only simple equations worked out with a calculator or with a modest spread sheet; and (iii) to ensure that such a procedure will give acceptable values of the chromatic variables. If the method requires little effort and is suitable for application by an untrained user, it will be utilized as one more of the routine determinations made in the laboratories of oil mills and bottlers to characterize olive oil samples.

EXPERIMENTAL PROCEDURES

Samples. A total of 45 different virgin and extra virgin olive oils, ranging in color from pale yellow to dark green, were used. These oils were, in general, blends of different varieties of olives or of different degrees of maturity within the same variety and were purchased at supermarkets in Spain in bottles of 75 cL. The 45 oils were divided randomly into two groups: 25 of these were used to obtain initial fitting functions to serve as the basis for the method; the other 20 were used to confirm the validity of these functions.

Since some oils are marketed without filtering, in accordance with consumer preferences, it was necessary to centrifuge three of these so that the lower end of their spectrum better coincided with the baseline. The centrifuge was a Selecta Centromix S-549, which was operated at $3120 \times g$ for 1 h.

Spectral acquisition. The spectra were obtained with an ATI Unicam UV4 spectrophotometer connected to and controlled by a personal computer. Polystyrene disposable cells of 1 cm thickness (Dispolab 1937; Kartell, Noviglio, Italy) were used, although many results were checked by repeating the spectra in Suprasil quartz cells (Hellma GmbH & Co. KG, Müllheim, Germany).

Basis of the method. In the visible spectrum of a virgin olive oil, certain bands in the ranges 430–480 nm and 660–670 nm stand out owing to the presence of various carotenoid and chlorophyllic pigments. Oils can be differentiated spectroscopically one from another by the intensity of

these bands. Two of these, one at either 450 or 480 nm, the other at 670 nm, are sufficiently specific for the value of their absorbance to identify, with almost no interference, the quantity of each group of pigments responsible for the total color. By measuring the absorbance of an oil at 480 nm (or at 450 nm) and at 670 nm, it is possible to obtain, approximately, the proportion by which each group of pigments participates in the final color of the oil (5,6). But at present, we are only interested in the absorbance values (or maximum heights) of these well-characterized peaks, because it is possible that the values of their absorbances—for instance, A_{480} and A_{670}—may be related by a simple equation to their chromatic coordinates, in the form: $J = f(A_{480}, A_{670})$, where $J = L^*$, a^*, b^*, or C and f is a first- or second-degree function.

To investigate the possible existence of such relationships, it was necessary to read the absorbances at each nanometer in the spectrum of each of the 45 oil samples to obtain the values of the CIE$L^*a^*b^*$ chromatic coordinates. The method, which we designate as the standard method of calculating L^*, a^*, b^*, and C is described elsewhere (3,7). This calculation was performed using a computer program developed in our laboratory, on the basis of absorbance values from the entire visible spectrum; this calculation may be made for the illuminants C and D65 and for the standard and supplementary observers without distinction.

Statistical procedure. This was performed using a commercial package (8). To compare samples, the Kolmogorov–Smirnov test (KS-test) and the Wilcoxon Mann-Whitney test were applied. The first is used to test whether two samples may reasonably be assumed to come from the same distribution; it does not require the assumption that the population is normally distributed, this test relies on the fact that the value of the sample cumulative density function is asymptotically normally distributed. The second is a nonparametric test for comparing two populations; it is used to test the null hypothesis that two populations have identical distribution functions, against the alternative hypothesis that the two distribution functions differ only with respect to location (median), if at all; this test was used because it does not require the assumption that the differences between the two samples are normally distributed.

RESULTS AND DISCUSSION

Figure 1 shows the L^*, a^*, b^*, and C values for the 45 oils, in which the symbols C and D65 represent the two illuminants and the numbers 2 and 10 represent the standard and supplementary observers, respectively. The profiles are similar in all the curves, except that the maxima of the L^* curves are the minima of the other curves. The change of illuminant and angle produces an almost linear shift of the chromatic coordinate values, with the difference between oils remaining fairly constant. Because the magnitude of a is smaller than that of the other variables, the effect of changing illuminant and angle can be appreciated better in the graph for variable a than in the other graphs. In general, across all the virgin olive oils, the change of angle affects the variables L^* and a^* more than the change of illuminant, whereas the reverse is seen for the variables b^* and C. Table 1 shows the average and SD of ΔL^*, Δa^*, Δb^*, and ΔC, in olive oils, due to change in solid angle of observation and due to change of illuminant. With the exception of the variable a^*, the relative effects of changing illuminant and angle may be considered small and, in the light of this observation, in the following analysis only those results for the illuminant C and for the CIE standard colorimetric observer (visual field of less than 4º) have been employed. In fact, the relative spectral radiant power distributions of illuminants C and D65 are similar in the visible zone (7).

The first group of 25 oils was taken as the determination set and the other group of 20 oils constituted the validation set. The values of variables L^*, a^*, b^*, and C obtained for the two groups, determination and validation, were compared with each other by means of various statistical tests to establish their homogeneity. The findings for each of the four variables were that there is no statistically significant difference between the two distributions and between the means, the SD, and the medians of the two samples at the 95% confidence level. In addition, to test for significant differences between the two sets of data, the respective data for variables L^*, a^*, b^*, and C were collected as pairs. In all four cases the shape of the paired differences indicates a normal distribution (the standardized coefficients of skewness and kurtosis are inside the range ±2.0).

The SD and medians of the two groups are very close and, in both sets, some 50% or more of the oils have values for the variables L^*, a^*, b^*, and C within a similar narrow range: $89 < L^* < 91$; $-11 < a^* < -9$; $118 < b^* < 128$; and $119 < C < 129$; the absolute ranges are larger, and they are given in Table 2 together with the averages, the SD, and the 95% confidence interval of the chromatic coordinates and chroma of the 45 olive oils for illuminant C and the standard observer.

Given that, for almost all the oils, their values for L^*, a^*, b^*, and C form analogous distributions, it is reasonable to assume that all these values are related in a similar way to the absorbance values of the most significant bands responsible for the colors. To confirm this assumption, polynomial functions in the form $J = (A)$ were fitted to the determination set values, where J was running among L^*, a^*, b^*, or C, and the oil absorbance A was successively A_{450}, A_{480}, or A_{670}. The R^2 statistic indicates that a second-order polynomial model, $J = f(A_\lambda^2, A_\lambda)$, explained the variability in each of the four variables better than a first-order polynomial.

In the ANOVA table, the second-order polynomials yielded $P < 0.01$ in all cases, using successively as the independent variable A_{450}, A_{480}, and A_{670}; therefore, there is a statistically significant second-order relationship to a confidence level of 99%. It was also found that this second-order polynomial as a function of the absorbance at either 450 or 480 nm explains more than 99% of the variability of b^* and C. This means that the coordinate b^* and the chroma C can almost be calculated solely from the measurement of the peak

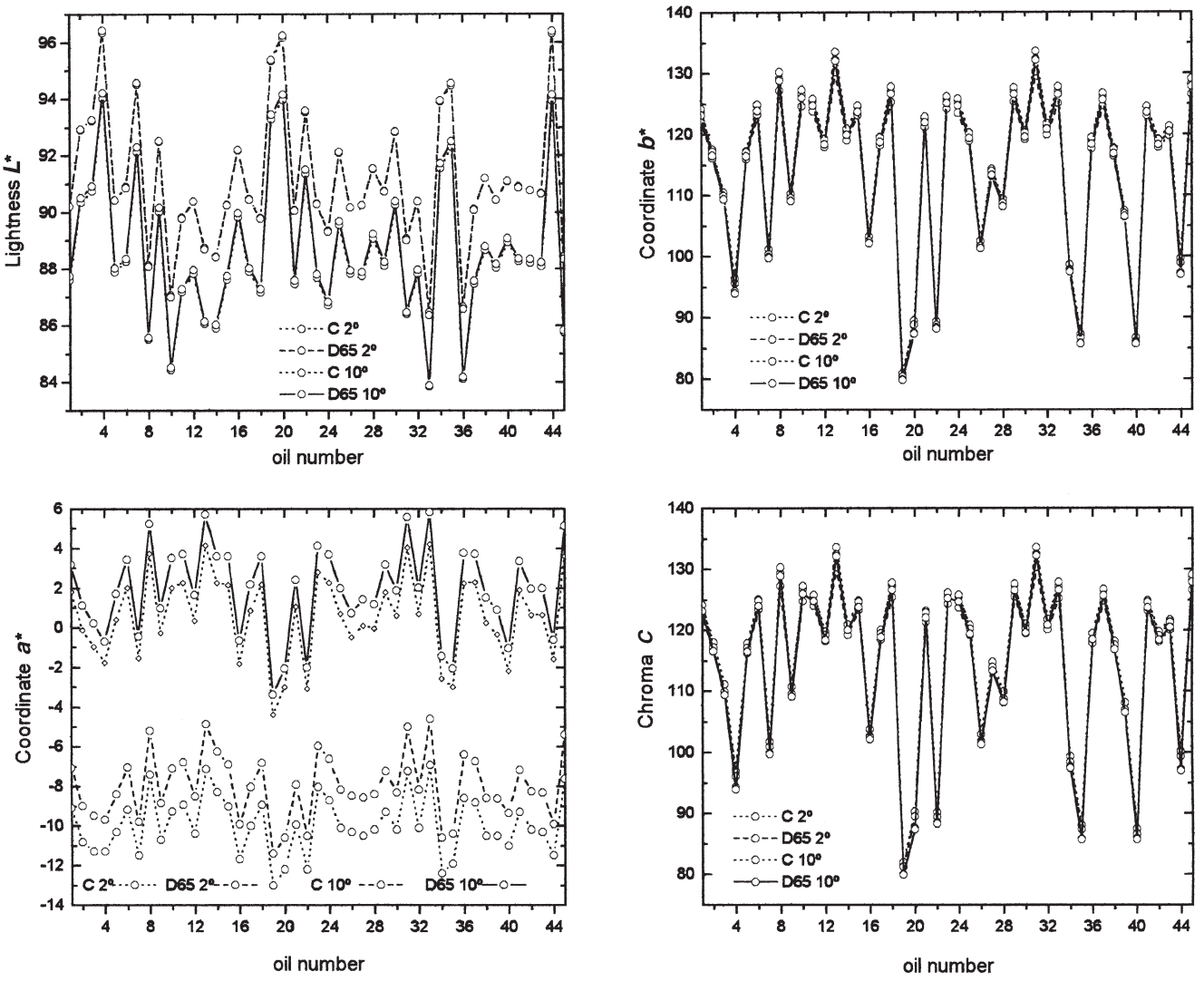

FIG. 1. Variation of *L**, *a**, *b**, and *C*, with the illuminants C and D65, and for the standard and supplementary observer.

absorbance of this band. The reason for this deduction is that the broad band at these lower wavelengths, attributable to carotenoid and chlorophyllic pigments, often exerts more influence on the total color of the oil than the other, narrower band of chlorophyllic pigments in the green zone of the visible spectrum. Nevertheless, it is considered more reliable to use an equation that also takes into account this absorption at 670 nm.

Although the variability of each of the four dependent variables is explained almost equally well by second-order polynomials as a function of the absorbance at either 450 or 480 nm, it was decided to use the data at 480 nm, which explain somewhat better the variability of *b*.

TABLE 1
Mean and SD of ΔL^*, Δa^*, Δb^*, and ΔC, in Olive Oils[a]

Parameter variation	Values for 4° minus values for 10°		Values for C minus values for D65	
	For illuminant C	For illuminant D65	For angle <4°	For angle ≥10°
ΔL^*	2.51 ± 0.16	2.37 ± 0.15	0.005 ± 0.043	−0.130 ± 0.041
Δa^*	−10.53 ± 0.74	−9.88 ± 0.70	−1.95 ± 0.20	−1.29 ± 0.18
Δb^*	0.17 ± 0.84	−0.38 ± 0.89	1.33 ± 0.44	0.78 ± 0.40
ΔC	0.62 ± 1.01	−0.11 ± 1.06	1.48 ± 0.42	0.76 ± 0.38

[a]Attributable to change in solid angle of observation (<4° or ≥10°) and to change of illuminant (C or D65).

TABLE 2
Range of Values of the Chromatic Coordinates and Chroma of Olive Oils for Illuminant C and Standard Observer

Variable	*L**	*a**	*b**	*C*
Range	86.5–96.4	–13.0– –6.9	81.0–132.3	82.0–132.5
Average	91.1	–9.9	115.0	115.4
SD	2.4	1.5	13.6	13.4
95% Confidence interval	0.72	0.45	4.09	4.04

Thus, the values of the variable *J*—i.e., *L**, *a**, *b**, or *C*—of the oils of the determination set were fitted to equations of the following type:

$$J = P_1 (A_{480})^2 + P_2 A_{480} + P_3 (A_{670})^2 + P_4 A_{670} + P_5 \qquad [1]$$

where A_{480} and A_{670} are the absorbances at 480 and 670 nm, and P_i are coefficients determined iteratively by the Gauss–Newton method. The values of P_i coincide with those obtained by the least squares method and differ only slightly from those obtained by the Marquardt method, but with this latter method, the R^2-test, involving multiple regression, had slightly lower values.

In Figure 2, open circles represent the values computed for the three chromatic coordinates and for *C*, obtained with the CIE*L***a***b** standard equations applied to 391 absorbance readings, as plotted against the predicted values calculated using only two measurements of absorbance and the quadratic equations previously deduced. The degree of agreement between the CIE*L***a***b** calculation and our approximation is measured by the separation with respect to the perfect correlation line. This separation is insignificant for variables *b** and *C*, and only a little larger for variable *L*; the greatest distances are observed in the graph for *a**, but the root mean squared error for *a** (RMS) is 0.40. Allowing for the different scales, the graphs for *C* and *b** are similar, since $C = (a^{*2} + b^{*2})^{1/2}$, and *b** is much greater than *a**. The mean of the differences between predicted and standard values is 0.00 for *a**, *b**, and *C* and is virtually zero for *L**.

As a test of the equations, they were applied to the valida-

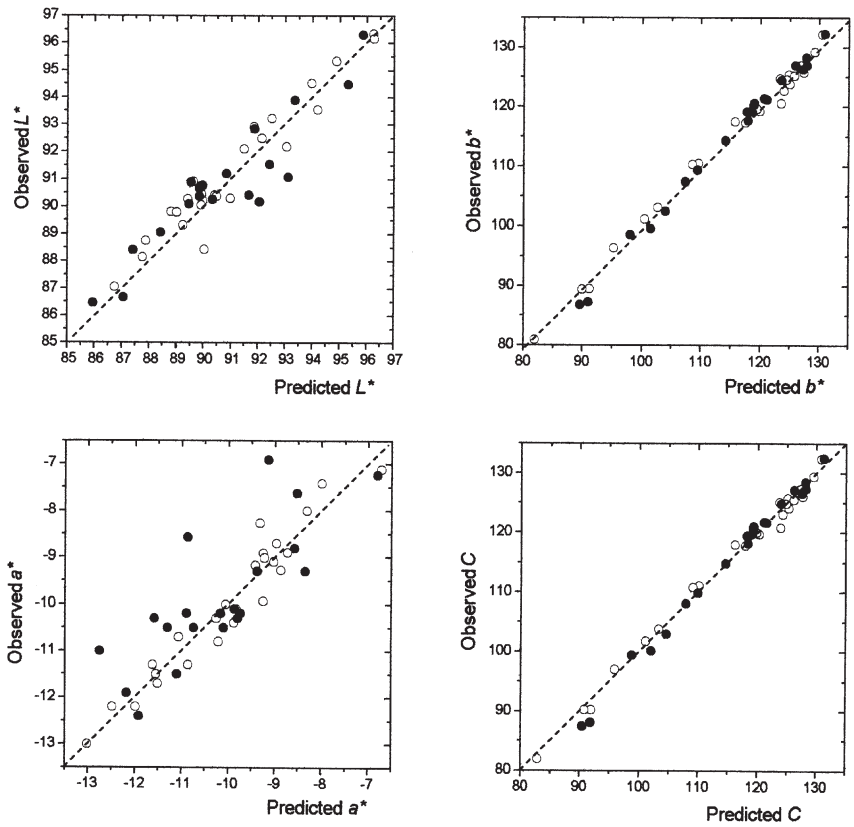

FIG. 2. Standard values obtained with the CIE*L***a***b** method and 391 absorbance values, plotted against the values obtained with a second-order polynomial using only two absorbance measurements. (○) Determination set; (●) validation set.

TABLE 3
Equations for Estimating the Chromatic Coordinates and the Chroma *C* of a Virgin or an Extra Virgin Olive Oil, Measuring the Absorbance at Only 480 and 670 nm

Equation	R^2	SEE[a]	RMS[b]
$L^* = 0.556458(A_{480})^2 - 2.51145A_{480} + 0.55504(A_{670})^2 - 8.53016A_{670} + 98.4089$	0.914	0.74	0.83
$a^* = 0.177372(A_{480})^2 + 2.1363A_{480} + 1.43254(A_{670})^2 - 0.789231A_{670} - 13.9246$	0.881	0.54	0.72
$b^* = -16.0277(A_{480})^2 + 79.8932A_{480} - 5.06558(A_{670})^2 + 3.36169A_{670} + 31.9405$	0.992	1.28	1.28
$C = -15.8439(A_{480})^2 + 78.9312A_{480} - 5.26784(A_{670})^2 + 3.56917A_{670} + 33.3927$	0.992	1.28	1.29

[a]Standard error of the estimate.
[b]Root mean squared error.

tion group of 20 oils. Again, the predicted values for L^*, a^*, b^*, and C were compared with the values obtained by applying the CIE$L^*a^*b^*$ method to each oil. This comparison is shown graphically as solid circles in Figure 2. As can be observed, the agreement is good except for some values of a that have enough influence to increase the RMS to 0.98, but the rough relative error—estimated as RMS/$|a|$—is less than 10%. Consequently it is considered that, overall, the use of these quadratic equations to obtain the chromatic coordinates is acceptable.

On the strength of this, a further set of quadratic equations was deduced but this time employing the data of the total group of 45 different oils. Such equations together with some of the statistical results are presented in Table 3; the values of the R^2 statistic indicate that the model as fitted explains the indicated variability of L^*, a^*, b^*, or C; the R^2 for a is not as high as for the other variables. The variability is satisfactory, in general, given that the experimental samples form a set of diversified oils of different compositions.

In the right-hand columns of this table are shown the standard error of the estimate (SEE) and the RMS of the differences between the predicted and standard values; the latter gives more weight to large errors. Therefore, the coordinate a^* has a RMS rather different from its SEE. On the whole, the measures of accuracy of L^*, a^*, b^*, or C are acceptable; this is a foreseeable finding in light of the elevated R^2 values; hence, it is considered that the proposed equations are reliable for use in the rapid estimation of the chromatic coordinates of any virgin or extra virgin olive oil.

Figure 3 shows the residuals, i.e., the predicted values less the standard values, plotted against the values predicted with these second-order equations. For variables L^* and a^*, the residuals fall approximately between +1 and −1, whereas the residuals of b^* and C mostly fall between +1.5 and −1.5. The mean of these residuals for each of the chromatic variables was again 0.00 with admissible SD: SD (L^*) = 0.71, SD (a^*) = 0.52, SD (b^*) = 1.22, and SD (C) = 1.22. These values and the previous ranges are considered acceptable for most purposes,

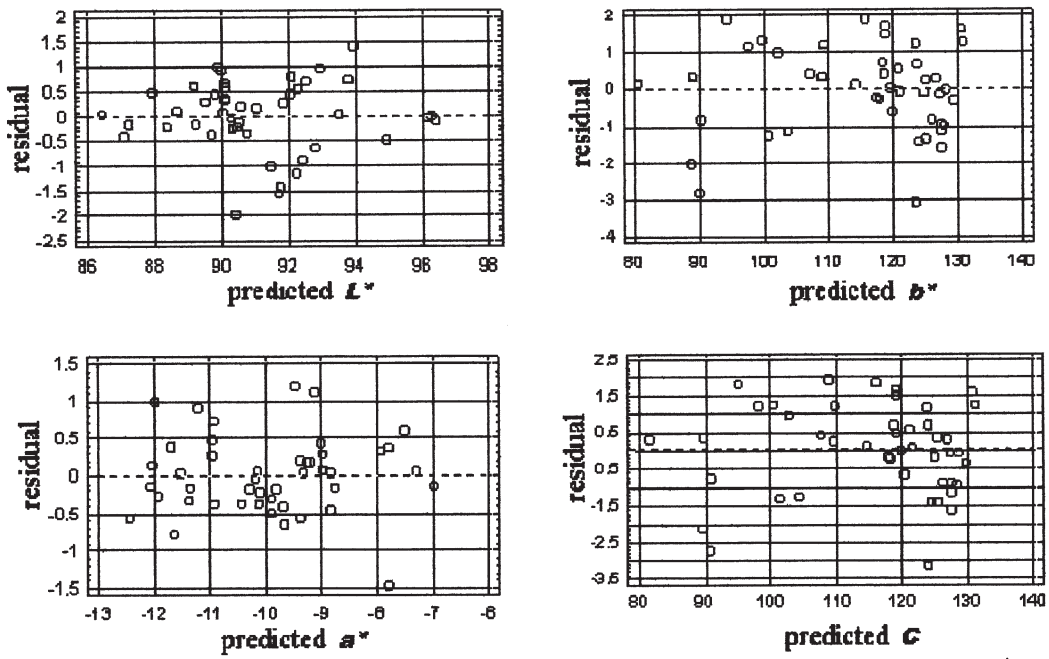

FIG. 3. Residuals (predicted values less standard values) compared with values predicted using the equations given in Table 3.

given the simplicity of the equations in Table 3 used to calculate the predicted values.

The form of the proposed equations in Table 3 is easily adapted to a calculator or a spreadsheet, or a simple computer program can be written to evaluate the chromatic coordinates and the chroma, using as input data the absorbance at 480 and 670 nm of pure virgin or extra virgin olive oil measured in a cell of 1 cm thickness.

The method proposed here should not be confused with methods that use only a few absorbance values to obtain the tristimulus values, *X*, *Y,* and *Z*, with which the chromatic coordinates are calculated using the equations of the CIE*L*a*b** method. These methods, which have been applied to such foodstuffs as wines and olive oils, and which are designated weighted or selected ordinate methods, are much more laborious than the method proposed here because they use all the necessary equations of the CIE*L*a*b** method; moreover, they provide rather different results for *L**, *a*,* and *b*,* depending on the number of absorbance values used in the calculation (3,5,9).

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