Reliability of the Bromthymol Blue Method for Color in Virgin Olive Oils

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ABSTRACT: The bromthymol blue (BTB) method is currently used for the assessment of color in olive and seed oils by visual comparison with standard solutions. Two BTB scales were prepared with 2 yr difference and compared, and the recent one was used to analyze 502 virgin olive oil samples, obtained by the Abencor® technique reproducing the industrial procedure. The temporal chromatic degradation of the BTB samples after 2 yr [3.93 Commission Internationale de l'Eclairage (CIE) 1976-(L*a*b*) (CIELAB) units, on the average], as well as the small percentage of virgin olive oils matching the colors of the samples provided by the BTB scales (13.1% with a suprathreshold color tolerance of 1.52 CIELAB units), indicates the limitations of the BTB method. Linear regression models are proposed in order to compute with acceptable accuracy the BTB indices from chromaticparameters. The use of CIELAB for the specification and future studies on color in virgin olive oils is recommended.

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KEY WORDS: Bromthymol blue method, CIELAB, color, tristimulus colorimetry, virgin olive oil.

Color is a fundamental characteristic in the quality of virgin olive oil, directly related to the chlorophyll and carotene contents, and it is influenced by a number of factors: olive variety, cultivar conditions, maturation index, oil extraction procedures, and conservation conditions (including humidity, temperature, illumination, and oxygen exposure). Therefore, virgin olive oils show colors ranking from dark green for the earliest olive oils of the harvest to pale yellow for overripe oils.

There are several methods proposed for the visual determination of color in oils and fats (1–9). Spain's official method for oil color analysis is based on the determination of the "bromthymol blue (BTB) index" through the comparison of the oil with visual standards (10). It establishes a scale of indices for the color determination of olive and seed oils, warning that red tonalities are not included; that is, the method is only advised for tonalities varying from yellow to green. Diverse standard solutions of BTB at different pH values are prepared for visual comparison with the sample. The method stipulates the preparation of nine standards with the same BTB concentration (15.4 mg/L) in mixtures of KH_2PO_4 and Na_2HPO_4 at different proportions. In addition, the method recommends, if necessary, preparing another series with the same pH scale but with different BTB concentrations to better simulate the color of the samples. This modification has been developed for virgin olive oils, suggesting a two-dimensional scale involving 10 different concentrations of BTB in six different mixtures of KH_2PO_4 and Na_2HPO_4; that is, a total of 60 standard solutions (11). This two-dimensional scale improves the sampling of colors of real virgin olive oils, providing better determination of dark green hues.

The solutions of BTB scales are visually compared with oil samples to assign the corresponding index. It is possible to use instrumental devices to facilitate comparisons; however, it is generally accepted that the method lacks sufficient precision and accuracy. For example, the illuminating source that must be used is not specified, nor are the characteristics of the "white" background against which the visual assessments are performed following this method. Thus, it has been previously stated that the BTB scales entail deficiencies concerning their validity in the characterization of virgin olive oils (12), due to the existence of metamerism (13) and also the visual variability of the observers. In fact, the assessment is frequently done by an untrained operator whose visual aptitude and normal color vision have not been previously tested, but the application of the method implies an important effort to achieve a relatively satisfactory visual match. Moreover, it is necessary to prepare a great number of solutions, which makes the procedure long and tedious.

Tristimulus colorimetry, developed by the International Commission on Illumination (Commission Internationale de l'Eclairage, CIE), has been demonstrated to be a valuable resource for solving the problems of objective analysis of color in foods, and diverse applications have been carried out with vegetable oils. Relations between olive variety or stages of ripeness with pigment content and chromaticity coordinates a*, b* have been found (14). Reciprocal conversions have been proposed between methods for evaluating the color of oils. In relation to plant bleaching experiments, Lovibond and CIE systems gave similar results (15). Tristimulus measurements have been carried out by diverse simplified methods in comparison with the CIE recommended procedure based on

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the whole visible spectrum, concluding that those simplified methods are only valid for certain purposes (16–18).

Since 1976 the CIE has recommended the use of CIE 1976-(L*u*v*) color space (CIELUV) and CIE 1976-(L*a*b*) color space (CIELAB) as approximately uniform color systems, with the primary goal of promoting uniformity among users in practical applications. In particular, the CIELAB system has been accepted worldwide in most industries (19,20), starting from the tristimulus values previously defined by CIE colorimetry (21). In looking for an improved correlation between perceived and measured color differences, a recent color-difference formula, based on CIELAB and designated as CIE94, has been proposed for industrial color applications (22)

The aim of this work is to determine the reliability of the BTB visual comparative method. Through tristimulus colorimetry, we have stated color differences between time-dependent spoiled and nonspoiled scales and color discrimination thresholds to match virgin olive oil samples with BTB standards. Mathematical models to obtain BTB indices from CIELAB parameters are proposed.

EXPERIMENTAL PROCEDURES

Samples (502) of virgin olive oils were obtained from diverse olive varieties collected at the most representative production zones in Andalucía, Spain, during the 1995–1996 harvest. Oils were extracted in the laboratory by the Abencor® method (23), reproducing the industrial procedure. This system reproduces the working process of a standard olive mill at small scale and produces oil with all its flavors and taste almost intact, which can be used to perform organoleptic tests. Olive fruits were transformed into a paste after milling in an electric mill. The resulting paste was mixed in a malaxator and centrifuged at 3500 rpm to obtain the oil.

The 60 standard solutions proposed by the BTB method were prepared with increasing volumes of BTB 0.04% solution in different mixtures of KH_2PO_4 1/15 M and Na_2HPO_4 1/15 M solutions, according to the established procedure (11). This produced BTB concentrations ranging from 0.0078 to 0.0667 mg/mL. Standards were stored in the dark at 20°C. By using this procedure, two series of standards were prepared; a 2-yr-old standard (scale A), and a fresher standard (scale B). Scale B solutions were used as reference standards in all the calculations made in this work

With respect to the color measurements of oil samples and BTB standard solutions, the spectral transmittances in the whole visible range (380–770 nm) were measured with an ultraviolet-visible light diode array spectrophotometer (HP8452; Hewlett-Packard, Palo Alto, CA), using quartz cells with a pathlength of 5 mm. To obtain tristimulus values, the weighted-ordinate method (constant intervals, $\Delta\lambda = 2$ nm) was applied (20), using as references the CIE 1964 Standard Observer, the CIE Standard Illuminant D_{65}, and *n*-hexane as the white reference solution for transformations to CIELAB.

Following the most recent recommendations made by the CIE, we used the CIELAB system (i.e., the tridimensional L*, a*, b* coordinates, or the variables related with color attributes: lightness, L*, chroma, C^*_{ab}, and hue, h_{ab}) for color specifications, and the CIE94 color-difference formula (recently recommended for industrial applications, and based on CIELAB) when a better correlation with visually perceived color differences was desired (22). Statistical analysis was carried out using Statgraphics 5.0 software (Rockville, MD).

RESULTS AND DISCUSSION

First, we analyzed the temporal degradation of color of the standard solutions provided by the BTB method previously described. The CIELAB color differences (ΔE^*_{ab}) between corresponding samples of two BTB scales prepared with a difference of 2 yr were, on the average, 3.93 CIELAB units. Figure 1A shows the CIELAB color difference found for each BTB sample, which can be split into three parts, lightness ΔL^*, chroma ΔC^*, and hue differences ΔH^*, as shown in Figure 1B.

In most samples, the storage time causes a significant color degradation, clearly perceptible by the human eye (the large difference shown by some of them, e.g., 5/10 or 7/7, could also be due to experimental errors in preparation of the scale). A CIELAB color difference of around 1.0 is usually considered greater than the visual threshold for a normal observer (24). Specifically, the BTB samples became darker with time (L* decreased in 51 of the 60 solutions), more saturated (C* increased in 59 solutions), and slightly reddish (hue decreased in 52 solutions). On the average, the main contribution to the measured color differences ΔE^*_{ab} was the change in chroma ΔC^* (68.6%), followed by the change in lightness ΔL^* (27.5%) and hue ΔH^* (3.9%). In particular we also observe that $\Delta C^*/\Delta L^*$ give the main contributions to ΔE^*_{ab} for most of the samples with low/high pH values, respectively (see Fig. 1B).

These results suggest that the color stability of the BTB samples must be checked before use, even though they were maintained under appropriate storage conditions. In fact, this is not a new finding because a maximal storage period of 6 mon was advised in the initial BTB method (10). This period could be considered in good agreement with our average 2-yr-comparison results ($\Delta E^*_{ab} = 3.93$), assuming both a unit threshold color-difference ΔE^*_{ab} and a linear dependence of color differences with time. However, in particular this last assumption should be checked with a greater number of measurements over time, bearing in mind that sometimes color degradation processes appear to be nonlinear in other materials (25,26).

In a second step, we analyzed color discrimination within our old and new BTB scales (scales A and B, respectively), as well as the possibility of achieving a visual match between the colors of virgin olive oils and BTB samples. Color discrimination results are usually reported in a given color space (usually x,y,Y or CIELAB) by ellipsoids: all color stimuli within a given ellipsoid cannot be distinguished from the one

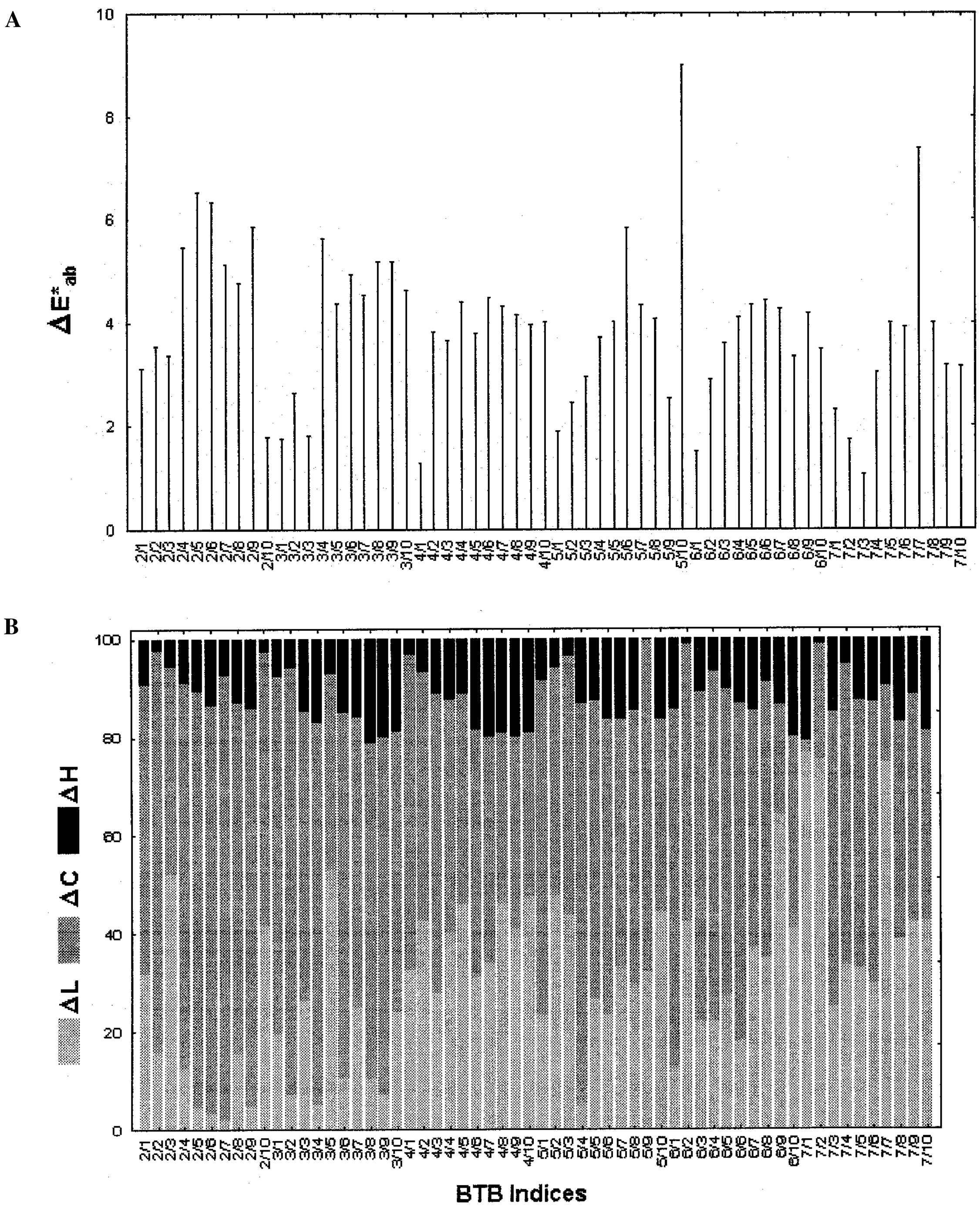

FIG. 1. (A) Commission Internationale de l'Eclairage (CIE) 1976-(L*a*b*) (CIELAB) color differences for each of the 60 bromthymol blue (BTB) samples over a period of 2 yr. (B) Lightness (ΔL^*), chroma (ΔC^*) and hue differences (ΔH^*) contributing to the whole CIELAB color difference ΔE^*_{ab}.

placed at its center by a normal observer. Here we used the discrimination ellipsoids predicted by the CIE94 color-difference formula with three different sizes, corresponding to just noticeable, threshold, and suprathreshold color differences. The sizes of these three types of color differences have been estimated as 0.23, 0.66 and 1.04 CIE94 units, respectively (27,28), starting from experimental research designed to study color differences with different sizes (29–31).

The results of our analyses are summarized in Table 1. As expected, all the samples of the new BTB scale have different colors if we use a just noticeable or threshold color difference. However, for the old BTB scale there are three pairs of samples that cannot be distinguished using a threshold tolerance (specifically the pairs 2/2–3/2, 2/9–2/10 and 4/1–5/1). The significant color differences between old and new BTB scales are also illustrated here by the fact that, for example, at a

TABLE 1
Pairs of Samples Which Can Be Considered Visually Identical from the Predictions Made by the CIE94 Color-Difference Formula[a]

Tolerance limits (CIE94 units)	Old–old BTB samples	New–new BTB samples	Old–new BTB samples	Oil–new BTB samples
Just noticeable (≤0.23)	0 (0%)	0 (0%)	6 (10%)	1 (0.2%)
Threshold (≤0.66)	3 (5%)	0 (0%)	11 (18.3%)	21 (4.2%)
Suprathreshold (≤1.04)	7 (11.7%)	8 (13.3%)	23 (38.3%)	66 (13.1%)

[a]Three tolerance limits (1st column) and four different pairs of datasets (2nd to 4th columns) are considered. BTB, bromthymol blue; CIE, Commission Internationale de l'Eclairage.

threshold tolerance, only 11 of the 60 samples (18.3%) of the old scale are within the threshold ellipsoids of the samples with the same designation in the new scale. The last column in Table 1 shows that only a small number of virgin olive oils can be matched with the samples provided by the new BTB scale: specifically, using the largest (suprathreshold) tolerance limit, only 13.1% of the 502 oil samples obtained.

The small proportion of oil samples matching BTB samples is another important shortcoming of this method, although this should also be expected from the use of any other color order system or color atlas with the same purpose. For example, a rough calculation in CIELAB of the ratio between the volume of the 60 ellipsoids associated with the BTB freshly prepared solutions (scale B) and the whole volume covered by these samples lead us to a probability lower than 3% for a visual threshold match. In addition, a more important question in our case is that the color gamuts corresponding to the BTB samples and our virgin olive oils do not completely correspond, as shown by Figure 2. It is apparent there are many oil samples with higher C* or b* than the solutions provided by the BTB scales. These samples would not be matched anyway by normal observers using BTB scales.

Aside from the problems of the BTB method previously mentioned, there is a relatively good correlation between BTB indices and different CIELAB parameters, as shown in Table 2. For example, high linear correlations were observed between the two BTB indices, pH and concentration, and the CIELAB parameters a* and C*, respectively. It is possible to obtain appropriate multiple regression models for the BTB indices (Table 3), using the stepwise method with backward selection (F-to-remove: 4.0). Models indicated as [1] and [4] are estimations from L*, a*, b*, while models indicated as [3] and [6] are estimations from L*, C*, h (deg). We provide these four models because although usually the CIELAB coordinates L*, a*, b* are used, some workers prefer the use of parameters L*, C*, h (deg), which are correlated with the

TABLE 2
Pearson's Product Moment Correlations Between Each Pair of Variables (BTB indices and CIELAB parameters)[a]

	BTB-pH	BTB-Conc.	a*	b*	L*	C*	h (deg)
BTB-pH		0.0[b]	–0.8468	–0.2176[b]	–0.3839	–0.2074[b]	0.7256
BTB-concentrate	0.0[b]		0.1896[b]	0.9405	–0.9039	0.9421	–0.6675
a*	–0.8468	0.1896[b]		0.3361	0.2383[b]	0.3270	–0.7499
b*	–0.2176[b]	0.9405	0.3361		–0.7733	0.9999	–0.8256
L*	–0.3839	–0.9039	0.2383[b]	–0.7733		–0.7788	0.3208
C*	–0.2074[b]	0.9421	0.3270	0.9999	–0.7788		–0.8194
h (deg)	0.7256	–0.6675	–0.7499	–0.8256	0.3208	–0.8194	

[a]The results shown in this table are from the freshly prepared BTB samples (scale B). CIELAB, CIE 1976 = (L*a*b*). a*,b*, chromaticity cooordinates; L*, lightness; C*, chroma; h, hue. See Table 1 for additional abbreviations
[b]Statistically nonsignificant correlation at the 95% confidence interval..

TABLE 3
Regression Linear Models for the Two Variables of the BTB Scale (concentration and pH) from Different CIELAB Parameters (stepwise backward selection; F-to-remove 4.0)[a]

Model	R^2 (%)	S.E.E.	M.A.E.
[1] Conc. = 36.0604 + 0.2605 a* + 0.0200 b* – 0.3612 L*	99.34	0.234	0.183
[2] Conc. = 59.3718 – 0.3215 L* – 0.2787 h	97.51	0.457	0.376
[3] Conc. = 62.9568 – 0.3341 L* – 0.0067 C* – 0.3007 h	97.48	0.460	0.377
[4] pH = 27.9560 – 0.1501 a* – 0.0642 b* – 0.2392 L*	82.66	0.717	0.498
[5] pH = –64.8776 + 0.0896 C* + 0.6660 h	98.21	0.230	0.164
[6] pH = –58.4833 – 0.0245 L* + 0.0778 C* + 0.6285 h	98.27	0.227	0.158

[a]The results shown in this table are from the freshly prepared BTB samples (scale B). R^2 (%), coefficient of determination adjusted for degrees of freedom; S.E.E., standard error of estimation; M.A.E., mean absolute error of the estimation. See Table 1 for other abbreviations.

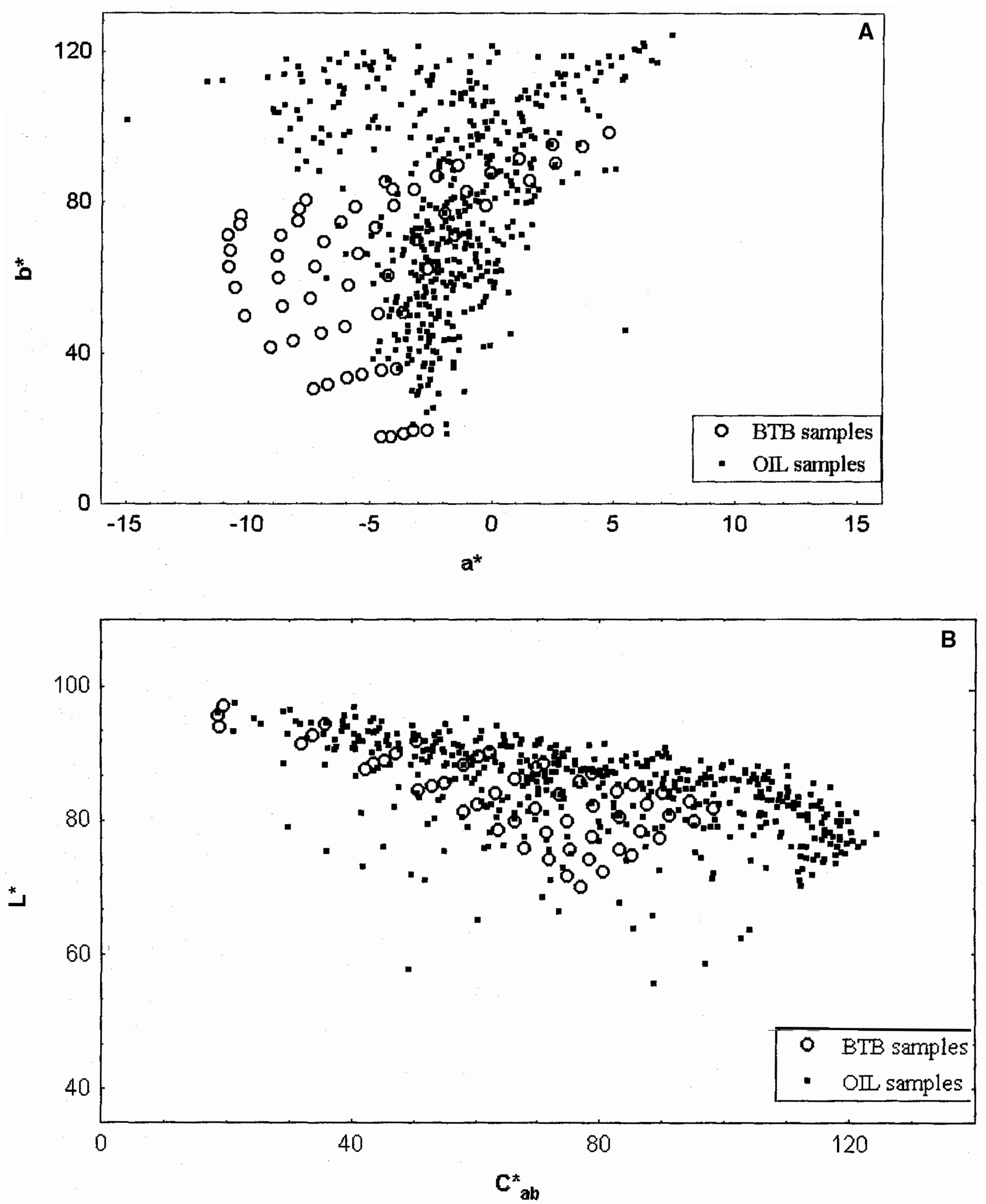

FIG. 2. BTB standard solutions and virgin olive oil samples measured in CIELAB color space: a*,b* (A) and C*,L* (B) diagrams. For abbreviations see Figure 1.

three classical attributes of color perception (lightness, chroma, and hue). In addition, we have also provided in Table 3 models [2] and [5], with very close performance to models [3] and [6], respectively, but using only two independent variables. The stepwise procedure employed to obtain the models uses the good correlation existing between L* and C* in this case (Table 2), achieving these two more simplified models. In particular, we would like to emphasize in Table 3 the good results provided by the models [1] and [6], where the mean absolute errors for the estimation of the BTB concentration and pH are 0.183 and 0.158, respectively.

Models provided in Table 3 could be useful in obtaining the BTB indices from color measurements in CIELAB, avoiding the preparation and potential chromatic degradation of the standard solutions indicated by the BTB method. In addition, bearing in mind that the BTB standard solutions are not uniformly distributed in CIELAB color space (see Fig. 2), we could design an improved distribution of L*, a*, b* coordinates and obtain from these models the corresponding solutions which should be prepared. To a certain extent the CIELAB space can be considered uniform, and the new BTB solutions obtained by this procedure could be better than the current ones for achieving a match with real oil samples.

This article shows some limitations to the use of the BTB method for the visual assessment of color in virgin olive oils. Although this method should be improved (for example, by fixing at least the illuminating and viewing conditions for the assessments, or increasing the number of standard solutions and their regular distribution in color space), we think that modern colorimetry requires the use of the CIELAB system, recommended by the CIE since 1976 and accepted worldwide in many other practical and industrial applications of color. An interim solution for practitioners with extensively customized use of the BTB method should be the use of the linear regression models proposed here, starting from accurate color measurements made with currently available instrumentation (which usually provides CIE tristimulus values and CIELAB coordinates).

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