Reproducibility of the Bromthymol Blue Standards Used for Color Specification of Virgin Olive Oil

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ABSTRACT: Three replicates were prepared for each of 60 BTB (bromthymol blue) standards, which are usually employed to determine the color of virgin olive and seed oils, and their colors were measured by spectrophotometric and spectroradiometric techniques on a monthly basis over a year. Although in principle both techniques are valid, their results are weakly correlated. The major color change of the BTB standards occurred soon after sample preparation; after 5 mon, the color stabilized at approximately 3.0 Commission Internationale de l'Éclairage 1976-(L*a*b*) (CIELAB) units, with respect to the initial values. Therefore, after preparation, a certain waiting period would be advisable before using the BTB standards. The color of the BTB standards changes over time in the sense of becoming lighter, more saturated, and less greenish. In the monthly periods after the fifth month, the average color change of the BTB standards was negligible, being slightly lower than the average variability of the three replicates (which is around 1.5 CIELAB units).

Paper no. J9707 in JAOCS 78, 265–270 (March 2001).

KEY WORDS: Bromthymol blue method, BTB method, CIELAB, color differences, color fastness, color of virgin olive oils, color reproducibility, oil-color measurement.

In any physical measurement, the spatial and temporal stability of the units used is a relevant question, given their influence on the accuracy and precision usually desired. Color measurement is not an exception to this rule (1), and thus, the instruments conventionally employed (e.g., spectrophotometer, spectroradiometer, and colorimeter) are calibrated and verified periodically using, for example, sets of sufficiently uniform and stable samples (2). Color specification through visual comparison with a set of standards is also a common type of measurement, owing to its simplicity and quickness. Certainly, the subjectivity of this last type of color measurement implies limited precision and accuracy, which can be improved by controlling the illumination and observation conditions as well as by using appropriate sets of color samples (e.g., samples regularly distributed in the color space and stable over time). Thus, for example, the Munsell atlas (3) is a good example of a rigorous collection of color samples, widely used and studied (4), reduced versions of which have been marketed for specific ends, such as determining soil color from a visual comparison (5). The bromthymol blue (BTB) method (6) is a modification of the current Spanish guidelines to determine virgin olive oil color (7). The procedure is also based on a visual comparison between the color of one oil sample and that of a series of preestablished solutions (BTB standards) so as to select the solution with the color most similar to that of the oil sample provided. For an appropriate determination of oil color, the BTB standard solutions should be both spatially and temporally stable.

Color is a basic criterion in assessing the quality of virgin olive oil. Although the measurement of this parameter is not currently required by rules set by the European Economic Community, it is a fundamental attribute in organoleptic evaluation (8). The quality of olive oil is determined by the set of properties or attributes that the oil possesses; these in turn determine the degree of consumer acceptance with respect to a particular use. In this context, the color of olive oil can be considered a key factor to take into account in quality control. Some authors have studied the relationship between color and quality in oils (9), proposing the color measurement of virgin olive oil as a characteristic of the quality of oils produced by different extraction methods (10), as a criterion for classifying oils according to origin and variety of the olive (11), or as a way of improving the characterization of oils (12).

The main goals of the current article are to report the variability in color of the BTB standards, as a consequence of the errors in the preparation of these standards and in the color measurement itself, and to analyze the color change of these standards with the passage of time. The temporal degradation of color in different materials is a known fact (13,14), which can also be assumed in the case of the BTB standards. In fact, it is recommended (7) that the color of the BTB standards be tested every 6 mon by comparison with recently prepared solutions, and significant color variations have been reported after a 2-yr period for a set of BTB standards (15). If the changes in color are real, then the loss of color fastness of the BTB standards may imply that, for rigorous specification of the oil color, the period of use of these standards should be limited to an appropriate time interval.

For our goal, we measured color with two different instruments. First, we used a spectrophotometer, an instrument that is easily accessible to oil-related industries, but which poses certain problems, such as the assumption of a reference illuminant (not exactly reproducible with real light sources), and the necessary manipulation of the samples to introduce them into

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their corresponding cells. Because the BTB method is based on a visual comparison, use of a spectroradiometer is appropriate for our study, since this instrument enables the evaluation of radiation truly reflected from an illuminated sample. Although spectroradiometric measurements require only minimal manipulation of the samples, careful control is needed for the geometry with which the samples are illuminated and measured and, because the samples are transparent, for the background behind them. With the spectrophotometer, we perform the color measurement basically by transmission (in fact it is the spectral transmittance that is measured), while with the spectroradiometer we measure the color corresponding to the light diffusely reflected by the sample. The comparison between the spectrophotometric and spectroradiometric measurements of the BTB standards over time forms part of the objective of the present work.

For this report, reproducibility is defined as the closeness of agreement between the results of successive measurements of the same test specimen (16). Conditions that changed and could be reflected in these measurements include operator, measuring instrument, laboratory, and time. The goal of this article is to study the reproducibility of the BTB standards looking for an improved method for color specification of virgin olive oils.

EXPERIMENTAL PROCEDURES

Separately, three replicates of each of the 60 standards making up the BTB scale were made, following the conventional procedure (6) and using automatic dosimeters to improve reproducibility. Each of the three replicates was divided in turn into two series of standards, one of which was measured by a spectrophotometer and the other by a spectroradiometer.

The spectrophotometer used was a Hewlett-Packard HP8452 (Palo Alto, CA) having quartz cells with a pathlength of 10 mm, assuming a daylight illuminant with correlated color temperature of 6500 K (D65), and the Commission Internationale de l'Éclairage (CIE) 1964 Standard Observer (17). The spectroradiometer used was a PR-704 of PhotoResearch (Chatsworth, CA), assuming CIE 1964 Standard Observer. For the measurements with this latter instrument, the different BTB standards (45 mL of solutions situated in identical cylindrical bottles 35 mm in exterior diameter) were situated in the interior of a Verivide CAC 120 cabinet (Frog Island, Leicester, United Kingdom) with a D65 light source, the spectroradiometer being focused (1° field) in the center of the sample. Given that the solutions measured were transparent, the Munsell gray mask (5) was used as a neutral and standard background the better to standardize the spectroradiometric measurements. The gray mask is preferable to the gray of the Verivide cabinet walls. (The gray color provided by the Verivide cabinet does not match with the Munsell gray mask, which is generally considered the standard background in colorimetric measurements.) Following the CIE recommendations (17), we referred all the color measurements and color differences in this work to the Commission Internationale de l'Éclairage 1976-(L*a*b*) (CIELAB) system. Thus, the coordinates of the Munsell gray mask situated behind the samples were $a^* = 1.1$; $b^* = 3.4$; $L^* = 93.9$. The reference white used to obtain the CIELAB coordinates was *n*-hexane, in the case of the spectrophotometric measurements, and, for the spectroradiometric measurements, a pressed barium-sulfate-powder plaque, supplied with the instrument. In both cases, the corresponding reference white was measured under the same experimental conditions, as were the BTB standards.

The color measurements of the different BTB standards were made immediately after preparing the solutions, 15 and 30 d after their preparation, and, from then on at monthly intervals for a year. Thus, 14 measurements were made over the experimental period, for three replicates of each of the 60 BTB standards, by two different methods (spectrophotometric and spectroradiometric); that is, a total of 5040 measurements. Between the successive measurements, the BTB standards were kept in darkness at room temperature and pressure, following the usual practice. The warm-up, verification, and other recommendations made by the manufacturers of our color measurement instruments were carefully tested before each session of experimental measurements.

RESULTS AND DISCUSSION

Comparison between spectrophotometric and spectroradiometric measurements. Figure 1 shows the coordinates a*, b* of the 60 BTB standards (average of three replicates) immediately after their preparation for spectrophotometric and spectroradiometric measurements. The corresponding BTB index (pH-concentration) is indicated together with each standard. Note that the characteristic triangular distribution of the BTB standards in the a*,b* plane from spectrophotometric measurements (15) disappears when the spectroradiometric measurements are used. A comparable result is found when considering the third dimension L* of the CIELAB space (i.e., using the representations in the a*,L* or b*,L* plane). However, these results are not surprising, since they must be attributed first, to the different thickness of the samples used (10 mm in the spectrophotometer; 35 mm in the spectroradiometer) and second to the different illumination/observation conditions in the two types of measurements: i.e., the transmitted light in the spectrophotometer vs. the light reflected diffusely by the sample plus the gray Munsell mask behind in the spectroradiometer.

Applying the Lambert-Beer law (16) to the absorption values, we referred the results of our spectrophotometric measurements to a 35-mm thickness. After computing the corresponding transmittances and tristimulus values, these results led to a certain similarity with the CIELAB coordinates obtained from our spectroradiometric measurement (Fig. 2A). Even so, there was a low correlation between any of the coordinates a*, b*, L* of the BTB standards obtained from the spectrophotometric and spectroradiometric measurements (in the best of cases, r =0.479, corresponding to the coordinate L*). The similarity between the two types of measurement slightly increased (Fig. 2B) when we referred to the spectrophotometric measurement at 35 mm, and compare it to the result of a new spectroradiometric measurement made without using the Verivide cabinet, but rather by simply situating one of the D65 sources of the cabinet behind the sample and the spectroradiometer in front

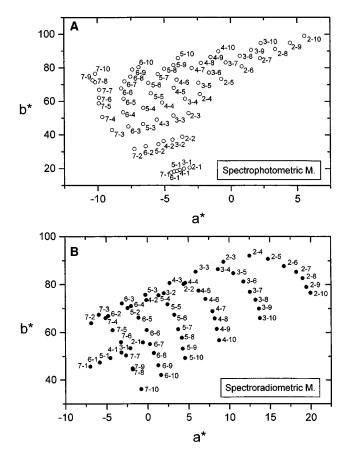


FIG. 1. Commission International de l'Eclairage 1976 (L*a*b*) CIELAB coordinates for 60 recently prepared bromthymol blue (BTB) standards from the spectrophotometric (A) and spectroradiometric (B) measurements (M) performed, as described in the text. The corresponding BTB index (pH–concentration) is indicated together with each BTB standard (mean of three replicates measured).

(i.e., eliminating the Munsell gray background, which was placed behind the samples in an attempt to simulate the measurement by transmission characteristic of the spectrophotometer). In any case, the correlation between any of the coordinates a^* , b^* , L^* of the 60 BTB standards by these two procedures was again poor (in the best of cases r = 0.491, corresponding also to coordinate L^*).

The foregoing indicates that spectrophotometric and spectroradiometric measurements were difficult to compare, since the experimental conditions were different in each case. Among the principal differences in experimental conditions between the two cases was the use of light reflected diffusely as opposed to transmitted light, and the presence or absence of the neutral gray background behind the samples. In addition, the optical glass cells with flat sides in the spectrophotometer, as opposed to cylindrical bottles of conventional glass in the spectroradiometer should be mentioned. It was confirmed thus that the color measurement was quite critical, and that it was significantly affected by the illumination/observation conditions under which the measurements were made. This observation does not invalidate the use of either of the two instruments (spectrophotometer or spectroradiometer) in the determination

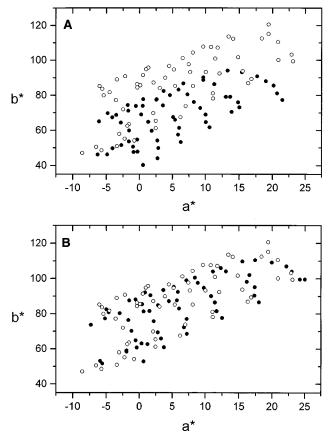


FIG. 2. Comparison of the CIELAB coordinates of the BTB standards, determined by spectrophotometric (\bigcirc) and spectroradiometric (\bigcirc) measurements. (A) Results for the two types of measurements in reference to the same sample thickness (35 mm); (B) in addition to the same thickness, the spectroradiometer used without the Verivide cabinet, attempting to simulate the measurement by transmission of the spectrophotometer. For abbreviations see Figure 1.

of color, rather it simply warns us of the need to use the appropriate instrument for the task to be undertaken. Thus, if we wish to evaluate the radiation that reaches the human eye from a BTB standard illuminated by a certain source, it is more appropriate to use the spectroradiometer situated in the same position as the eye. On the other hand, if we wish to verify whether an oil sample has the same color as a certain BTB standard, a spectrophotometer can correctly resolve the problem.

Color variability for different BTB replicates. With respect to the variability of color among the three replicates of each BTB standard, the manipulation inherent in the spectrophotometric measurements, obligating the opening of the containers to extract the quantity of solution that should be placed in the measurement cell, causes a clear deterioration of the replicates in some cases. Thus, anomalous data appeared (outliers), which were discarded by application of a simple data-rejection test, in this case based on $\pm 10\%$ of the median. Specifically, the replicates in which some of the tristimulus values exceeded 10% difference with respect to the central value were rejected, thus affecting roughly 10% of the spectrophotometric measurements. After the rejection, we calculated the standard deviation

TABLE 1
Color Variability Corresponding to the Three Replicates of the BTB
Standards ^a

Average standard		o
deviation of CIELAB	Spectrophotometric	Spectroradiometric
parameters	measurements	measurements
L*	0.40	0.44
a*	0.24	0.23
b*	0.53	0.89
C*	0.52	0.89
h (deg)	0.26°	0.20°

^aThe standard deviations are indicated for different CIELAB parameters (average of those calculated in the 14 series of measurements made over the experimental period for the 60 BTB standards) measured by spectrophotometric and spectroradiometric techniques.

of the coordinates a*, b*, L* of the three replicates of each BTB standard for the spectrophotometric and spectroradiometric measurements. Table 1 shows the results, as an average of the 60 BTB standards and the 14 series of measurements made over time. This average is reasonable, since no outstanding trend in color variability was discernible between different BTB standards, nor over time (only one slight increase in variability with time, which was negligible in our case). Table 1 also indicates the standard deviations corresponding to the parameters chroma (C*) and hue-angle (h), since it is often more intuitive to refer in CIELAB to the variables L*, h, C* (approximately correlated with the classical color attributes: lightness, hue, and saturation, respectively) than to the coordinates L*, a*, b*.

The fact that the color variability of the three replicates is on average less in the spectrophotometric measurements than in the spectroradiometric ones (see Table 1) should be interpreted with caution. The variability of the spectroradiometric measurements indicated in Table 1 is in fact exactly the average of the experimental measurements of the three replicates, but, in the spectrophotometric measurements, some results that show a high degree of outliers were rejected by the aforementioned test, thereby somewhat reducing the scatter of these measurements.

In any case, Table 1 illustrates the colorimetric variability, that we should admit in the BTB standard, as a consequence of the errors committed in their preparation and measurement. This is of interest when determining the color fastness of the BTB standards that we measured over 1 yr. We find the highest variability in coordinate b* (which has values similar to those of C*, as might be expected according to Fig. 1). Consequently, this variability in the CIELAB coordinates causes certain color differences between the three replicates of the same BTB standard, which, on the average, prove to be 1.7 and 1.3 CIELAB units, for the spectroradiometric and spectrophotometric measurements, respectively. Taking into account the rejection test of the data applied to the spectrophotometric measurements, we could estimate that the variability of the color of the three replicates has a value of not less than 1.5 CIELAB units (average of 60 BTB standards).

Temporal evolution of color of BTB standards. Figure 3 indicates the temporal evolution of the color of the BTB standards. Specifically, the average color differences with respect

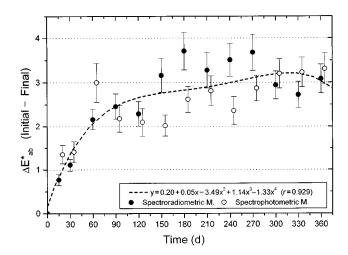
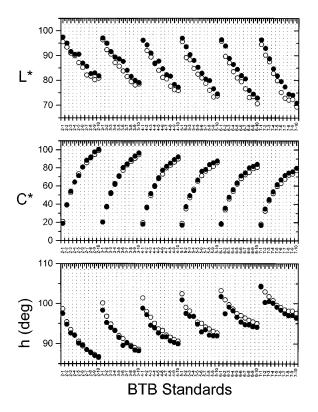


FIG. 3. Color differences (CIELAB units) of the BTB standards over 1 yr, with respect to the color of recently prepared standards. The points indicate the mean values for the 60 BTB standards (with their three corresponding replicates), and error bars 0.5 times the standard deviation. The results are distinguished for the spectrophotometric and spectroradiometric measurements, having adjusted the average of the two by a fourth-degree polynomial (broken line). For abbreviations see Figure 1.

to the initial values (corresponding to the recently prepared solutions) are represented for the spectroradiometric and spectrophotometric measurements. The average indicated refers to the 60 BTB standards and to the three replicates of each standard, the error bars indicating the corresponding scattering. The temporal evolution and degradation of the color in the spectrophotometric and spectroradiometric measurements are relatively similar, and thus the mean values for both have been adjusted to a fourth-degree polynomial (broken line, Fig. 3). The samples initially underwent a major color change and, from a certain point on (approximately 150 d), the color remained relatively stable, with a mean color difference of about 3.0 CIELAB units with respect to the initial values. It thus appears that the color of the BTB standards changes immediately after their preparation, in a way that reminds one of the color change that occurs when a painted surface dries, which is taken into account when making painted color samples in the laboratory (18). For this reason, we measured the BTB standards only 15 d after their formulation, continuing afterward at 30 d, and then in successive periods of 30 d for a year.

This overall color difference with the passage of time (3.0 CIELAB units) did not greatly surpass the variability corresponding to the measurement of the three replicates at any given moment (approximately 1.5 CIELAB units, as indicated above); that is, the BTB standards did not undergo strong color variations. This result was previously pointed out (7) and is reasonable, considering that a concerted effort was made to preserve the standards (e.g., storage in darkness and in laboratories where ambient conditions were relatively stable). In any case, the color of the BTB standards immediately after their preparation appears to be somewhat unstable. Therefore, it is advisable to allow a certain amount of time to pass before their use (at least 3 mon, according to Fig. 3).



80 60 L* 40 20 ᇇᆂᄵ൴ഺ൴ൕൎ൳ഺ൮൛**ᆂ൴൴൛**൴ൕൎ൳ 100 80 C* 60 40 100 ուսերերերերերերերերերերեր 90 h (deg) 80 70 **BTB Standards**

FIG. 4. Results for the spectrophotometric measurement $(\bigcirc$, initial; \bullet , final) in relation to the variation of the parameters lightness (L*), chroma (C*) and hue-angle (h), for each of the 60 BTB standards, and for 1 yr beginning at the preparation of the standards. For abbreviation see Figure 1.

In relation to the type of color change that the BTB standards underwent over time, Figures 4 and 5 show for each standard the differences in L*, C*, and h (degrees) over a period of 1 yr for the spectrophotometric and spectroradiometric measurements, respectively. Again, we found a certain similarity in the temporal evolution according to the two types of measurements: in general, the samples became lighter (greater L*), more saturated (greater C*), and less greenish (less h) with the passage of time. Figures 4 and 5 also show the regularity with which the color parameters varied with concentration (between 1 and 10) for each pH (between 2 and 7), since six similar welldifferentiated branches appear (one for each pH value). In particular, these figures also reveal the values of the third coordinate, L*, of the BTB standards, which for simplicity was not indicated in the initial representations (Figs. 1 and 2).

Nevertheless, comparing Figures 4 and 5, we also found some differences between the results of the spectrophotometric and spectroradiometric measurements that were not well correlated. For example, according to the values of the ordinate axes of both figures, we see that, for a given standard, both lightness (L*) and hue-angle (h) were greater in the spectrophotometric than in the spectroradiometric measurements. Also, L* and h evolved similarly on changing the BTB index in the spectrophotometric and spectroradiometric measurements, but chroma (C*) evolved differently. Specifically, in the latter mea-

FIG. 5. Results for the spectroradiometric measurement (\bigcirc , initial; ●, final) in relation to the variation of the parameters lightness (L*), chroma (C*) and hue-angle (h) for each of the 60 BTB standards, and for 1 yr beginning at the preparation of the standards. For abbreviation see Figure 1.

surements (Fig. 5), for each pH value the C* presented a maximum at a certain intermediate concentration; this did not occur in the former measurements (Fig. 4). This difference is possibly attributable to the effect of the gray Munsell background used only for the spectrophotometric measurements.

As an example, Figure 6 shows the temporal evolution of the color of an intermediate sample of the BTB scale (standard 5-5), according to the spectroradiometric measurements. Results of this type were also found for other BTB standards and for the spectrophotometric measurements. We found that with the passage of time this BTB standard increases in lightness (L*) and chroma (C^*) , and the hue-angle (h) diminishes. The color change was most significant in the first 3 mon after the preparation of the samples. However, in successive months the color changes were of a similar order of magnitude to the variability between the three replicates of this BTB standard (designated in Fig. 6 by error bars for the initial and final instances of the measurement). Thus, with the exception of the initial months, the average color changes of the 60 BTB standards in temporal intervals of 1 month are negligible, being comparable to the color variability of the three replicates (about 1.5 CIELAB units).

General comment and suggestion. The color change in BTB standards was tracked over a year by spectrophotometric and spectroradiometric measurements of three replicates of each of the standards. The principal results of this experiment can be

FIG. 6. Temporal evolution of the color of BTB standard 5-5 over a year, according to spectroradiometric measurements. The CIELAB planes C^*, L^* (A) and h,L* (B) are distinguished; in each, the numbers 0–12 indicate the number of months since the preparation of the standard. The error bars centered in the initial and final measurements (0 and 12) indicate the standard deviation corresponding to the measurements of the three replicates of this BTB standard at these times.

summarized as follows: (i) The spectrophotometric and spectroradiometric measurements of the BTB standards do not closely correlate because of the different experimental conditions under which the measurements were made. Though both measurements separately can be considered completely valid, one or the other should be chosen according to the specific practical application intended. (ii) The measurements of the three replicates of each BTB standard show a certain scattering, attributable to errors in the preparation of the standards as well as in the measurement itself. Thus, the standard deviation of coordinate b* (or of chroma C*) was the greatest, being slightly less than 1.0 CIELAB units. Among the three replicates for the same BTB standard, there was a color difference that, on average, was roughly 1.5 CIELAB units. (iii) Over a 1yr period, we found that the principal color change of the BTB standards occurred in the months immediately following the preparation of the standards, the evolution of these stabilizing after about 150 d, with an average color difference of around 3.0 CIELAB units with respect to the initial value. Therefore, a 3-mon waiting period would be advisable after the preparation of the BTB standards. Thereafter, in 1 mon the color change of the BTB standards is around 1.0 CIELAB units and therefore negligible, given the variability between different replicates of the BTB standards described above.

Given the great economic importance of olive oil, particularly in southern Spain, rigorous quality control should be encouraged. This would justify a reassessment of the current methods of specifying the color of olive oil, as has been initiated in the present study others (15,19).

ACKNOWLEDGMENTS

This work has been in part supported by research projects BFM2000-1473 and OLI96-2157-C02-01, Dirección General de Investigación Científica y Técnica (DGICYT), Ministerio de Ciencia y Tecnología (Spain).

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[Received July 20, 2000; accepted November 4, 2000]

