Simulation of the Visible Spectra for Edible Virgin Olive Oils: Potential Uses

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The photodegradation technique was used to record the visible spectra for carotenoids and chlorophylls in virgin olive oil. Principal component analysis of the visible spectra for 81 samples of this type of oil with widely variable composition revealed that only two components contribute more than 99.7% of the spectral information. The Varimax factors and the absorbances of the oil pigments are linearly related. The joint use of these spectra and the absorbances at 455 and 670 nm allows the accurate reproduction of the visible spectrum for any virgin olive oil sample. Therefore, one can question whether a commercially available product is actually virgin olive oil if a comparison of its experimental and simulated absorbance values gives $R^2 < 0.995$ and RMSD > 0.006. The simulated spectra provide CIE$L^*a^*b^*$ chromatic coordinates that describe the color of the samples with little difference from those provided by the spectra for real samples.

Index Headings: **Simulated spectra; Principal component analysis–Varimax; Photodecolorization; Olive oil carotenoid spectrum; Olive oil chlorophyll spectrum; Olive oil pigment spectra.**

INTRODUCTION

The color of virgin or extra virgin olive oil, and hence its visible spectrum, results from two pigment groups present in olives, namely, carotenoids, which give golden to orange hues, and chlorophylls, which give green hues. These two groups are the only ones identified in virgin olive oil and include pigments naturally occurring in fresh olives and others formed during the oil extraction process.[1–4] The pigment fraction of virgin olive oil typically consists of 37.36% lutein, 12.73% β-carotene, 8.59% minor carotenoids, and 41.32% pheophytin *a*; on the other hand, oil obtained from overripe olives picked late in the season contains as much as 61% lutein.[2,5,6] More details about the constituents of olive oil can be found elsewhere.[7,8]

The visible spectrum for virgin olive oil exhibits a strong band with several peaks between 380 and 500 nm that result from overlap of the bands for the constituent carotenoids. The absorption maxima for the carotenoids are at identical or very similar wavelengths, so the presence of a specific compound cannot be ascertained by numerical analysis of the spectral profile, and a chromatographic separation is therefore required.[2,8,9] The presence of chlorophyll pigments is signalled by two typical bands, one at approximately 420 nm, which is quite strong and overlapped with the carotenoid band, and the other at approximately 670 nm. The visible spectra for commercial samples of virgin olive oil contain the bands for both pigment groups, their relative intensities depending on the particular fruit variety and ripeness.[7–9]

The literature contains spectral data for various carotenoids and chlorophylls that have been obtained using the typical solvents for UV/VIS spectroscopy.[2,10,11] If such spectra could be digitized and combined—with provision for their composition—the resulting visible spectrum would be similar to that for oil, albeit shifted to some extent. This procedure, however, would be rather labor-intensive and yield dubious results. The aim of this work was to obtain the combined spectrum for the components of each pigment group without the need to isolate its components, using a medium highly similar to oil as solvent. The two types of spectra thus obtained can be used to simulate the spectra for actual virgin olive oil samples in order to assess their quality. Oil adulteration, which has been the subject of much research involving a variety of experimental techniques in combination with chemometric computations,[7,12,13] was beyond the scope this work.

One way of isolating the two groups of pigments in the oil is by photodegradation; in fact, their photodecomposition rates are rather different. Light rapidly reduces to an identical extent the intensity of the bands for chlorophyll pigments; by contrast, carotenoids take much longer to degrade.[6,14] The visible spectrum does not expose the influence of light on oil components other than pigments as no colored products are formed during the photodegradation process.[6] The presence of such products reflects in other spectral regions e.g., that of vibrations.[15] In this work, we used two of the above-described favorable facts, namely (1) that the visible spectrum for virgin olive oil is provided by two types of pigments only; and (2) that the components of each pigment group have very similar spectra and are similarly affected by irradiation.

Principal component analysis is among the factor analysis methods most widely used for the chemometric processing of spectra. In our case, it might be of help to identify potential common components in virgin olive oils from their bands in the visible spectral region. The results can be made highly representative by using a wide variety of samples and hence pigment compositions.

EXPERIMENTAL

Samples. We used 81 commercially available samples of virgin olive oil, most of which were "extra" grade, from a variety of origins in Spain, Greek, Italy, and Portugal. The samples spanned a variety of colors (i.e., a wide range of pigment compositions). Most were mixtures of oils from different olive varieties. Also, those marketed as single-variety had actually been obtained from mixtures of the same olive variety but in different degrees of ripeness. Spectra were obtained from freshly opened packages, which, however, were also used to fill

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five topaz bottles of which three were stored frozen for use as reference and in other types of tests, and the other two refrigerated for subsequent checks.

Spectra. Spectral data were obtained from pure, undiluted samples, using 1-cm-thick disposable polystyrene cells and an ATI Unicam UV4 interfaced to a PC computer. A few non-transparent samples were centrifuged to lower their spectral background to the baseline in order to avoid spurious variations in chromatic coordinates.[16] Each spectrum was routinely recorded at least three or four times to check for potential operational errors and sample deterioration. Differences between recordings for the same sample were so small that they overlapped with the baseline. The maximum noise level was 1×10^{-3} AU (absorbance units) and the mean square noise level was 4.94×10^{-4} AU (i.e., very small, as usual for the visible spectra of liquids).

Data Treatment. Noise and statistical parameters were calculated using a spreadsheet. The goodness of the simulation was measured in terms of proximity (via the coefficient of determination, R^2_{xy}) and distance (via the root mean square deviation, RMSD, or standard error of prediction, SEP, which is given by RMSD = $\{[\Sigma(X_i - Y_i)^2]/n\}^{1/2}$). In our case, X_i was the absorbance for an oil sample and Y_i is that for its simulated spectrum, both at the same λ, and $n = 196$ (viz. the number of absorbance values measured at 2-nm intervals from 380 to 770 nm).[17,18]

Principal component analysis (PCA) is used to reduce the number of data and eliminate irrelevant random variables. We implemented both PCA and Varimax orthogonal rotation using commercially available software.[19] The variables used were the visible spectra for the 81 samples, each of which contained 196 data; therefore, the initial data matrix contained 15 876 data. Using a larger number of absorbance values resulted in no appreciable improvement. The first PC accounts for most of the total variance, the second for most of the remaining variance, and so on. Because these factors are wavelength dependent, they can be used to reconstruct the spectrum for any variable or oil. However, the factors are orthogonal (i.e., uncorrelated and independent of each other), but correlated to each individual spectrum, for the 81 samples. In order to better expose wavelength dependence, we used a Varimax rotation to obtain new factors, linear combinations of the previous ones, with which the variance was maximal for some initial variables (spectra) and near zero for others. Each new factor was thus more closely related to specific wavelength ranges and the other with different regions. Further details about PCA can be found in a textbook on multivariate analysis.[20]

The color of the samples was described in terms of the CIE$L^*a^*b^*$ chromatic coordinates, using illuminant D65 and the supplementary standard observer. Computations were done using software developed by the authors that processes spectra in ASCII format. The software has various computation capabilities and graphical outputs, and it provides a number of color-related variables, including Chroma.[21,22] The use of a large number of absorbance values results in better color definitions for oils; we therefore used 391 spectral ordinates per sample (viz. the equivalent of measuring the absorbance at 1-nm intervals over the range 380–770 nm).[23]

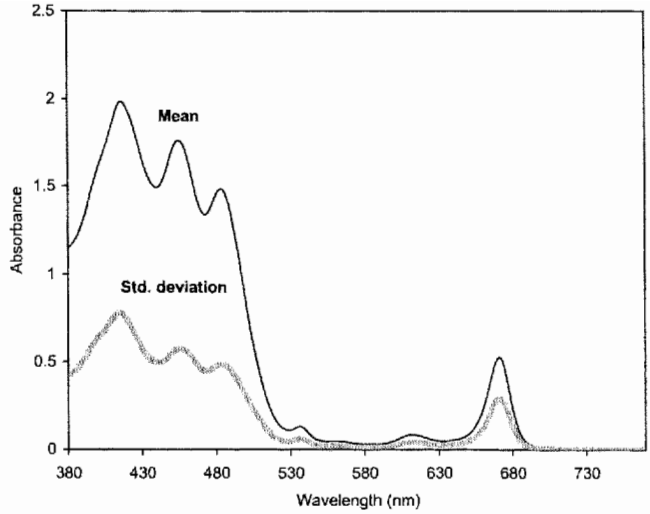

FIG. 1. Mean and standard deviation of the visible spectra for the 81 virgin olive oil samples.

Pigments. The obtainment of the oil solution of each pigment group was based on their different photodegradation rates.

Photochemistry. We used an Osram 125 W mercury vapor lamp to irradiate samples, which were held in quartz cells 1 cm thick, from a distance of 15 cm. The assembly was housed in a ventilated cabinet, away from direct external light. The sample temperature increased with increasing exposure time, but never exceeded 42 °C. Other authors had previously obtained similar results and conclusions using different light sources.[6,14]

RESULTS AND DISCUSSION

Figure 1 shows the mean and standard deviation spectra for all 81 samples. The standard deviation was high at all wavelengths, thus reflecting the large differences in pigment composition among samples. Similar results have previously been obtained in other spectral regions.[24] The spectral standard deviation at any wavelength was much greater than the mean square noise level. Figure 2 shows the variation of Lightness (L^*) with Chroma (C^*); as can be seen, points were highly scattered, as expected for the wide variety of color, hue, and lightness values encompassed by the oil samples. The L^* and C^* values obtained are typical for olive oil and depend on olive variety and harvest time; in fact, some oils had high carotenoid contents and low chlorophyll contents, and vice versa.[2,7,8,16] As can be seen from Fig. 2, some samples exhibited a different Lightness but a similar Chroma, as samples with a different color or different a^* and b^* chromatic coordinates can have a similar Chroma ($C^* = [(a^*)^2 + (b^*)^2]^{1/2}$).

The photochemical process was monitored by periodically recording the spectrum as shown in Fig. 3. The samples took a yellowish-orange color after 1 h of irradiation, which changed to a pale yellow after 16 h and to virtually colorless after 48 h, the time by which the spectrum nearly touched the baseline. These times vary slightly among samples. Note that chlorophyll pigments virtually disappeared within 1 h; the remainder, caroten-

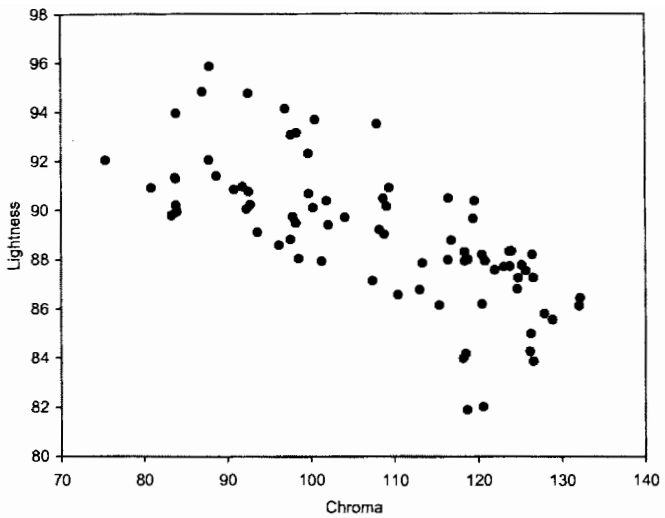

FIG. 2. Lightness vs. Chroma plot for the 81 virgin olive oil samples.

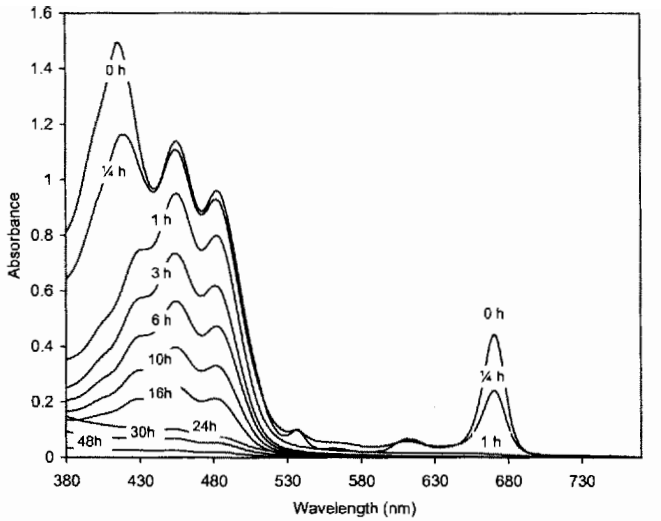

FIG. 3. Variation of the spectral profile for virgin olive oil with the irradiation time (in hours).

oid pigments, exhibited a slightly decreased intensity at the absorption maximum (455 nm) relative to the initial sample. This large difference in photodegradation rate between chlorophylls and carotenoids was previously observed by other authors.[6,14] The weakening of the band at 455 nm was a result of its overlap with the band for chlorophylls at 420 nm (which disappeared), and the band of carotenoids being slightly degraded all at once.[6] This is clearly apparent from Fig. 4, in which spectrum *c* is the result of multiplying all the absorbances in spectrum *b* (or spectrum 3h in Fig. 3) by 1.54. The profile of this spectrum is quite consistent with that for olive oil between 440 and 510 nm; it is somewhat weaker at approximately 520 nm as a result of the presence of a small band for chlorophylls.

Based on these results, all carotenoids are degraded to the same extent during the first 3 h of irradiation, at least under our experimental conditions. The situation holds much longer, with no change in the position of absorption maxima. Like other authors, we detected the formation of no colored substances during the photodecomposition process.[6] Therefore, it suffices to multiply the visible spectrum for the oil, once chlorophylls have disappeared, by an appropriate factor to obtain the *visible spectrum for carotenoids* in virgin olive oil. Subtraction of such a spectrum from that for the initial sample will then yield the *visible spectrum for chlorophylls* in virgin olive oil.

Our photodegradation results are consistent with a number of chromatographic results for pigments in olive oil.[1–4] Therefore, the visible spectrum for olive oil consists exclusively of the bands for its constituent pigments; also, the differences between spectra for virgin olive oil samples arise from differences in the proportion of each pigment group.

The photodegradation of five samples of oil differing widely in color and olive variety gave rise to very similar spectra that differed in band intensity only. We thus normalized each spectrum by assigning one absorbance unit to the maximum with respect to the band at 670 nm for chlorophylls and that at 455 nm for carotenoids. These two maxima lie at the position of a typical band for each pigment group. Other authors have used very similar wavelengths to describe the visible spectral bands for olive oil.[2,16,24] The average normalized spectrum for each pigment group is shown in Fig. 5; normalization substantially boosted the band at 420 nm for chlorophylls, which is usually weak except in deep green colored oils.

Despite the experimental evidence, oil might contain some colored compounds other than chlorophylls and carotenoids, varying among the samples. However, they would have to be present at low concentrations as no colored component other than the pigments has so far been identified.[1,7,8] In order to search for components potentially present in all samples, we applied multivariate PCA to the body of absorbance values. The results are summarized in Table I, the first column of which lists potential components and the second lists the percent variance accounted for by the factors. As can be seen, the first two factors account for more than 99.7% of the in-

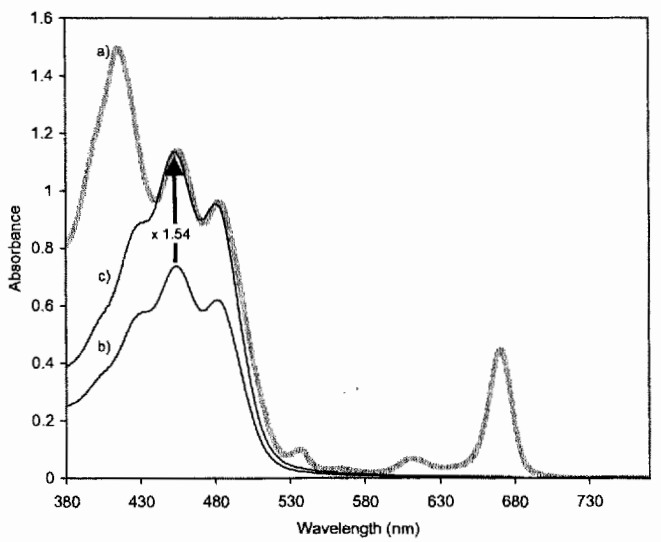

FIG. 4. (*a*) Visible spectrum for a virgin olive oil sample. (*b*) Spectrum for the same sample after 3 h of irradiation (i.e., containing carotenoids as the sole pigments). (*c*) Spectrum b multiplied by a factor of 1.54.

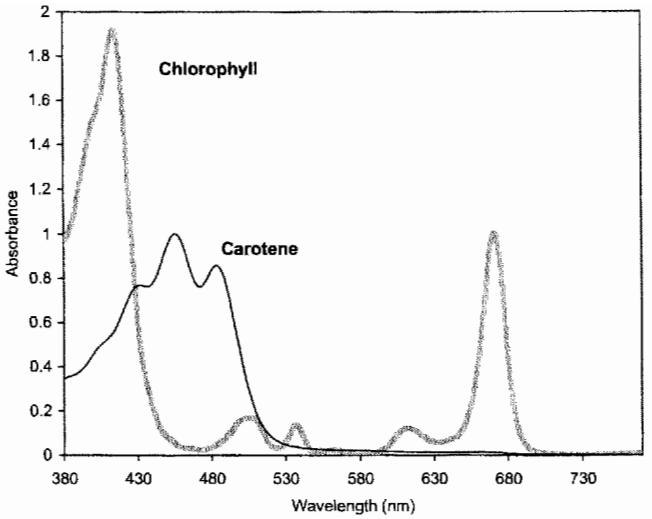

FIG. 5. Normalized spectrum for carotenoids (thin line) and chlorophylls (bold line) as recorded in photodecolorized virgin olive oil.

formation contained in the body of spectra for the samples, whereas the third factor accounts for less than 0.3% of it. Such a low percentage might have been the result of noise in the spectra or, rather, of the truncation of absorbance data, but not of the presence of additional colored components. Therefore, the visible spectrum for olive oil can be expressed as the combination of the spectra for two of its principal components only. Based on the PCA results, pigments must be considered in groups that constitute two components that vary among samples. Application of Varimax rotation to such factors yielded another two that are plotted against wavelength in Fig. 6. The graph reflects the fact that upon Varimax rotation, each factor is more markedly correlated to some spectra than to others. The curve profiles resemble those for the pigments in Fig. 5. The curves in Fig. 5 represent the experimental data, whereas those in Fig. 6 represent the chemometric data for the body of samples; however, the curves in both figures are related by simple linear equations, namely:

$$N_{\text{carotenoid}}(\lambda) = 0.31754 \cdot \text{factor1}(\lambda) + 0.073140 \cdot \text{factor2}(\lambda) + 0.23216 \quad (R^2 = 0.997) \quad (1)$$

$$N_{\text{chlorophyll}}(\lambda) = 0.042045 \cdot \text{factor1}(\lambda) + 0.46843 \cdot \text{factor2}(\lambda) + 0.27732 \quad (R^2 = 0.956) \quad (2)$$

where $N_{\text{carotenoid}}(\lambda)$ and $N_{\text{chlorophyll}}(\lambda)$ are the absorbances of the normalized spectrum for each pigment group (Fig. 5), and factor1(λ) and factor2(λ) are the results of the Varimax rotation (Fig. 6).

The existence of these linear relations further confirms

TABLE I. Statistical factor analysis.

Component	% Variance
1	95.978
2	3.7240
3	0.1322
4	0.0944
5	0.030

TABLE II. RMSD range spanned by the 81 virgin olive oil samples.

RMSD range	Number of samples	%
0.001–0.002	22	27.16
0.002–0.003	41	50.62
0.003–0.004	15	18.52
0.004–0.005	2	2.47
0.005–0.006	1	1.23

that the carotenoid and chlorophyll bands obtained upon photodegradation are the sole components of the visible spectrum for virgin olive oil. Therefore, the spectral profile $S_{\text{olive oil}}(\lambda)$ for any sample of virgin olive oil can be simulated as the combination of the spectra for its two types of constituent pigments, i.e.:

$$S_{\text{olive oil}}(\lambda) = A_{455} \cdot N_{\text{carotenoid}}(\lambda) + A_{670} \cdot N_{\text{chlorophyll}}(\lambda) \quad (3)$$

where A_{455} and A_{670} are the only two absorbances that need be measured, using a pure sample in a 1-cm-thick cell. Equation 3 is a result of the additivity of the absorbances of the components at any wavelength. Figure 7 shows its application to two oils not used to record the spectra for chlorophylls and carotenoids. As can be seen, the actual and simulated spectra are very similar, which confirms the validity of the Eq. 3. The figure also shows the difference profile between the two spectra, and the R^2 and RMSD values. The difference line varies between the two samples, so it cannot conceal the spectral profile for some colored component potentially formed in significant amounts during the photochemical process and hence present in the spectrum for the pigments, but not in that for the samples.

The coefficient of determination obtained in the linear least-squares fitting of the actual versus simulated absorbances can be used as a measure of consistency between the actual and simulated spectra. The results of the linear fitting for the 81 samples studied are shown as a histogram in Fig. 8. Although the samples were highly varied in composition, R^2 was always greater than 0.995 and was 0.9985 on average. Such high R^2 values testify to the high

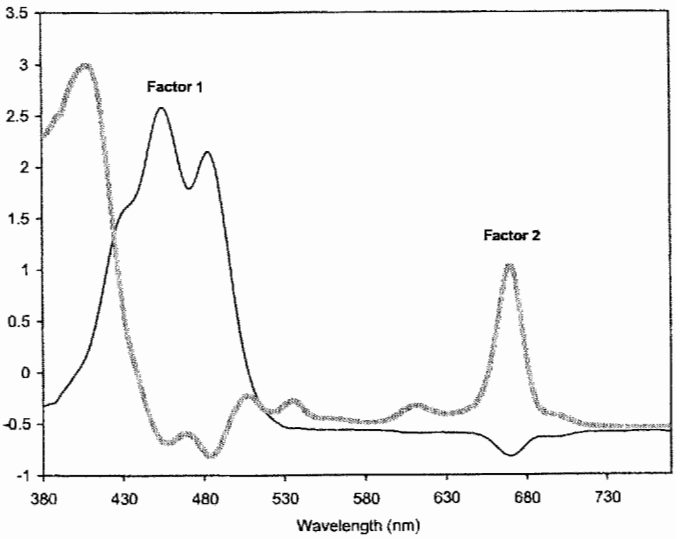

FIG. 6. Graph of the factors obtained upon Varimax rotation of the first two principal components.

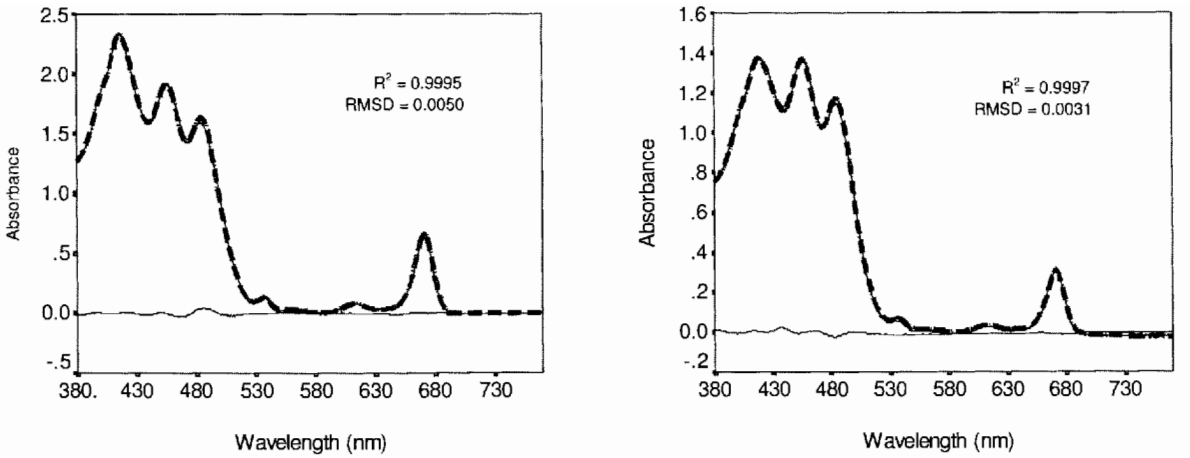

FIG. 7. Actual (bold line) and simulated spectra (thin line) for two virgin olive oil samples. The lower line represents the difference between the two.

accuracy of the simulated spectrum for the samples studied. The goodness of simulation was also assessed in terms of the distance parameter Root Mean Squares Deviation (RMSD). As can be seen from Table II, although most samples exhibited an RMSD between 0.002 and 0.003, all simulated spectra had an RMSD less than 0.006, so we used the latter value as the threshold for goodness of simulation. Spectral noise was very low, so its influence on the results can be assumed to be insubstantial. Because the samples examined were so variable in composition, any other virgin olive oil samples can be expected to behave similarly. Therefore, one can question the quality of a virgin olive oil if the linear fitting between the absorbances of its actual and simulated spectrum provides an R^2 value less than 0.995 or an RMSD value greater than 0.006. While simulated spectra could be used to detect oil adulteration, this should be confirmed by using other detection methods.[7–9,13]

The UV/VIS spectrum is one of the most simple, affordable, and frequently used preliminary tests for virgin olive oil. However, the visible spectrum cannot reflect incipient deterioration in a virgin olive oil. As can be seen from Fig. 9, one cannot state that the sample spectrum shown corresponds to an oil type other than virgin olive oil, at least at first sight. However, a comparison with the simulated spectrum exposes some differences that are confirmed by the values of the statistical parameters, namely: $R^2 = 0.976$ (and thus less than 0.995) and RMSD = 0.0314 (and thus greater than 0.006). One can therefore conclude that the sample in question might be slightly deteriorated, or adulterated. In fact, it was an oil sample that had been stored over a long period of time. Based on the difference spectrum, one might think that the sample lost some carotenoids and that pheophytins had started to transform; however, such a conclusion would be purely speculative and would require confirmation with standard tests.[25] Figure 10 exposes the large difference between the spectrum for a non-virgin olive oil and its simulated spectrum obtained from Eq. 3: the spectrum it should have if it were virgin olive oil. In this case, $R^2 = 0.808$ and RMSD = 0.0721, which departs markedly from the values corresponding to virgin olive oil.

The visible spectrum for a sample can be used to cal-

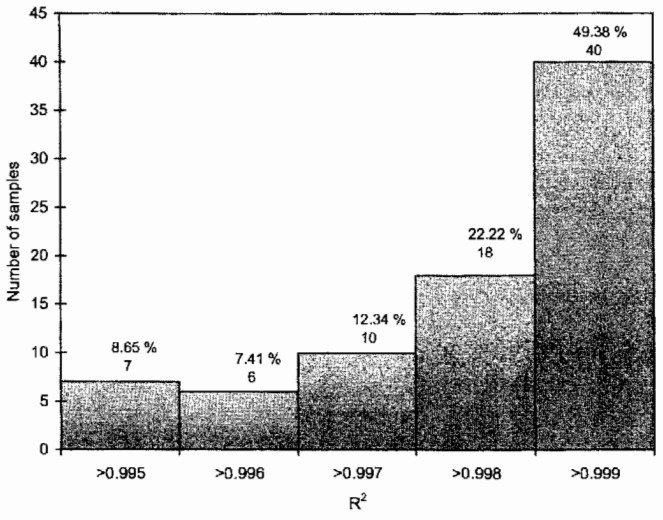

FIG. 8. Histogram of R^2 values. The figures at the top of each bar denote the number of samples and their fraction of the total.

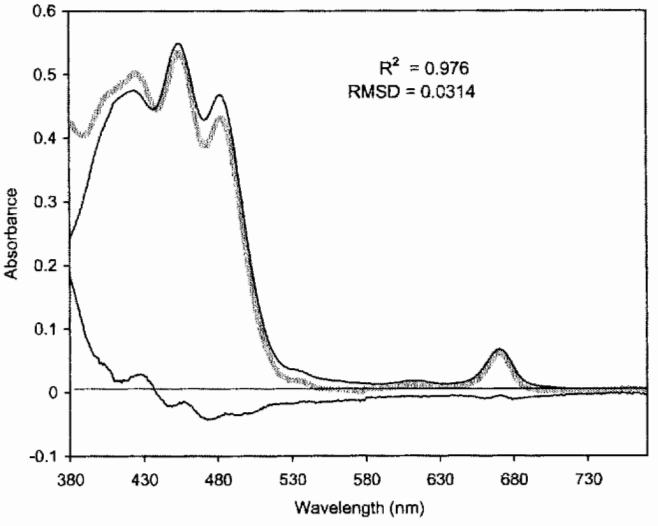

FIG. 9. Actual (bold line) and simulated spectrum (thin line) for a sample marketed as virgin olive oil. The lower line represents the difference between the two.

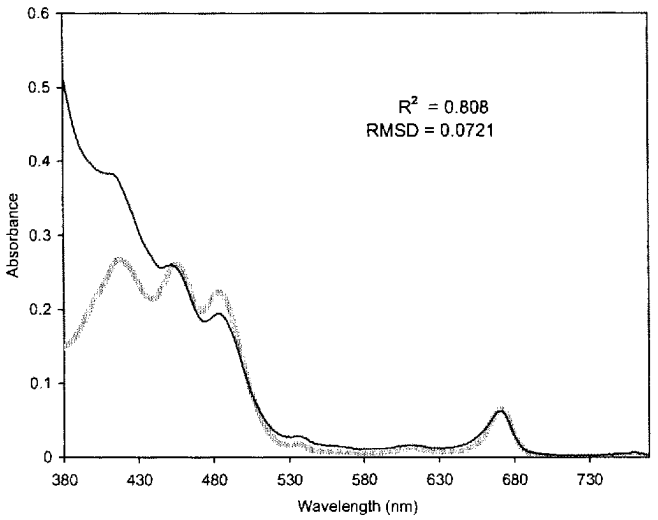

FIG. 10. Actual (thin line) and simulated spectrum (bold line) for an olive oil sample. The latter would coincide with the actual spectrum if the sample were virgin olive oil.

culate the CIE$L^*a^*b^*$ chromatic coordinates that define its color at a given time.[23,26] If the sample is in fact virgin olive oil, then its actual and simulated spectra will be virtually identical, so there will be little difference between the chromatic coordinates obtained from the two. Such is indeed the case in Fig. 11, which shows the Lightness and Chroma for the 81 samples studied, all of which gave very high R^2 values. Therefore, the color of virgin olive oil will be natural (i.e., exclusively due to its chlorophyll and carotenoid pigments) if its chromatic coordinates coincide with those calculated from its simulated spectrum. This is therefore one other potential application of the spectra for chlorophylls and carotenoids in oil: the estimation of reliable CIE$L^*a^*b^*$ chromatic coordinates simply by measuring the sample absorbance at 455 and 670 nm, constructing the simulated spectrum, and calculating L^*, a^*, and b^* or any other chromatic parameters.[16,21]

CONCLUSION

Normalized spectra for the two types of pigments present in virgin olive oil can be used for the expeditious obtainment of the visible spectral profile of any oil sample in this grade as the spectra for the pigments were obtained in their natural medium: oil. The actual and simulated spectra are highly consistent and the linear fitting of their respective absorbances yielded high R^2 and RMSD values. Because the samples used had widely variable pigment contents, any other sample can be assumed not to be virgin olive oil if $R^2 < 0.995$ and RMSD > 0.006. Also, because the actual and simulated spectra are so highly consistent, both must give very similar values for the chromatic coordinates of an oil unless its color is not exclusively due to the natural pigments it contains. Therefore, the absorbances at 455 and 670 nm for virgin olive oil can be used to reliably calculate its CIE$L^*a^*b^*$ chromatic coordinates from its simulated spectrum. The proposed method can also be applied to other pigment-containing substances.

A listing in ASCII or JCAMP format of the normalized spectra for carotenoids and chlorophylls in oil, $N_{\text{carotenoid}}(\lambda)$ and $N_{\text{chlorophyll}}(\lambda)$, as well as other spectral data obtained in this work, can be obtained upon request from the authors.

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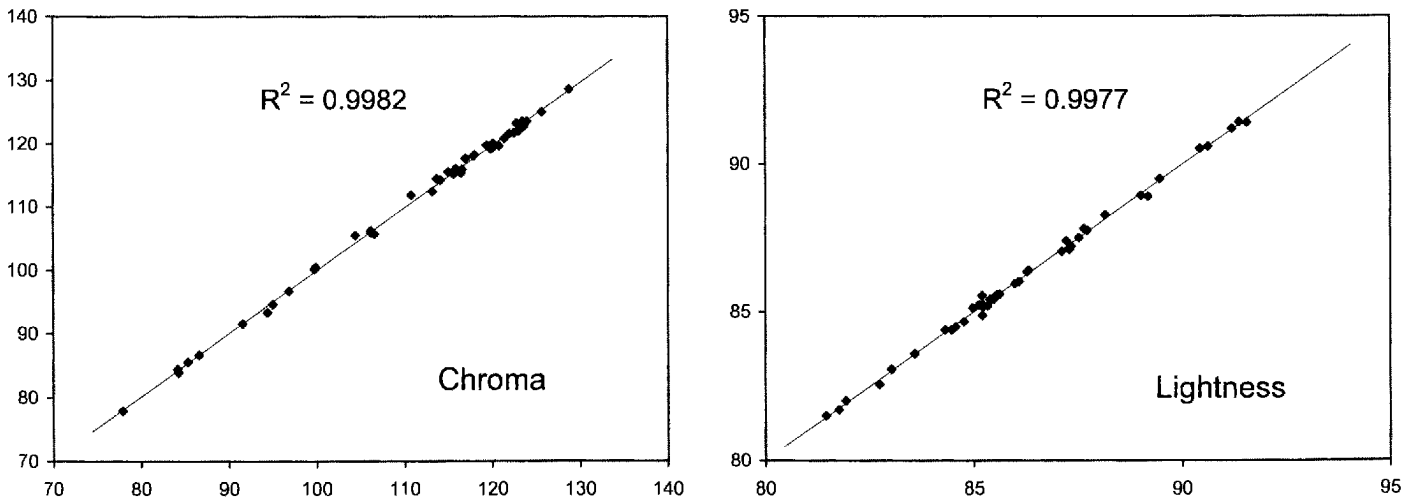

FIG. 11. Actual vs. simulated Chroma (C) and Lightness (L) values for virgin olive oil (CIE$L^*a^*b^*$, illuminant D65, Supplementary Standard Observer).

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