Application of Laser-Induced Breakdown Spectroscopy (LIBS) and Neural Networks to Olive Oils Analysis

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The adulteration and traceability of olive oils are serious problems in the olive oil industry. In this work, a method based on laser-induced breakdown spectroscopy (LIBS) and neural networks (NNs) has been developed and applied to the identification, quality control, traceability, and adulteration detection of extra virgin olive oils. Instant identification of the samples is achieved using a spectral library, which was obtained by analysis of representative samples using a single laser pulse and treatment by NNs. The samples used in this study belong to four countries. The study also included different regions of each country. The results obtained allow the identification of the oils tested with a certainty of more than 95%. Single-shot measurements were enough for clear identification of the samples. The method can be developed for automatic real-time, fast, reliable, and robust measurements, and the system can be packed into portable form for non-specialist users.

Index Headings: **Laser-induced breakdown spectroscopy; LIBS; Neural networks; Olive oils; Edible oils.**

INTRODUCTION

Control of adulteration, together with authentication and traceability, is an important problem in the quality control of olive oils. In particular, adulteration with lower-price oils is one of the most difficult situations to detect and is a serious problem in the olive oil industry, because of the similar composition of the adulterant oils used.

The high price in the market and the known health benefits attributed to olive oil[1,2] make this product a target for different types of fraud, such as adulteration by blending with other oils of different sources. This is especially true for extra virgin olive oil, which fetches much higher prices in international markets than any other vegetable oil.

Besides the intentional addition of oils from different sources to extra virgin olive oil during the production process, there is also a possibility of unwanted contamination. Different analytical tests are described in the literature to detect small additions of low-quality olive oil,[3,4] hazelnut oils,[5] and seed oils[6] in the extra virgin product.

The use of techniques such as gas chromatography,[7] reverse-phase high-performance liquid chromatography,[8,9] gas chromatography coupled to mass spectrometry,[10,11] and nuclear magnetic resonance (NMR)[12–14] provides authentication and detection of adulterants in quantities less than 1% of foreign oils in extra virgin olive oil. Although these analytical techniques are widely used in authentication, quality control, and adulteration detection of edible oils, they have the drawback of being expensive or involve a time-consuming derivatization step.

Vibrational spectroscopy such as Fourier transform infrared spectroscopy,[15,16] mid-infrared spectroscopy,[17] and Raman techniques[18,19] has been used to detect adulterant in olive oils for many years.[20,21] The use of chemometric methods for data analysis has greatly improved the results obtained using these techniques.[17,18,22] In combination with principal component analysis (PCA), partial least squares, mode recognition, or neural network (NN) techniques, Raman spectroscopy has been successfully used to identify and quantify adulteration of olive oils with corn oils, soybean oils, olive pomace oils, hazelnut oils, etc.[22–24] Although many of the spectral features are similar, there are nevertheless sufficiently large differences in the Raman spectra for unambiguous identification.[25] However, Raman spectroscopy is not routinely used in many situations because of high background scattering and time-consuming sample alignment procedures.[26,27]

Laser induced breakdown spectroscopy (LIBS) has been a subject of research for the past few decades because of its unique features and the wide variety of applications in various fields.[28,29] In recent years, LIBS has become a powerful analytical tool because of its ability to carry out a rapid qualitative and quantitative analysis of different samples.[30,31] LIBS analyzes a sample by direct measurement of the atomic emission of the elements from a laser-induced plasma generated by the ablation of the sample, providing an immediate spectral fingerprint that is representative of its elemental composition.[32] Moreover, the technique requires little or no sample preparation. Although there is a loss of molecular information in plasma, this technique has provided excellent results for the identification of many polymer organic compounds,[33] explosives,[34] and bacteria.[32] The intensities of C, H, N, and O lines and the C/H, C/O, and C/N intensity ratios have been used for the classification of organic compounds.[34]

The aim of this paper is to use a simple and direct method to identify and discriminate edible oils based on LIBS and NNs. The method developed relies on the instantaneous identification of the oil sample using a unique feature of LIBS, which is its ability to generate a spectral "fingerprint" of the sample because of the nature of the emission spectra, representative of the main elements that constituted the sample. Thus, LIBS provides a unique spectrum, corresponding only to the sample under analysis. Using a correlation procedure, the developed LIBS-NN system, can be trained to recognize spectra from different samples, evaluating the similarity of unknown spectra against a spectral library of classified samples.

Several chemometric methods used to process LIBS data have been evaluated by different research groups, such as PCA,

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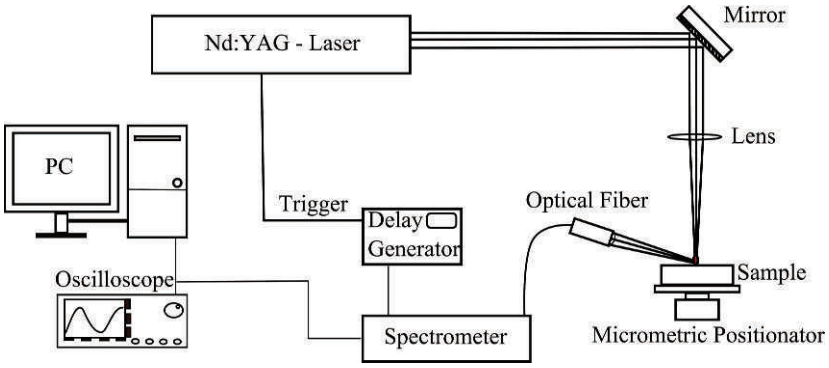

FIG. 1. The experimental setup, including a Nd : YAG laser, a delay generator, a micrometric positionator, a 1 m optical fiber, and an optical charge coupled device spectrometer.

soft independent modeling of class analogy, and partial least-squares discriminant analysis. These methods are not able to give satisfactory solutions to many practical problems attributed mainly to uncertainty in identification, which can be even higher than 30%.[35,36]

A comparison of some of these methods with respect to NNs has been performed,[37,38] showing that NNs provide better results. The aim of this work was to improve the recognition capacity of extremely similar samples that have few physical and spectral differences between them. For this reason NNs were selected because the technique combines a significant identification capability with a relatively simple implementation.

EXPERIMENTAL PROCEDURES

LIBS Setup. The LIBS setup has been described elsewhere.[32] Figure 1 shows a schematic view of the experimental setup. Experiments were performed using a Q-switched Nd:YAG laser (Brio model, Quantel) operating at 1064 nm and 1 Hz, with a pulse duration of 4 ns full width at half-maximum, 4 mm beam diameter, and 0.6 mrad divergence. The laser beam was focused onto the sample surface with a 100 mm focal-distance lens, producing a spot of about 250 μm in diameter and fluences of about 20 J/cm^2. The samples were introduced in a 100 mL open vessel, at atmospheric pressure, allowing each pulse of laser impinging on the same place. It is well known that plasma generation in liquids presents several problems, for example, the strong splashing and sloshing of the liquid produced by the shock waves generated by powerful laser pulses.[39] In the present study the distance from the sample to the focusing lens was optimized to avoid the oil droplets generated by splashing of the liquid modifying the transmission of the lens. No significant losses of oil or variations in the volume were observed when the measurements were taken. All samples were measured at room temperature. The emission from the created plasma was collected with a 4 mm aperture, 7 mm focus-fused silica collimator, placed at a distance of 5 cm from the sample and at 60° with respect to the surface normal. The collected light was focused into an optical fiber of 1000 μm core diameter and 0.22 numerical aperture, which was coupled to the entrance of the spectrometer. Although splashing mostly occurs normally to the surface and in the direction of the laser beam, no splashing was observed on the detection fiber using this configuration. The spectrometer system was a user-configured miniature single-fiber system (EPP2000, StellarNet, Tampa, FL) with a linear CCD detector (2048 pixels). It was used with a grating of 300 L/mm and a 7 μm entrance slit, allowing a spectral resolution less than 0.5 nm in a range from 200 to 1000 nm. The detector integration time was set to 1 ms. To prevent the detection of bremsstrahlung, the detector was triggered with a 5 μs delay time between the laser pulse and the acquired plasma radiation using a digital delay generator (Stanford model DG535). The spectrometer was computer controlled using an interface developed with Matlab, which allowed for data processing and real-time NN analysis.

Oil Samples. Commercial edible oils, with different brands and grades (olive, sunflower, hazelnut, and corn oils), produced in different countries were used. Olive oils were all purchased at local markets from Spain, Italy, Greece, and Argentina for a total of 20 oils. Some extra virgin olive oil corresponds to appellations of origin, including olive varieties such as Picual (Cambil), Picual and Royal (Coosur), Hojiblanca (Hojiblanca), Picudo, Lechín, Chorrúo, Hojiblanca, and Picual (Nuñez del Prado), and Rotondella, Carpellese, Frantoio, Nostrale, and Leccino (Pregio). There were also three edible oil samples of sunflower seed oil, corn oil, and hazelnut that were purchased at local markets in Spain.

Four sets of olive oils samples were blended with fixed-volume percentages of sunflower or hazelnut oils used as adulterants, obtaining a total of 20 blended samples. These samples were prepared by mixing the respective pure oils with the percentages ranging from 1 to 5% v/v of adulterating oils as detailed in Table I and agitating continuously using a magnetic stirrer for 15 minutes. The rest of the samples were used with no further preparation. This means that the samples were poured directly from their commercial packaging into the glass vessel used for measurements. The edible oil samples were numbered from 1 to 43. As shown in Table I, the first 23 samples were pure oils, and the last 20 were blended samples. A total of 118 samples were analyzed, including a minimum of 2 replicate tests for each sample. A detailed list of the samples information is given in Table I.

LIBS Measurements and Spectral Libraries. Each sample was irradiated with 100 laser pulses. For each pulse the generated plasma spectrum was acquired and stored as a column on a dataset. The dataset contains the intensity at different wavelengths in rows and the spectra in columns. Thus, our dataset has 2048 rows (one for each wavelength) and 100 columns or spectra for each sample. Each of these individual worksheets containing the spectra for a specific

TABLE I. Sample description.

Sample	Origin	Class	Brand	Grade	No. replicates analyzed
1	Spain	1	Coosur batch 1	Extra virgin olive oil	4
2	Spain	1	Nuñez del Prado	Extra virgin olive oil	3
3	Spain	1	Cambil	Extra virgin olive oil	3
4	Spain	1	Hojiblanca	Olive oil	3
5	Spain	1	Carbonel	Olive oil	3
6	Spain	1	Ybarra	Olive oil	3
7	Spain	1	Koipesol	Sunflower oil	3
8	Spain	1	La Masia	Corn oil	3
9	Spain	1	Coosur batch 2	Extra virgin olive oil	3
10	Spain	1	Guinama	Hazelnut	3
11	Italy	2	De Marco	Extra virgin olive oil	3
12	Italy	2	Pregio	Extra virgin olive oil	3
13	Italy	2	Diesis Torretta	Extra virgin olive oil	3
14	Greece	3	Kretiko	Extra virgin olive oil	3
15	Greece	3	Kanakis	Olive oil	3
16	Argentina	4	Cocinero	Extra virgin olive oil	3
17	Argentina	4	Altavia	Extra virgin olive oil	3
18	Argentina	4	Gretvalue	Extra virgin olive oil	3
19	Argentina	4	Indalo	Extra virgin olive oil	3
20	Argentina	4	Lira	Extra virgin olive oil	3
21	Argentina	4	Nucete	Extra virgin olive oil	3
22	Argentina	4	Oleovita	Extra virgin olive oil	3
23	Argentina	4	Toscana	Extra virgin olive oil	3
24	Spain	–	Coosur + 1% sunflower oil	Blended sample	3
25	Spain	–	Coosur + 2% sunflower oil	Blended sample	2
26	Spain	–	Coosur + 3% sunflower oil	Blended sample	2
27	Spain	–	Coosur + 4% sunflower oil	Blended sample	2
28	Spain	–	Coosur + 5% sunflower oil	Blended sample	3
29	Spain	–	Cambil + 1% sunflower oil	Blended sample	3
30	Spain	–	Cambil + 2% sunflower oil	Blended sample	2
31	Spain	–	Cambil + 3% sunflower oil	Blended sample	2
32	Spain	–	Cambil + 4% sunflower oil	Blended sample	2
33	Spain	–	Cambil + 5% sunflower oil	Blended sample	3
34	Spain	–	Coosur + 1% hazelnut oil	Blended sample	3
35	Spain	–	Coosur + 2% hazelnut oil	Blended sample	2
36	Spain	–	Coosur + 3% hazelnut oil	Blended sample	2
37	Spain	–	Coosur + 4% hazelnut oil	Blended sample	2
38	Spain	–	Coosur + 5% hazelnut oil	Blended sample	3
39	Italy	–	Pregio + 1% hazelnut oil	Blended sample	3
40	Italy	–	Pregio + 2% hazelnut oil	Blended sample	2
41	Italy	–	Pregio + 3% hazelnut oil	Blended sample	2
42	Italy	–	Pregio + 4% hazelnut oil	Blended sample	2
43	Italy	–	Pregio + 5% hazelnut oil	Blended sample	3

sample constitutes a spectral library. Fifty spectra were used to create the fingerprint, and the remaining spectra were used to test the identification model. To avoid data variations due to changes in the laser pulse energy, each spectrum was normalized by the most intense emission line of nitrogen assigned as N(II)[40,41] at 500 nm as shown in Fig. 2. The acquisition of these 100 spectra is very fast, less than 2 min, taking into account the integration time and 1 Hz laser pulse repetition. Although the data matrix could be considerably large, the computation time in the training of the NN was always below 10 s.

NN Model. For the identification model a broad spectral range was used to cover the greatest number of spectral characteristics of the samples. Reducing the variables in the training of NNs using the shorter spectral ranges with few peaks, selected by PCA, shows that the model's recognition ability decreases.[42] The NN model consisted of three layers (input, hidden, and output), a topology widely used to model systems with a similar level of complexity.[43] In particular, the input layer consisted of 2048 nodes (intensity values in the 200–1000 nm wavelength range). The output layer comprised J neurons (where J = number of reference samples used) for estimating the similarity between the reference sample spectra and the testing sample spectrum. The identification process was based on the ability of the NN to detect the degree of similarity between the new spectrum and each of the reference spectra used in the learning process.

The training set was composed of the library of each oil sample used as a reference. During the training process, each sample used as a reference was associated with an identification number (the same number assigned to the sample) in the output layer. Thus, a perfect identification was obtained if the output from the NN model for the test sample matched the identification number assigned to the reference. It is possible to use more than two identification numbers simultaneously, e.g., when analyzing a large number of samples. Zero was always used to indicate no match at all.

NN training was achieved by applying the back-propagation algorithm, based on the conjugate gradient method,[44] one of the general-purpose, second-order techniques that helps minimize the goal functions of several variables. Second-order indicates that such methods use the second derivatives of the error function, whereas a first-order technique, such as standard back-propagation, uses only the first derivatives. To determine

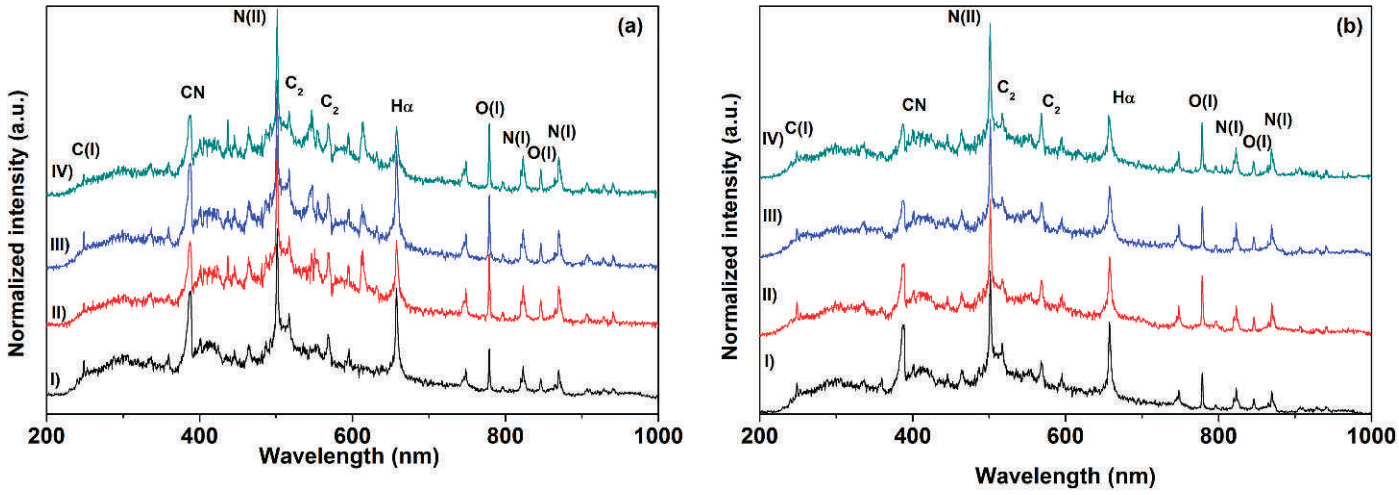

FIG. 2. Normalized LIBS single-shot spectra: (**a**) Pure edible (***I***) olive oil, (***II***) sunflower oil, (***III***) corn oil, and (***IV***) hazelnut oil. (**b**) Four edible olive oils of Class 1, (***I***) Coosur batch 1 olive oil, (***II***) Nuñez del Prado olive oil, (***III***) Cambil olive oil, and (***IV***) blended sample (Coosur olive oil + 5% v/v with sunflower oil). Plots have been shifted for better observation.

when the training should be stopped, an early stopping criteria based on the test set was used.[45] The number of epochs was not relevant in this case. To avoid an over-fitting of the NN model, the learning process was repeated while the verification mean square error (MSE), defined in Eq. 1, was decreased:

$$\mathrm{MSE} = \frac{1}{N}\sum_{k}^{N}(r_k - y_k)^2 \qquad (1)$$

where N, y_k, and r_k are the number of input data, the response from each output neuron, and the real output response, respectively. A detailed description of the calculation process is provided in the literature.[45]

Accuracy (A) is the main parameter of a recognition procedure for decision making, and the reason the metrics for assessing detection processes are so important. These involve the relative frequency of correct and incorrect identification obtained from the results and can be calculated according to Eq. 2:[46,47]

$$A = TP + TN/(TP + TN + FP + FN) \qquad (2)$$

where TP, TN, FP, and FN are true positive, true negative, false positive, and false negative, respectively. The receiver operating characteristic (ROC) curve is the standard tool for plotting all possible combinations of sensitivity and specificity for a screening process. ROC plot analysis was developed to evaluate classification performance.[47] The true positive rate (TPR) is used as the y axis in an ROC plot, and the false positive rate (FPR) is used as the x axis. An ROC curve is at the ideal operating point when $TPR = 1$ and $FPR = 0$. It is widely accepted[46] that the area under curve (AUC) provides a better measure than accuracy for evaluating the predictive ability of the classification. This can be calculated using Eq. 3:[48]

$$AUC = \frac{S_{o-n_o}(n_o + 1)/2}{n_o n_1} \qquad (3)$$

where n_0 and n_1 are the numbers of positives and negatives, respectively, and $S_o = \sum r_i$ where r_i is the i_{th} positive number.

The confidence of the prediction can be expressed by a conditional probability, i.e., the rate of correct classification within the classified spectra (accuracy). The confidence was estimated by a match index. The higher the match index, the better the efficiency of the network to identify an oil sample.

The robustness of the model was tested by assessing the ability of LIBS-NN to estimate the correct result when an unknown sample was input into the network model. In other words, the higher the robustness, the better the efficiency of the NN model for identifying a sample not included in the training step as an "unknown," and not identifying it as another oil.[49] To test this, one dataset (input data spectra) from the training set was completely removed. The results obtained with the remaining ones were then checked, in terms of the probability of correct identification. This was alternately repeated for each oil sample.

The greater the number of spectra used in the fingerprint of a sample, the better is the capacity of recognition of the method. A more thorough study of how the recognition affected model identification is shown in the results section.

RESULTS AND DISCUSSION

The NN was trained for spectral characteristics using the input data of known samples. The subsequent NN model parameters were then handled by an identification program that performs real-time identification during data acquisition. Figure 2a shows normalized single-shot spectrum for pure oils: olive (sample 1), sunflower (sample 7), corn (sample 8), and hazelnut (sample 10). The composition of these samples produces significant variation between the emission intensities of spectral line that can produce the identification of these pure edible oils. Figure 2b shows normalized single-shot spectra for three pure olive oils: samples 1, 2, and 3 and a 5% v/v blended sample (sample 28) prepared by adulterating pure olive oil with sunflower oil. The compositions of these samples were very similar, and their spectra look the same, making visual identification difficult. Due to very fast testing conditions, and bearing in mind the high spectral similarity between the samples (for which a single shot might be the only sampling event), the spectra were not averaged.

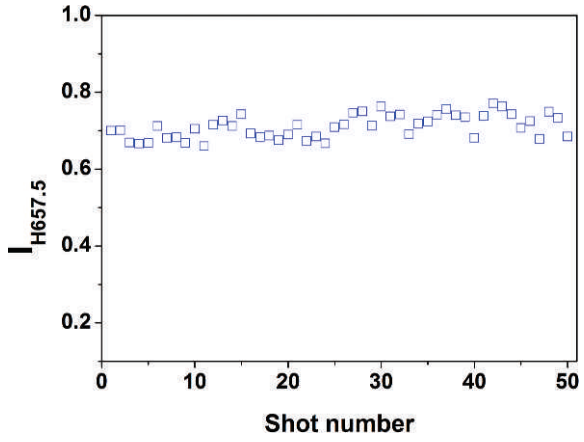

FIG. 3. Shot to shot variation of emission intensity of Hα for Cambil olive oil sample.

Previous studies have demonstrated that the experimental parameters in LIBS processes that affect the overall intensity of the spectrum must be assumed to change from shot to shot due to the chaotic nature of this interaction.[50–52] When an ablation event is recorded pulse-to-pulse, many parameters can affect the spectra obtained, such as laser-matter coupling, distance lens displays, pulse energy, generated crater depth, etc. Furthermore, the plasma is generated from evaporated and ionized sample materials mixed with ambient gas,[53] some spectral lines from air are present in the spectra. On the other hand, it has been demonstrated[54] that spectra from air can be correlated with the use of a simple multiplicative scaling factor, thus demonstrating that changes in relative spectral contributions from oxygen and nitrogen are not occurring. Also the temperature T must be assumed to vary from shot to shot, especially when extensive sample preparation is avoided. In this work the evaluation of shot-to-shot variation of emission intensity of Hα was done and is shown in Fig. 3. The deviation of plot is very little for this liquid oil sample (<4% RSD) and confirmed the good stability and repeatability of the experiment.

First Test Process. The LIBS/NN correlation method was first applied to the identification of oils of the same class. A collection of single-shot spectra of each sample were analyzed and contrasted with the spectra of reference samples of the same class, in a binary manner. In each binary analysis, part of the library of the sample was used as a reference. As an example, Fig. 4a shows the analysis of sample 1 and 2 of class 1. Fifty spectra of each sample were used as reference sample set, where 1 and 2 were assigned as identification numbers, respectively. Fifty spectra for samples not included in the reference were tested. Thus if the NN has an output equal to 1, this corresponds to the identification of oil 1, and if the NN output has a value equal to 2, this corresponds to the identification of oil 2. With the first laser shot, the network recognizes the spectrum as belonging to a reference dataset, assigning the respective NN output value, because the NN model cannot "see" the difference between the analyzed sample and the reference library.

Most of the spectra for this sample were assigned correctly, and only one deviated from the expected behavior (sample 1), giving values between the two assigned. This behavior strongly affects both the match index and the *AUC*, which were 99.0% and 0.9976, respectively. Given that the spectra analyzed came from a single laser shot, the disturbance in only 1 out of 50 spectra is not only more than acceptable, it is essential for taking into account the match index. Exactly the same results were obtained when using the data set for all other oil samples from different classes as a reference, and updating the NN parameters, as shown in Table II.

Figure 4b shows the ROC plot and match index (Eq. 2) obtained for this case and the capacity of the LIBS/NN to identify those olive oils. Only the difference between oils can cause identification. Moreover, despite the contribution of emission lines from air in the spectrum, a high match index was achieved, indicating a correct identification of the samples, which permits us to measure the samples at room conditions,

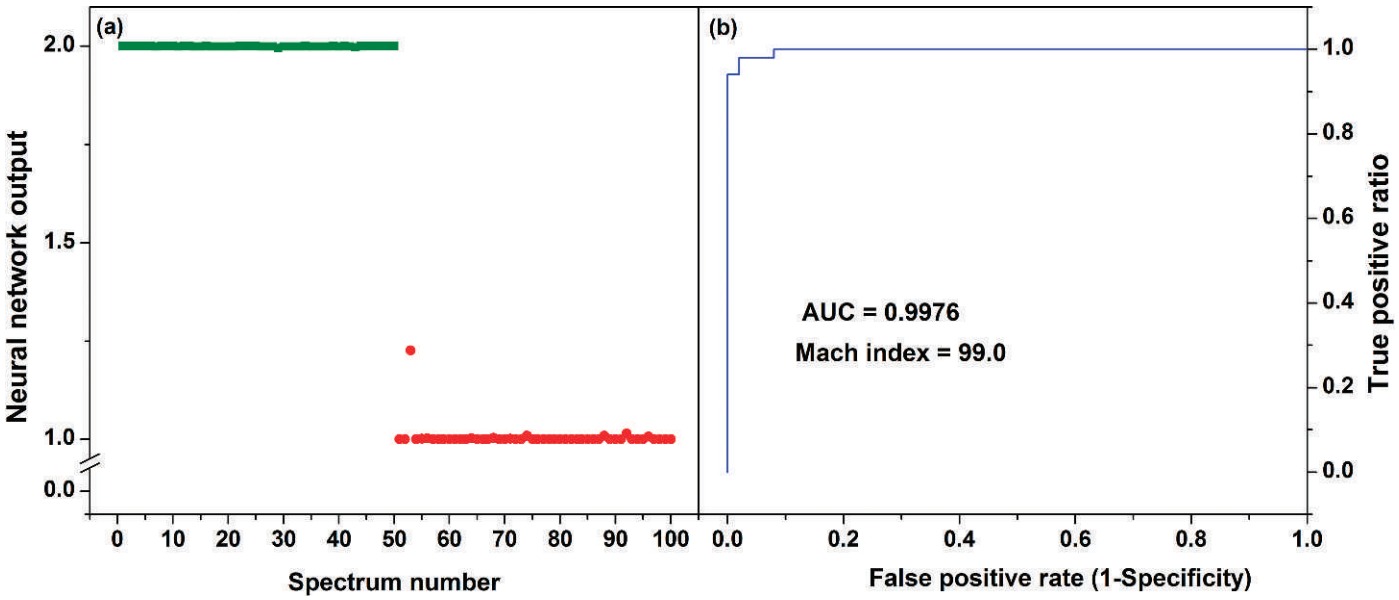

FIG. 4. (**a**) LIBS-NN correlation method applied to the identification of class 1. (●) Sample 1. (■) Sample 2. (**b**) ROC plot calculated as discussed in the text, for samples 1 and 2 (AUC = 0.9976, match index = 99.0%).

TABLE II. Classification results from neural networks analysis for olive oils and lower adulterated blended samples.

	Classification results (%)					
	Identification test				Robustness test	
Olive oils	Match index	Unidentified	Misidentified	Correct identification	Misidentified	Correct as unknown
Coosur batch 1	97.4	0	0	100	0	100
Nuñez del Prado	98.2	0	0	100	0	100
Cambil	98.0	0	0	100	0	100
Hojiblanca	99.0	0	0	100	0	100
Carbonel	100	0	0	100	0	100
Ybarra	100	0	0	100	0	100
Koipesol	99.2	0	0	100	0	100
La Masia	99.0	0	0	100	0	100
Coosur batch2	96.6	0	0	100	0	100
De Marco	100	0	0	100	0	100
Pregio	99.2	0	0	100	0	100
Diesis Torretta	98.4	0	0	100	0	100
Kretiko	96.4	0	0	100	0	100
Kanakis	96.8	0	0	100	0	100
Cocinero	100	0	0	100	0	100
Altavia	100	0	0	100	0	100
Gretvalue	98.0	0	0	100	0	100
Indalo	98.4	0	0	100	0	100
Lira	97.2	0	0	100	0	100
Nucete	100	0	0	100	0	100
Oleovita	100	0	0	100	0	100
Toscana	98.4	0	0	100	0	100
Coosur + 1% sunflower oil	97.8	0	0	100	0	100
Cambil + 1% sunflower oil	100	0	0	100	0	100
Coosur + 1% hazelnut oil	100	0	0	100	0	100
Pregio + 1% hazelnut oil	98.4	0	0	100	0	100

decreasing the analysis time without significantly affecting the model's discrimination capacity.

Second Test Process. As a part of a tough test for evaluating the model's capacity to identify adulterated olive oils, the spectra of each oil sample were introduced simultaneously as references within NNs. The mathematical procedure followed was similar to the training and test process described above. As an example, Fig. 5a shows the analysis of pure olive oils (sample 1) and adulterated with 1, 3, and 5% of hazelnut oil. Fifty spectra of each sample were used as a reference sample set, where 1, 2, 3, and 4 were assigned as identification numbers, respectively. Twenty spectra for samples not included in the reference set were tested. As shown individual identification and comparison with pure oils provide a satisfactory classification that permits the discrimination of the adulteration. Results also showed the NN capacity to simultaneously work with more than one fingerprint, without increasing significantly the computing time. As has been mentioned previously, the training time was always below 10 s.

Third Test Process. To complete the test of the prediction capability of the optimized NN, a third independent test set was carried out to test the model's ability to identify unknown samples as unknown. To test the robustness of the model, the spectra of samples from class 1 were used as the library reference, and one oil dataset was removed and alternately repeated for each olive oil.

The NN compares the analyzed sample spectra with those stored as a reference (like two fingerprints). Therefore, if they match, the output from the network is satisfactory, and the value assigned to the reference and NN output match. If the fingerprints (spectra) differ slightly, the NN output must be zero. This means that the test sample is not present in the training set and is therefore unknown. As an example, Fig. 5b shows a possible stronger test. In this case all samples from class 1 were used as a reference, and the spectra of sample 3 (Cambil) were completely removed. The NN output for 20 spectra from each sample in class 1 (sample 1: Coosur batch 1, sample 2: Nuñez del Pardo, and sample 3: Cambil) were tested. All samples were correctly identified. Even sample 3 was correctly identified as unknown and not as another oil sample included in the training set, which demonstrates the robustness of the method used. Complete results of this test for all pure oils and blended samples (1% v/v) are shown in Table II.

For this analytical methodology, in the case of an unknown olive oil sample suspected for adulteration, the analyst does not have access either to the original olive oil samples or to the adulterant, which might be considered the reality that the analyst has to cope with. As is well known in all analytical techniques, it is necessary to have a sample reference. This is, for example, the case of chromatography, where the retention time of an analyte is verifiable only with the use of a pattern of such an analyte. In addition, in the case of techniques such as NMR or MS the high cost of these analyses restricts its application. The success of the current study paves the way for further investigations of other, more complex, algorithms based on unsupervised systems, without any need for reference spectra, such as genetic NNs.

Finally, we would like to remark that the usefulness of the method developed is evident, for example, for implementation of the oil quality control and traceability of food products. Thus, this model is appropriate to identify these chemical compounds on line, for quality and control processes in the food industry, with adequate accuracy and without pretreatment of samples.

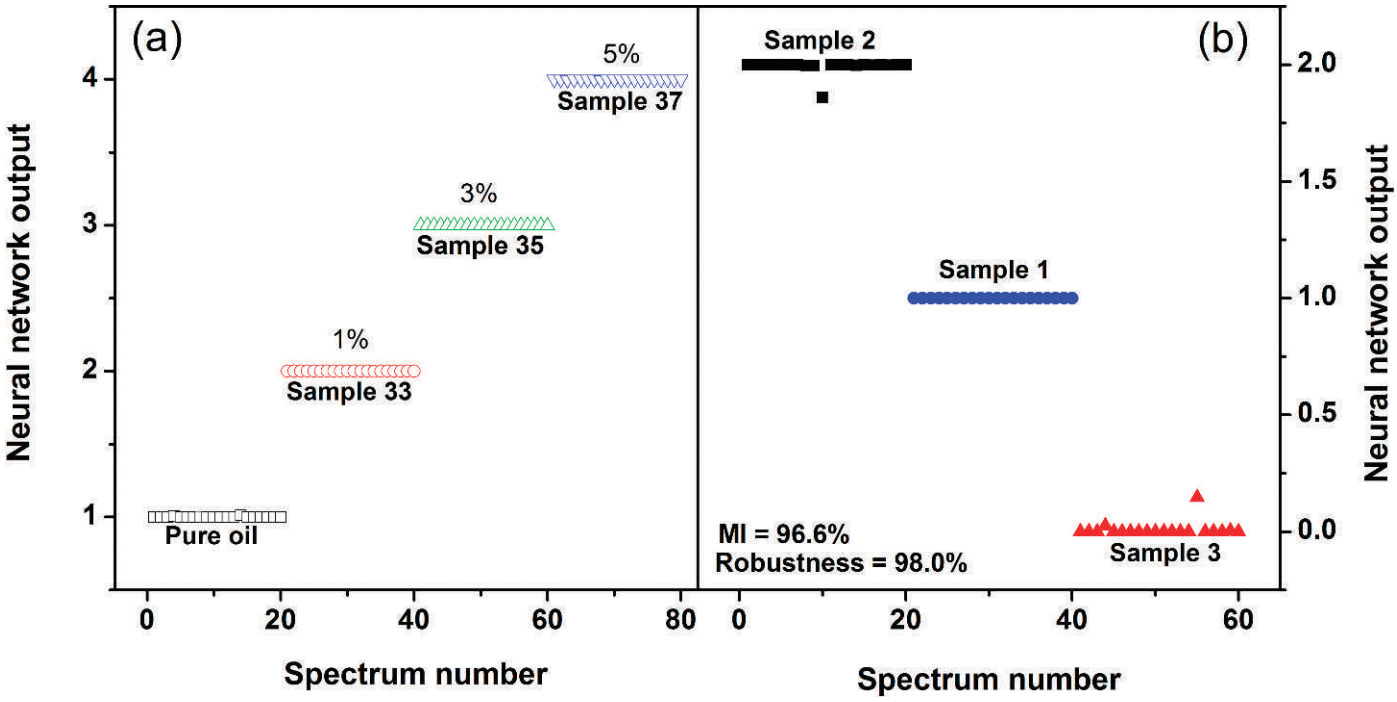

FIG. 5. (**a**) NN output for 20 spectra of sample pure Coosur olive oils and the same sample adulterated with hazelnut oil, with 1, 3, and 5%, respectively (not included in the training). A correct match between NN outputs with the reference assigned number are shown. Both individual identification and comparison with pure oils report a satisfactory discrimination. (**b**) NN outputs for samples 1 (Coosur batch 1), 2 (Nuñezdel Prado), and 3 (Cambil), where the last corresponds to a sample set not included in the training set. Thus this sample 3 was unknown for the NN model. Sample 3 was correctly assigned zero, the identification number assigned to unknown samples. The match index and robustness was 96.6 and 98.0%, respectively.

Number of Spectra Used in the Training Set. Finally, the optimum number of spectra used in the training process and the variations in robustness as a function of the number of spectra used in the training matrix was studied. Figure 6 shows a plot of these results. As can be observed, robustness increases rapidly with the number of spectra. Even for very low numbers (eight spectra), the robustness is acceptable.

Another important result is obtained from this test, and some interesting modes of identification can be observed. To improve the correct identification rate, the number of spectra in the training set for each sample needs to be increased to improve the match index. It is therefore necessary to select a high number of spectra, almost 50, and because each spectrum comes from a single laser pulse, the time taken to collect the data (1 s for each

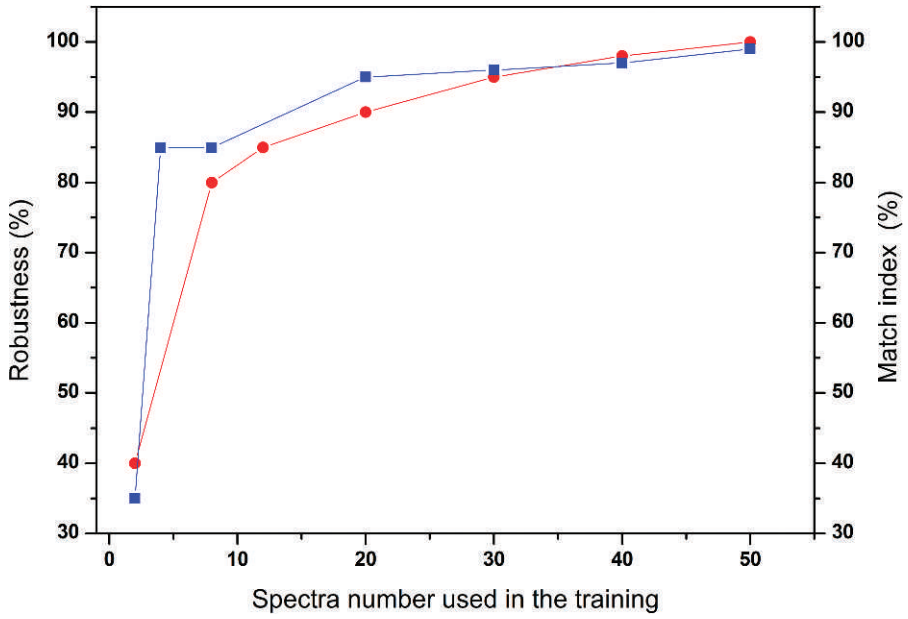

FIG. 6. Match index for sample 1 (●) and the robustness obtained for sample 11 (■), which is not part of the reference samples, as a function of the number of spectra used in the process of training the NN.

spectrum) is not an issue. The broad spectral range used also plays a key role in the correct sample identification.

Testing the spectral data obtained from the oil samples measured every 15 days over a period of two months, using the reference libraries from the first day, showed that the identification ability of generated NN models was stable over a long period. This confirmed the temporal validity of the libraries, at least during this study.

The time required to obtain the spectra is very fast. Once stored, they are selected for further analysis in real time. Analysis carried out with different types of oils showed that the libraries were adequate for correct identification of the edible olive oils.

Single-shot measurements were sufficient for clear identification of the olive oils studied. Taking this into account, the optimized NN model provides reliable results (sample identification) for all samples analyzed. This result is the best indicator of the capacity of the methodology presented.

Although studies with concentrations less than 1% can be interesting, they have not been made because of low economic significance in a deliberate adulteration.

The results obtained demonstrate that although the intensity of the spectra can change from pulse to pulse and from day to day, it does not affect the system's ability to identify the sample. Despite that there are not significant variations in the spectra of the oil samples from which the oil can be easily discriminated, from a mathematical point of view each oil sample can be discriminated based on its complete spectral fingerprint. The full sets of variables (intensities at each wavelength) that constitute the sample spectrum are important in the process of comparison performed by the NN, which constitutes the basis of their ability to carry out discrimination. The NN is able to compute internal parameters (weights and bias) in the training process for classifying a given set of input variables as belonging to particular oil, with a high tolerance for noise and the presence of outliers.

CONCLUSIONS

It has been shown that accurate sample analysis can be obtained using LIBS/NN. Tests performed on edible oil demonstrated 100% reliable identification of both known and unknown samples with very similar spectral characteristics.

In addition, in studies where the only variation was the type of olive oils, the identification was correct. Only the difference between oils can cause identification. Despite the possible contribution of emission lines from air in the spectrum, a correct identification can be achieved, which helps to decrease the analysis time without significantly affecting the model's discrimination capacity.

The application of LIBS/NN to extra virgin olive oil samples coming from different regions of the same country and collected in different harvesting periods resulted in their being correctly identified. The same happens with samples extracted from different olive varieties. These results can be considered the most rigorous test conducted for the developed method.

The identification analysis was steady over a long period. Minor changes in experimental conditions, such as the intensity of the LIBS single-shot regime and continuum background, were not relevant for sample identification. The system was able to perform a correct identification even with a single laser shot. The most important conclusion is that in the 200–1000 nm range, each spectrum is a true fingerprint of the sample, allowing a correct differentiation of each olive oil using the NN model.

Multivariate techniques are known to be efficient methods for sorting and classifying data. However, the results of this study show that better reproducibility data and the introduction of advanced statistical models are needed to produce robust classification models.

The verification test emphasized that the methodology used in this work can provide a measure of classification confidence that may have practical significance. The study will be extended to use unsupervised systems such as genetic NNs, and further analysis could be necessary to validate the used methodology for larger samples and time robustness. This work is currently underway in our laboratories.

Taking into account the speed of the measurement, the method developed in this work can be used for automatic, reliable, and robust real-time measurements.

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