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Detection of the Presence of Refined Hazelnut Oil in Refined Olive Oil by Fluorescence Spectroscopy

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The fluorescence spectroscopy technique has been tested as regards its ability to differentiate between refined hazelnut and olive oils. Classification of these oils based on their excitation−emission fluorescence spectra data (spectral range 300−500 nm of the excitation spectra at λ_{em} = 655 and spectral range 650−900 of the emission spectra at λ_{ex} =350 nm) was performed using principal component analysis and artificial neural networks. Both methods provided good discrimination between the refined hazelnut and olive oils. The results have also pointed out the possibilities of a spectrofluorimetric method joined to multivariate analysis, to differentiate refined oils, and even to detect the presence of refined hazelnut oils in refined olive oils at percentages higher than 9%.

KEYWORDS: Fluorescence; refined olive oil; refined hazelnut oil; multivariate analysis

INTRODUCTION

The ability to authenticate olive oil is, nowadays, of major concern to food safety and quality. Chromatographic techniques have been widely used for oil analysis due to their high selectivity and low detection limits for many relevant compounds (*1*). They often involve extraction and preconcentration steps that make them unable for on-line analyses besides their requirements of high purity solvents and full-trained analysts. The alternative to chromatography is spectroscopy. The latter is gaining popularity as it can produce rapid and inexpensive analytical methods. The main disadvantage of the spectroscopic methods is, however, their lack of selectivity. It means that they need advanced multivariate procedures to analyze the spectra. Pattern recognition routines, based on either statistical methods or artificial neural networks, have been the most used for quantitative analysis (*2*).

Fluorescence spectroscopy is one of the most promising spectroscopic techniques with increasing importance in complex food analyses. Instrumental improvements—such as the availability of new sample handling accessories, computer facilities, and existence of software specially designed to extract and to use the information contained in spectra—have contributed to the extensive application of fluorescence spectroscopy in chemistry, biochemistry, and environmental analysis, and more recently, in food science. Among the benefits of fluorescence spectroscopy is its enhanced selectivity as compared to other spectroscopic methods, the high sensitivity to a wide array of potential analytes, and in general, the avoidance of consumable reagents and of an extensive sample pretreatment (*3*).

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Several papers have reported the potential of fluorescence in the analysis of food products. Fluorescence spectroscopy has been used in the sugar industry process (*4*), the analysis of fish oil (*5*) and frying oil (*6*) among others.

The papers published on vegetable oil fluorescence are mainly focused on the characterization of several crude vegetable oils (*7*−*15*). Vegetable oils are complex mixtures of fluorescent and nonfluorescent compounds. Fluorescence spectra of vegetable oils are usually constituted by a series of bands corresponding to the fluorescent components. Usually, crude oils have different spectra than refined ones due to the fact that refining gives rise to the loss of minor compounds, and in some cases, the production of new compounds with fluorescent properties. This fact has been previously used to detect the presence of crude hazelnut oils in virgin olive oils (*16*). Detection of adulteration is, however, more difficult when blends of refined oils are used since their composition in minor components is very similar.

It has been reported that quantities of hazelnut oil (*Corylus avellana* L.) are being imported into the European community, without proper declaration to Customs and Excise, and it is suspected that hazelnut oil is being used to adulterate olive oils bottled within the community (*17*). Three kinds of adulteration of olive oil with other edible oils can be found: blends of virgin oils, blends of virgin and refined oils, and blends of refined oils. The first kind of adulteration does not seem to be lucrative due to the low odor threshold of the filbertone, the hazelnut oil marker. The alternative would be to carry out the blend with lampante olive oil that might mask the hazelnut oil odor (*18*); however, the lampante olive oil must be refined prior to being sold to consumers, which means that the real problem is the detection of the presence of refined hazelnut oil in refined olive oil. The addition of refined hazelnut oil to virgin olive oil can be detected, even at very low percentages of the adulterant, by

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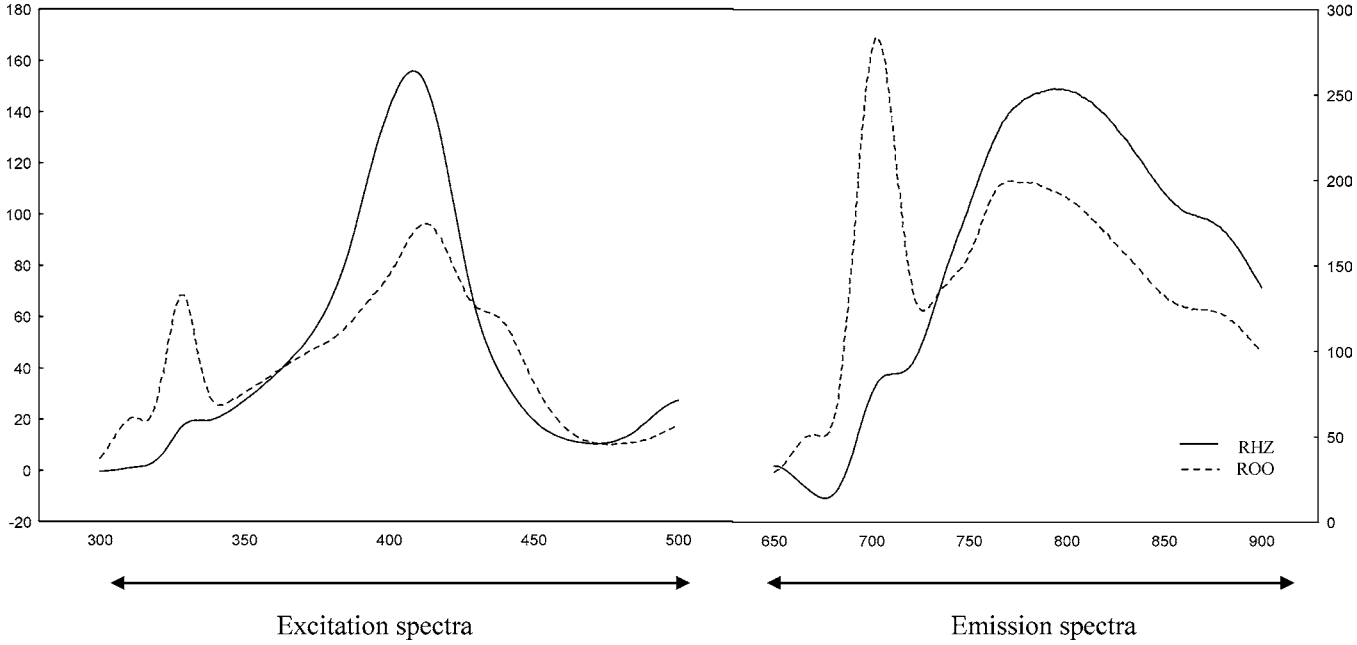

Figure 1. Excitation (λ_{em} = 655 nm) and emission (λ_{ex} = 350 nm) spectra of refined hazelnut oils (solid line) and refined olive oils (dotted line).

the standard method based on quantification of stigmastadienes (*19*). However, there is no standard that detects the presence of refined hazelnut oil in refined olive oil or olive oil (blend of refined and virgin olive oils) at percentages lower than 20 (*20*). Only fluorescent compounds produced in the step of thermal deodoration (*21*) could be quantified to detect this kind of fraud. The aim of this study was to distinguish refined hazelnut oils from refined olive oils and to evaluate a method to detect the presence of refined hazelnut oil in olive oil, an adulteration that is still uncontrolled at low percentages (*22*).

EXPERIMENTAL PROCEDURES

Samples. Thirty-eight samples from several geographical origins and varieties were analyzed. Thirteen refined olive oils were collected in Turkey (var. Yag Çelebi, Memecik, Ayvalik, and Egriburun), Tunisia (var. Chetoui), Spain (*v*ar. Hojiblanca), Greece (var. Koroneiki), and Italy (var. Moraiolo), and seven refined hazelnut oils were collected in Turkey (var. Foça, Kara Findik, Palaz, and Tombul), Spain (var. Barcelona), and Italy (var. Mortarella). Eighteen mixtures were prepared adding refined hazelnut oils to refined olive oils from 3 to 30% (3, 5, 7, 7.5, 8, 9, 10, 12, 15, 25, and 30), 11 of these mixtures being edible oils produced in Turkey as this country is the major producer of hazelnut oil.

Samples were kept at 4 °C while they were not in use and were analyzed without any pretreatment (e.g., solvents) to avoid potential interferences.

Instrumentation. All the spectrofluorimetric measurements were performed with a RF-1501 Shimadzu spectrofluorophotometer (Shimadzu Corporation, Kyoto, Japan) equipped with a continuous 150 W xenon lamp, excitation and emission monochromators, and a photomultiplier. Fluorescence emission spectra (360−900 nm, 1 nm interval) were collected at 350 nm excitation wavelength, and the excitation spectra (220−645 nm, 1 nm interval) were collected at 655 nm for the objective of this work. Excitation and emission slits were both set at 10 nm. Samples were scanned using a 3 mL nonfluorescent cell (10 mm path length). After each series of measurements, the cuvette was cleaned using detergent, followed by a rinse with deionized hot water and acetone to dry and eliminate the rest of the fat. Each sample was analyzed in triplicate.

The spectrofluorophotometer was interfaced to a computer for spectral acquisition and data processing. Only the spectral range of 300−500 nm of the excitation spectra at λ_{em} = 655 and the spectral range of 650−900 of the emission spectra at λ_{ex} = 350 nm were used for the data analysis. Raw spectra were used as acquired without any pretreatment.

Statistical Analysis. The Brown−Forsythe test was used to perform the univariate analysis as it gives quite accurate error rates even when the underlying distributions for the raw scores deviate significantly from the normal distribution (*23*). This first screening was followed by the multivariate procedure of principal component analysis (PCA) to reduce the data considerably and to determine the percentage of the explained variance that allows distinguishing the refined oils. Once we knew that it was possible to distinguish refined hazelnut oil from refined olive oil, the next step was to determine if the selected fluorescent information might detect the addition of hazelnut oil to olive oil. ANN was used because of its ability to handle nonlinear and complex data (*24*). ANN was run with back-propagation (50 epochs) and conjugate gradient descent (234 epochs) algorithms (*25*) to minimize the prediction errors (rms).

Microsoft Excel 2000 (Microsoft Corporation, Redmond, WA) and Statistica 6.0 (StatSoft, Tulsa, OK) were, respectively, used for building the database and carrying out the statistical analyses.

RESULTS AND DISCUSSION

There are only small differences between the refined oils if the whole spectrum is considered, although there are several zones with remarkable differences if a detailed study is carried out. These zones were reduced from 1800 raw data to 452. **Figure 1** shows the selected zones of the excitation−emission spectra of a refined olive oil (ROO) and a refined hazelnut oil (RHZ) that represent all the edible oils analyzed. ROO excitation spectra (λ_{em} = 655 nm) exhibit two main bands centered at 328 and 414 nm, both of them having a shoulder. On the contrary, RHZ oil spectra showed only a main band centered at 408 nm with a weak shoulder at 333 nm. Emission spectra (λ_{ex} = 350 nm) of both oils were also different, ROO having two main bands at 703 and 777 nm and RHZ at 794 nm. The major intensity band in the ROO spectra appears at the zone attributed to the pigment fraction of virgin olive oil (*26*, *27*), compounds that may be not completely removed during the refining process.

To select the variables showing major differences between the refined edible oils according to the spectra, the Brown−

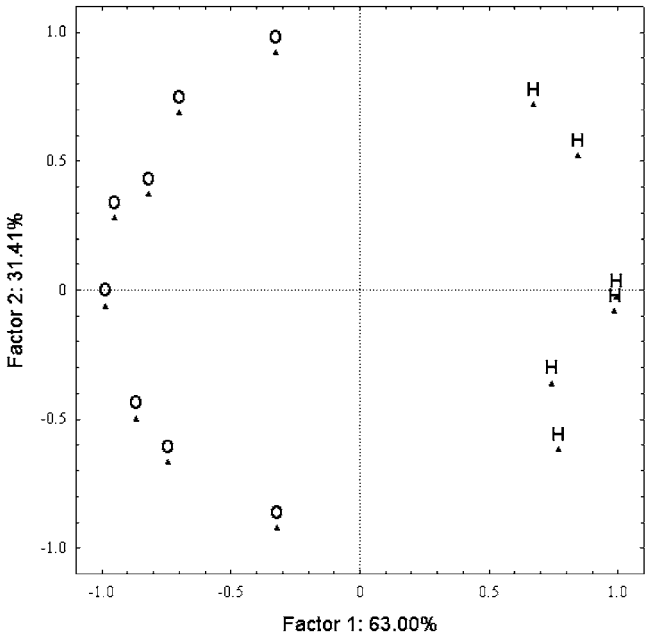

Figure 2. Principal component analysis of pure refined olive (O) and refined hazelnut (H) oil samples.

Table 1. Values of the Parameters of the Neural Network Equations

	hidden layer				output layer			
	w_{i1}	w_{i2}	w_{i3}	w_{i4}		output units		
a_i	7.55	7.26	0.34	17.35	a_j	−14.67	6.65	10.01
x_1	−5.51	−31.39	−23.43	−33.80	w_1	−20.59	−22.22	14.39
x_2	−0.72	−46.92	−16.47	−31.53	w_2	−28.12	31.73	−33.01
x_3	21.06	−13.22	9.35	3.04	w_3	30.40	−6.94	−22.67
x_4	19.55	−15.75	20.71	3.68	w_4	−30.88	3.58	23.27

Forsythe test for homogeneity of variances ($p < 0.05$) was applied. Thus, the initial number of variables (452 wavelengths) was reduced to a small number (40 wavelengths). Once again, the spectrum zones selected corresponded to pigments (390−410 nm of excitation spectrum at $\lambda_{em} = 655$ nm and 692−765 nm of the emission spectrum at $\lambda_{ex} = 350$ nm) (*11*, *28*).

To evaluate the possible presence of pigments in the samples, the oils were analyzed to determine the kind of fluorescent compounds that were not removed in the refining process. Pigment fraction analysis was carried out by the method suggested by Gallardo-Guerrero et al. (*26*), and the result was the presence of pyropheophytins, whose total concentration was higher in the refined hazelnut oils than in refined olive oils. These kinds of fluorescent compounds are thermal degradation products of chlorophylls and pheophytines induced by heating during the refining process where the oil temperature might reach 220 °C (*21*).

Once it was determined that the selected fluorescent bands may have a chemical explanation, the data matrix constituted by pure samples (eight ROO and six RHO) was normalized by a *Z*-score prior to using PCA. The first two factors explained 94.41% of total variance (63.00 and 31.41%), but obviously, a part of the variance was common to both kinds of edible oils. Only the first factor allowed a correct separation between the samples (**Figure 2**). This factor correlates positively with the hazelnut oil samples and negatively with the olive oil samples. The factor loadings showed that the wavelengths in the range of 390−410 and 700−718 nm mainly explains factors 1 and 2, respectively. Thus, the fluorescence intensities registered at the former wavelength range (e.g., 400 nm) were higher in hazelnut oils, while those in 700−718 nm were higher in olive oil.

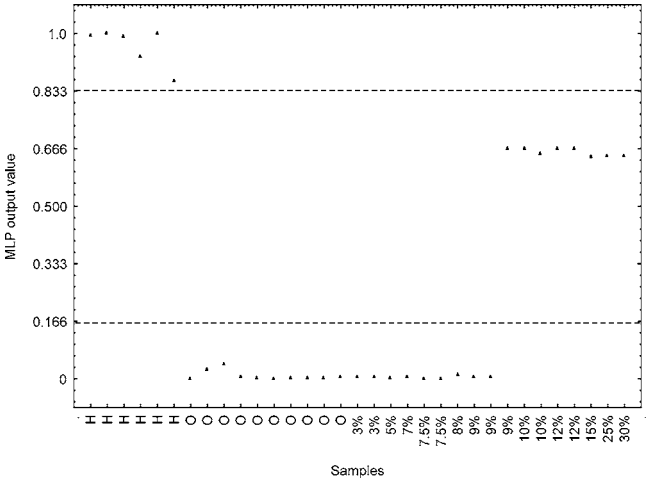

Figure 3. Classification of the pure refined olive oils, refined hazelnut oils, and blend samples by ANNs.

The result of the PCA unsupervised statistical procedure seemed to indicate that the information from these fluorescent zones might be used to detect the presence of refined hazelnut oils in refined olive oils but by means of more sophisticated mathematical procedures. An artificial neural network was applied to a matrix of 17 pure samples (eight for the training set, three RHZO and five ROO, and nine for the internal test set, three RHZO and six ROO) with the aim of distinguishing the samples of refined olive oils from the refined hazelnut oils.

A genetic algorithm was first applied to the data to choose the most discriminating wavelengths. The mathematical algorithm selected four wavelengths (inputs) from the initial set of 40 variables. The set of selected wavelengths (714, 718, 737, and 740) belonged to the emission spectra ($\lambda_{ex} = 350$ nm). Several ANN architectures were then designed with these four wavelengths. An ANN architecture 4:3:1 allowed classifying the samples with a 6% error.

To know the possibilities of the method to detect the adulteration of refined olive oils with refined hazelnut oils, several ANN architectures were designed using the same wavelengths selected previously. The matrix used for this aim was formed by a training set of 10 samples (three RHZO, four ROO, and three blends) and an internal test set of 15 samples (three RHZO, eight ROO, and four blends). The best designed model ($rms_{error} = 0.26$) corresponded to a multilayer perceptron (MLP) of three layers (4:4−4-3:1), defined by the following equation:

$$y = f(\sum_j w_j f(\sum_i w_{ij} x_i + a_i) + a_j)$$

where y is the output variable, x_i is the input variable, w_{ij} and w_j are the weights for the connections from the input layer to the hidden one and from the hidden layer to the output, respectively, and a_i and a_j are constants that operate as bias values in the network. The values of all these parameters are shown in **Table 1**. The output values for each node use the sigmoidal activation function (f):

$$f(x) = \frac{1}{1 + e^{-x}}$$

This network allowed classifying all the samples of the training set used in PCA analysis, correctly improving its results.

Furthermore, all the blends were classified between the genuine oils, so showing the ANN model might detect the presence of refined hazelnut oil in refined olive oil, although at percentages higher than 12.

A set of 13 samples was then used to test the model and also to determine its limit of detection tentatively. **Figure 3** shows, in two dimensions, the results from the classifications of the training and test samples in the predetermined clusters (refined hazelnut oils, refined olive oils, and a blend of these edible oils). All the blends were correctly classified with the exception of the samples with percentages of refined hazelnut oils lower than 9.

The information from this band of the spectrum seems to be useful to distinguish refined hazelnut oils from refined olive oils and potentially to detect the adulteration of refined olive oil with refined hazelnut oil.

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