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AUTHENTICATION OF OLIVE OIL: DETECTION OF ADULTERATION WITH HAZELNUT OIL BY DIRECT COUPLING OF HEADSPACE WITH MASS SPECTROMETRY, AND MULTIVARIATE REGRESSION TECHNIQUES

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FORO DE LA INDUSTRIA OLEÍCOLA Y LA CALIDAD

ABSTRACT
Due to its higher price, compared to other edible oils, control of adulteration of the olive oil is one of the main aspects to be evaluated in its quality control. Adulteration with hazelnut oil is one of the most difficult to be detected due to the great similarity existing in the composition of hazelnut and olive oils; both virgin olive oil and olive oil are subjected to that kind of adulteration. The main objective of this work was to develop an analytical method able to detect adulteration of virgin olive oils and olive oils with hazelnut oil by means of its analysis by a headspace autosampler directly coupled to a mass spectrometer used as detector. No chromatographic separation of the individual components of the samples exists, so, a broad single signal of the sample is obtained and employed for its characterization as a chemical fingerprint. Multivariate regression techniques (Partial Least Squares and Principal Components Analysis) were applied in this context to generate adequate regression models. Good values were obtained in both techniques for the parameters employed (SEP and PRESS) to evaluate its goodness. Once validated, the method was applied to the detection of such adulteration in commercial olive oil and virgin olive oil samples.

INTRODUCTION
Despite its high price, olive oil is highly appreciated by consumers due to its pleasant flavour and nutritional benefits, Thus, its adulteration with other cheaper oils can lead to large economical profits. In this way, authentication of virgin olive oils has become an interesting subject from both commercial and health perspectives [1]. Nowadays, one of the most concerning adulterations found in virgin olive oil (VOO) is carried out with hazelnut oil (*Corylus Avellana L.*), on account of their similar composition as regards triacylglycerol, total sterol and fatty acid profile, rich in mono- and polyunsaturated fatty acids, specially oleic and linoleic [2,3]. EU authorities have expressed concern about quality control of olive oil, specially its adulteration with hazelnut oil [4].

Filbertone [(E)-5-methylhept-2-en-4-one] has been identified as responsible for the flavour of hazelnut oil [5]. Its absence in VOO makes it ideal as a marker of adulteration. Analytical methods described for the detection of filbertone in VOO, include multimodal LC-GC separation [6], stable isotope dilution [7] and GC coupled to different sample preparation techniques [8,9]. Authentication and characterization of hazelnut oil, and its use as adulterant in VOO, has been reported by using NMR [10], mid-IR [11] and RAMAN [12] spectroscopies and multivariate statistical techniques.

Recently, the direct combination of headspace sampling and mass spectrometry (HS-MS) has been proposed as a competitive fast-response analytical tool for the characterization of edible oil samples, especially olive oil [13]. That system enables to obtain a chemical « fingerprint » of the sample by the direct analysis of the whole volatile fraction, what can be used for its authentication and to control its purity. Several applications of the system can be found in the literature concerning the use of HS-MS for olive oil authentication. Marcos-Lorenzo et al. [14] employed this system to develop a methodology to detect adulteration with suflower and olive-pomace olive oil, by means of linear discriminant analysis as chemometric approach for data treatment. Our research group has also proposed some methods to control VOO quality focused on classification of the three main types of olive oil (virgin olive oil, olive oil and olive-pomace olive oil) by using several pattern-recognition techniques [15], determination of hexane residues in olive-pomace olive oil with two multivariate regression techniques [16] and screening of volatile benzene-hydrocarbon residues in VOO [17].

The aim of the present work was to develop a new methodology to detect and quantify adulteration of virgin olive oil and olive oil with crude hazelnut oil through direct analysis of oil samples by headspace-mass spectrometry and various multivariate pattern-recognition and regression techniques for data treatment: Clusters Analysis (CA), Soft Independent Modelling of Class Analogy (SIMCA), Partial Least Squares (PLS) and Principal Components Regression (PCR).

EXPERIMENTAL SECTION

Apparatus. Oil analyses were performed with a ChemSensor 4440 (Agilent Technologies, Palo Alto, CA) system, which comprises two modules. The first one is a headspace autosampler for 10-mL headspace vials which included a robotic arm for direct introduction of the sample into the module; it also includes an oven for headspace generation and a six-port injection valve with a 3-mL loop. The second module is a quadrupole mass spectrometer used as detector which operates in full scan mode (*m/z* range 65-135) and electron impact ionization (70 eV). An inert transfer line directly connects both modules. Helium (5.0 grade, Air Liquide, Seville, Spain), regulated by a digital pressure and flow controller, was used for both pressurizing the vial and carrying the formed headspace, containing the sample volatiles, directly into the detector. The transfer line, source and quadruple temperatures were maintained at 130ºC, 200ºC and 150ºC, respectively. Statistical treatment of the data was done by means of Pirouette data evaluation software, provided by Infometrix Inc. on a Pentium II computer that also controlled the whole system.

Oil samples. Four different pure refined and virgin olive oil samples were provided by a Spanish oil manufacturer company. Four different crude hazelnut oil from Turkey were kindly supplied by the *Instituto de la Grasa* (Consejo Superior de Investigaciones Científicas, CSIC, Seville, Spain). Working oil samples were prepared on a daily basis by mixing appropriate amounts of crude hazelnut oil with refined or virgin olive oil, and stored in a cold dark place for samples not to go rancid before analysis.

Analytical Procedure. The schematic procedure is depicted in Fig. 1. Aliquots of 6.0 g of each type of oil were added to 10-mL headspace vials and placed into the autosampler. The robotic arm took each vial and placed it into the oven, where the sample was heated at 120ºC, for 30 min, in order to enrich the gaseous phase in the volatile compounds of the oil. Then, (Fig. 1A) the vial was pressurized for 12 s using an helium stream; opening the vent valve allows the 3-mL loop (125ºC) to be filled with the formed headspace. In a second step (Fig. 1 B), the injection valve is switched and a second helium stream transports the loop contents directly to the mass spectrometer, via the transfer line heated at 130ºC. As no chromatographic separation exists, the signal obtained from the detector is a single broad peak, characteristic of each analyzed sample, which can be considered as its chemical fingerprint for classification purposes. See Figure 1.

CHEMOMETRICS

Hazelnut oil has non toxic effects on consumer's health, but its lower price makes adulteration an economic fraud more than a risk for human health. The objective of the present work was to detect the adulteration of virgin and refined olive oil samples with crude hazelnut oil, as refined one contains no volatile components and its detection with the proposed instrumentation is not possible.

Analytical performance of the method. Partial Least Squares (PLS) and Principal Components Regression (PCR) techniques were employed to generate adequate regression models for both refined and virgin olive oil samples. These models were created by the analysis of oil samples adulterated with variable amounts of crude hazelnut oil (between 3-50% w/w), by using the procedure described under Experimental section. The model for refined olive oil was created by analyzing a mixture of virgin and refined olive oil (20% w/w, commonly marketed as olive oil). The goodness of each model was evaluated by means of four multivariate parameters, namely: Prediction Residual Error Sum of Squares (PRESS), Standard Error of Calibration (SEC), percentage of explained variance, and correlation coefficient (*r*). For each type of olive oil (refined or virgin), the training set was composed of a total of 140 objects (oil samples) and 71 variables (*m/z* ions from 65 to 135). Meancentering and autoscaling were assayed as pre-treatment techniques to improve the results obtained.

A preliminary evaluation of the data yielded by the instrument was performed by the CA dendrograms showed in Fig. 2. The best discrimination among the different adulteration percentages was obtained

with a previous meancentering of the data. As can be seen, refined oil samples (Fig. 2A) with adulteration up to 7% were grouped together with non-adulterated samples, and clearly separated from other oil samples of higher adulteration; however, in the case of virgin olive oil samples (Fig. 2B), there is no such clear discrimination of samples with an adulteration higher or lower than 7%, and up to 15% there is not so good separation from those non-adulterated oils. These results were confirmed by the "*interclass distance*" parameter provided by the application of SIMCA to each data set; such parameter, for oil samples with adulteration between 0 and 7%, offered a higher value in the case of refined olive oil (6.5) than in the case of virgin olive oil (3.2). It could be explained by the higher similarity existing in the composition of hazelnut and virgin olive oil, compared to refined olive oil, as it contains lower concentration of volatiles. See Figure 2

PLS and PCR regression models were created upon mean-centered data as yielded better analytical features than autoscaling or no pre-treatment. Figures of merit of the calibration graphs are summarized in Table 1. As can be seen, both models offered good values for the different multivariate parameters; PLS model provided lower SEC values than PCR, as well as higher percentages of explained variance from the original data. Results on virgin olive oil were slightly worse as the similarity between the volatile profiles of both samples makes discrimination more difficult. Plots for both refined and virgin olive oil of measured versus predicted adulteration percentage values are shown in Fig. 3. Again, the best results were obtained when the crude hazelnut oil was added to the refined oil matrix. See Figure 3 and Table 1

Validation of the proposed methods. A validation step of each regression model created was performed by analyzing several quality control samples of olive oil and virgin olive oil adulterated with crude hazelnut oil at eight different percentages: 7, 11, 14, 16, 19, 21, 23 and 36% w/w. The samples were all run in quintuplicate (n = 5), and direct calibration transfer algorithm was employed to minimize the signal instability that could lead to variations in sensitivity [18]. Mean predicted values by using each regression model are listed in Table 2. As can be seen, good agreement between the amounts added and those found were obtained in general. Standard errors of prediction (SEP) and PRESS parameters were employed to evaluate the goodness of the validation. Prediction on olive oils gave slightly better results than on virgin olive oils. For olive oil, better results were obtained with PLS model (1.3 and 78.0 for SEP and PRESS, respectively) than with PCR (1.4 and 93.5); on the other hand, for virgin olive oil, they were also better with PLS (1.3 and 81.3, for SEP and PRESS) than with PCR (1.4 and 89.9). The accuracy expressed by the cited results, together with the simplicity and high sample throughput of the proposed method, makes it adequate in the task of quality control of olive oil. See Table 2

Finally, PLS model was applied for the detection of adulteration of commercial oil samples with crude hazelnut oil purchased at various local markets. 30 samples of virgin olive oil and olive oil, were analyzed in quintuplicate (n = 5). None of the virgin olive oil samples offered positive results; however, as can be seen in Table 3, six olive oil samples yielded adulteration percentages between 23 and 45 % w/w. See Table 3

CONCLUSIONS

The great concern existing nowadays about oil authentication has lead to the need of the development of new methodologies capable of detecting fraud by adulteration, being hazelnut oil one of the most concerning adulterants. It has been proved that the proposed methods allow the correct detection and quantification of crude hazelnut oil in virgin and refined olive oils. The direct analysis of the oil samples by coupling headspace autosampling with mass spectrometry detection, offers the advantages of rapidity and reliability but also exist the disadvantages of the need of multivariate statistical techniques for data treatment, and the absence of discriminated information of the sample composition. Within a practical point of view, the minimum adulteration levels reached by the proposed methods (7 and 15% for refined and virgin olive oils, respectively) are low enough to permit the detection and quantification of adulterations in commercial olive oil.

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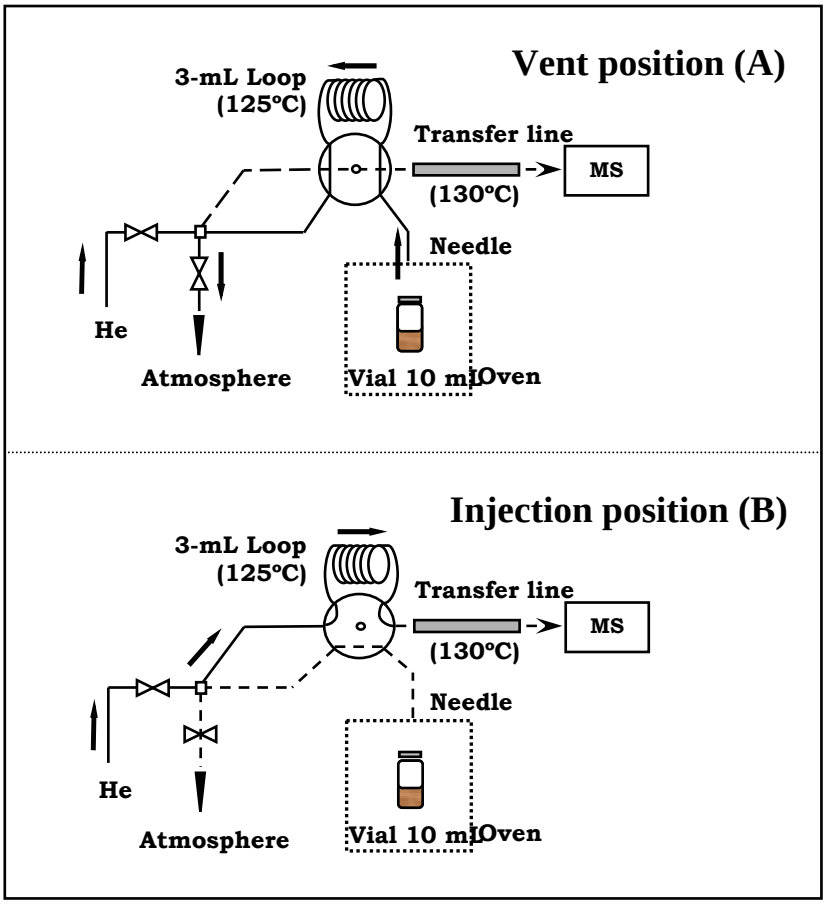

Fig. 1. Scheme of the headspace generation unit. MS, mass spectrometer.

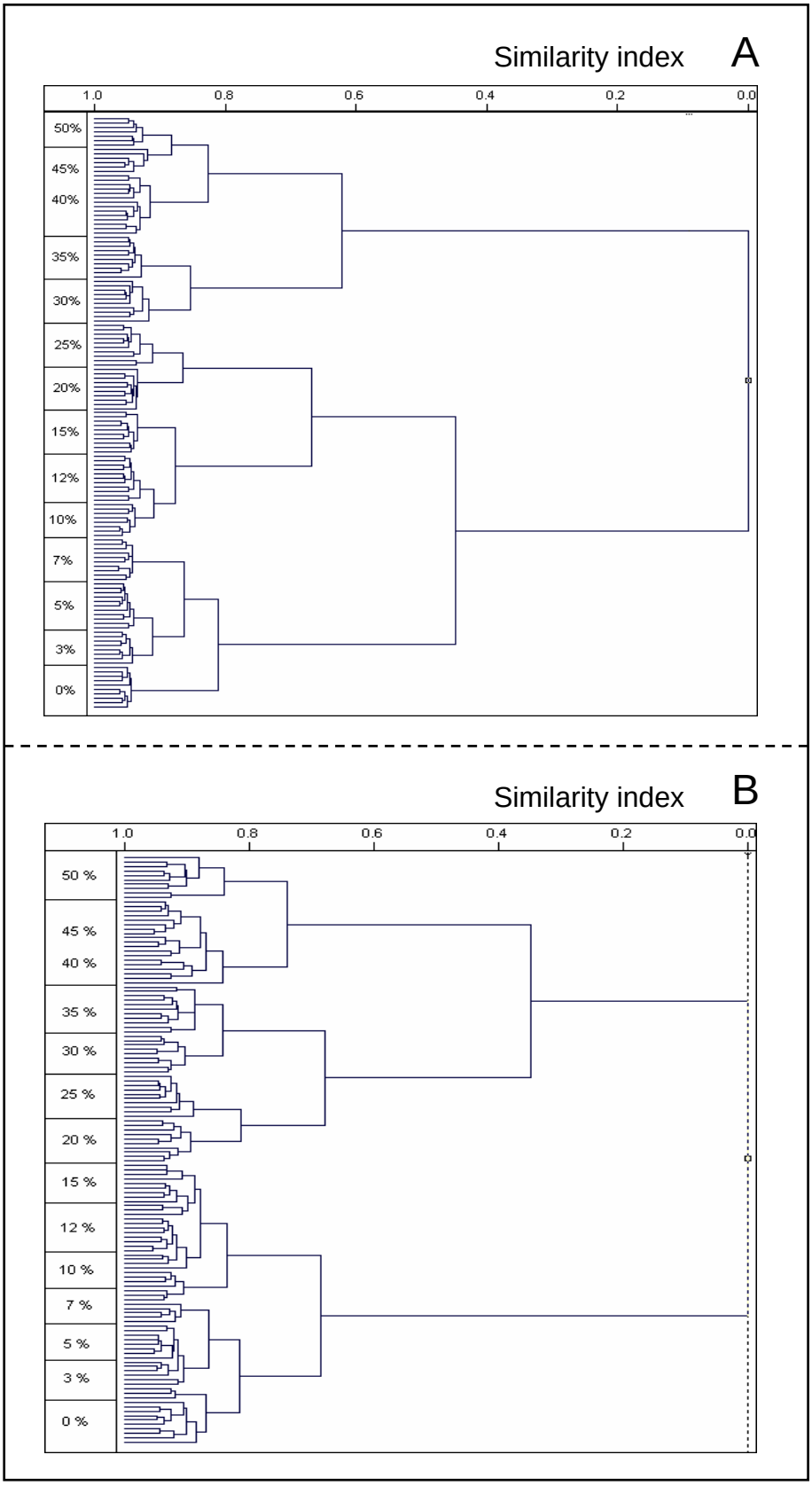

Fig. 2. Dendograms of the Clusters Analysis (CA) for both refined (A) and virgin (B) olive oil samples.

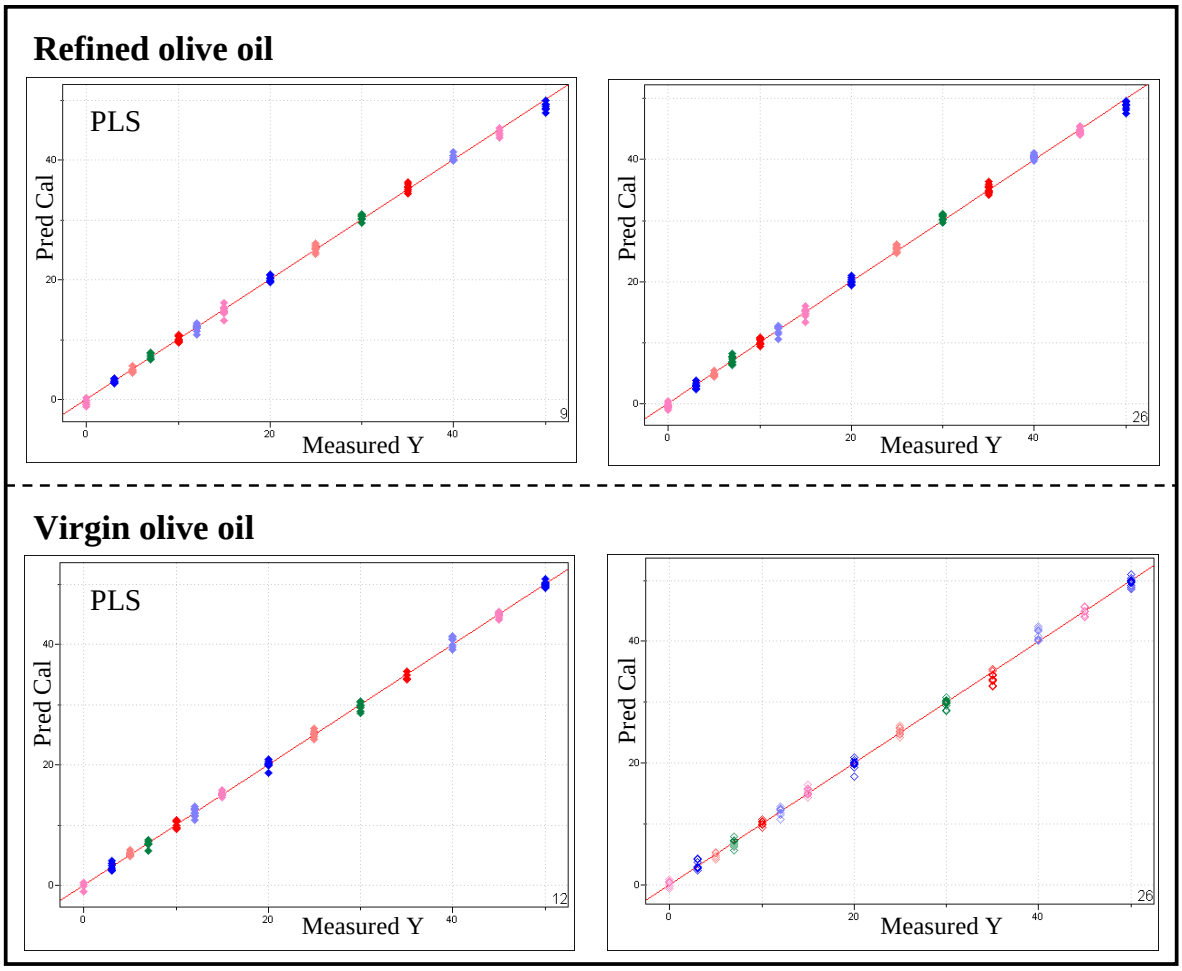

Fig. 3. Measured versus predicted adulteration plots for PLS and PCR models, by using both refined and virgin olive oil samples. At the right bottom corner of each plot, the number of factors employed is indicated.

Table 1. Figures of merit of the proposed PLS and PCR multivariate regression models

	PRESS [a]	SEC [b]	Explained variance (%)	Correlation coefficient (r)
Refined olive oil				
PLS	51.4	0.6	99.9	0.999
PCR	82.9	0.9	99.6	0.999
Virgin olive oil				
PLS	44.8	0.6	99.5	0.999
PCR	90.1	0.9	98.3	0.998

[a] Prediction residual error sum of squares

[b] Standard error of calibration

Table 2.Validation of both PLS and PCR methods for olive and virgin olive oil

Crude hazelnut oil added (%)	Refined olive oil [a,b]		Virgin olive oil[a]	
	PLS	PCR	PLS	PCR
7	7 ± 1	7 ± 1	7 ± 1	8 ± 1
11	11 ± 1	11 ± 1	11 ± 1	12 ± 1
14	13 ± 1	14 ± 1	13 ± 1	13 ± 1
16	16 ± 1	15 ± 1	16 ± 1	15 ± 1
19	20 ± 2	19 ± 2	20 ± 1	19 ± 2
21	21 ± 1	21 ± 1	22 ± 2	22 ± 2
23	23 ± 1	23 ± 1	24 ± 2	24 ± 2
36	37 ± 2	37 ± 2	35 ± 2	35 ± 2

[a] Crude hazelnut oil found (%)
[b] R [b] Refined olive oil blended with 20 % w/w of virgin olive oil

Table 3. Percentages of hazelnut oil found in real olive oil samples

Sample	Percentage of hazelnut oil found (%)	
	PLS[a]	PCR[b]
1	34 ± 2	34 ± 2
2	41 ± 3	40 ± 3
3	23 ± 1	25 ± 1
4	31 ± 2	32 ± 2
5	38 ± 2	39 ± 3
6	45 ± 3	45 ± 3

[a] Partial least squares regression

[b] Principal components regression