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Direct olive oil authentication: Detection of adulteration of olive oil with hazelnut oil by direct coupling of headspace and mass spectrometry, and multivariate regression techniques

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Abstract

Control of adulteration of olive oil, together with authentication and contamination, is one of the main aspects in the quality control of olive oil. Adulteration with hazelnut oil is one of the most difficult to detect due to the similar 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 (ChemSensor). As no chromatographic separation of the individual components of the samples exists, a global signal of the sample is obtained and employed for its characterization by means of chemometric techniques. Four different crude hazelnut oils from Turkey were employed for the development of the method. Multivariate regression techniques (partial least squares and principal components analysis) were applied to generate adequate regression models. Good values were obtained in both techniques for the parameters employed (standard errors of prediction (SEP) and prediction residual error sum of squares (PRESS)) to evaluate its goodness. With the proposed method, minimum adulteration levels of 7 and 15% can be detected in refined and virgin olive oils, respectively. Once validated, the method was applied to the detection of such adulteration in commercial olive oil and virgin olive oil samples.

Keywords: Hazelnut oil; Adulteration; Olive oil; Headspace; Mass spectrometry

1. Introduction

Virgin olive oil (VOO) is highly appreciated by consumers due to its pleasant flavour and nutritional benefits, despite its high price. 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]. Authentication covers many different aspects, including adulteration, mislabelling, characterization and misleading origin [2]. Several methods have been proposed for the monitoring of adulteration of virgin olive oils with other edible oils. In the last 10 years, technology and knowledge have undergone a great advance in the fight against adulteration; however, in the same way, knowledge of defrauders has also been increased, what enables them to prepare more sophisticated adulterations that make useless those methodologies proposed to detect them. Those oils normally added to VOO can be, either olive oils of lower quality (e.g. olive-pomace olive oil or virgin olive oil obtained by second centrifugation of the olives), or seed oils (e.g. corn, soybean, palm or sunflower oil, among others). Nowadays, one of the most concerning adulterations found in 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 [3,4]. EU authorities have expressed concern about quality control of olive oil, specially its adulteration with hazelnut oil [5].

Filbertone [(*E*)-5-methylhept-2-en-4-one] has been identified as the most important volatile compound in hazelnut

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oil, responsible for its flavour [2]; 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 [6,7], stable isotope dilution [8] and GC coupled to different sample preparation techniques [9–11]. Other methods use oil constituents as markers of adulteration, namely fatty acid profile [4,12], tocopherols [12,13], triglycerides [13,14], phospholipid fraction [14], vitamins [12,15] and sterols [12,13,16]. Fatty acid composition has also been isotopically characterized by carbon isotope analysis [17]. Authentication and characterization of hazelnut oil and its use as adulterant in VOO has been reported by using ^{13}C-NMR [18], ^{1}H-NMR [19], mid-IR [20] and Raman [20,21] spectroscopies and multivariate statistical techniques.

Recently, the direct combination of headspace sampling to mass spectrometry (HS–MS) has been proposed as a competitive fast-response analytical tool for the characterization of edible oil samples, especially olive oil [22]. That system enables to obtain a chemical "fingerprint" of the sample by the analysis of the whole volatile fraction, what can be used for its authentication and to detect the presence of any adulterant. 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. [23] developed a novel methodology to differentiate non-adulterated virgin olive oil from that adulterated with sunflower and olive-pomace olive oil by HS–MS and linear discriminant analysis as chemometric approach for data treatment. Our research group has also proposed some methods for VOO authentication by analysis of olive oil samples by headspace–mass spectrometry 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 [24], determination of hexane residues in olive-pomace olive oil with two multivariate regression techniques [25] and screening of volatile benzene–hydrocarbon residues in VOO [26].

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 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 modeling of class analogy (SIMCA), partial least squares (PLS) and principal components regression (PCR).

2. Experimental

2.1. Chemicals and standards

Four different pure refined and virgin olive oil samples were provided by a Spanish oil manufacturer company. Four different crude hazelnut oils 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.

2.2. Apparatus

Experiments were carried out by using a ChemSensor 4440 (Gerstel, Mülheim an der Ruhr, Germany) system which is composed of a Hewlett-Packard HP7694 headspace autosampler and a Hewlett-Packard HP5973 mass spectrometer. The autosampler consists of an oven to heat the samples and a carousel (with capacity for 44 vials) equipped with a robotic arm to place the vials inside the oven; also, the headspace sampler included a 3 ml loop connected to a six-port injection valve and an inert transfer line. Helium (5.0 grade purity, Air Liquide, Seville, Spain), regulated by a digital pressure and flow controller, was used for both pressurize the vial (18 psi) and transfer of the loop content directly to the detector (4 psi). Every tubing of the system has been passivated to ensure the inertness. The detector, an HP5973 quadrupole mass spectrometer, working under electron impact ionization mode (70 eV), was operated in full scan mode, with a scanned mass range from *m/z* 65 to 130. The transfer line, source and quadrupole temperatures were maintained at 130, 230 and 150 °C, respectively. Total ion current chromatograms were acquired and processed using G1701BA Standalone Data Analysis software (Agilent Technologies) on a Pentium II computer that also controlled the whole system.

The 10 ml glass flat-bottom vials for headspace analysis with 20 mm polytetrafluoroethylene/silicone septa caps and a crimped aluminum closure (Supelco, Madrid, Spain) were also employed. Vials and septa were heated at 100 and 70 °C, respectively, overnight, prior to use.

2.3. Analytical procedure

Aliquots of 6.0 g of commercial olive oil sample, or refined oil standard containing variable amounts of crude hazelnut oil, were placed into a 10 ml headspace vial and tightly sealed. Once placed in the headspace carousel, the robotic arm transferred them sequentially into the oven, where they were heated at 120 °C for 30 min; during this time, the volatile compounds of the sample enriched the headspace of the vial. By means of a needle connected to the injection valve (IV) set in vent position (see Fig. 1), a helium stream entered the headspace of the sample and pressurized the sample vial for 12 s; then, by opening the vent valve for 9 s, and thanks to the pressure difference inside the vial and at the end of the tubing (atmospheric pressure), the headspace fraction containing volatile fraction residues was released from the vial and filled the 3 ml loop of the IV, previously heated at 125 °C. In a second step, IV was switched to injection position (see Fig. 1) and a second helium stream carried the loop content directly to the mass spectrometer via the transfer line, heated at 130 °C.

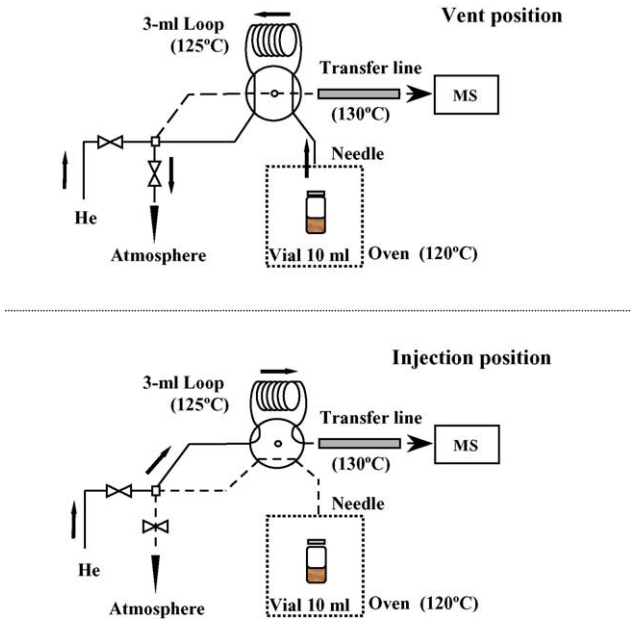

Fig. 1. Scheme of the headspace generation unit. MS, mass spectrometer.

2.4. Multivariate analysis

The global response obtained from the detector is a single, broad signal that corresponded to the whole volatile fraction of the oil sample, as no chromatographic separation exists in the system. This signal can be assumed as the chemical fingerprint of the sample, and therefore can be used for its characterization by multivariate techniques. Firstly, the different percentages of adulteration present in the oil samples were distinguished by an exploratory technique as it is CA; the quantification of the degree of separation achieved among the different percentages of adulteration was made by using the "interclass distance" parameter from the technique SIMCA. Finally, PLS and PCR were employed as multivariate regression techniques to estimate the percentage of adulteration of olive oil by hazelnut oil. Both techniques were based on multiple linear regression (MLR), where the response variable is dependent on some independent variables (predictor variables), and principal components analysis (PCA), where new and independent variables are obtained by linear combination of the original ones [27]. The regression algorithm of PLS searches for the direction adequate to explain the maximum variance among the variables, but weighting the variables upon their higher or lower correlation with the response variable. PCR firstly applies a PCA algorithm to the original data, raw or preprocessed, and then generates a MLR calibration model from the obtained scores; it just tries to explain the maximum variance among variables, but without any relation with the response variable.

All chemometric analyses were performed by means of the statistical software 'Pirouette: Multivariate Data Analysis' (v. 3.01), developed by Infometrix (Woodinville, WA, USA).

3. Results and discussion

Adulteration with crude hazelnut oil can not only be performed on virgin olive oil, but also on refined oil, which is later blended with virgin olive oil to form the so-called *olive oil*. For this reason, the adulteration on both types of matrices was studied by using a ChemSensor. Hazelnut oil has not 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 the adulteration with crude hazelnut oil as refined one contains no volatile components and its detection with the proposed instrumentation is not possible. For this reason, the described method is only useful for the detection of adulteration with crude hazelnut oil.

3.1. Optimization of the ChemSensor variables

To achieve the best separation of the different percentages of adulteration, and so, higher analytical properties of the regression method, the chemical and instrumental variables of the system involved in the headspace generation step, were optimized. With this aim, samples of non-adulterated virgin olive oil and adulterated with 20% (w/w) of crude hazelnut oil were analyzed by the proposed system. Optimal values of each parameter were chosen according to the degree of separation achieved among adulterated and non-adulterated virgin olive oil; such separation was estimated by means of CA (visual evaluation) and SIMCA ("interclass distance" parameter).

Sample amount was the first chemical variable optimized; its importance on the analytical signal derives from the marked influence it has on the volatiles concentration in the headspace of the vial; the amount of oil was varied from 4.0 to 7.0 g and analyzed by the ChemSensor according to the previously described procedure. Slightly better separation between adulterated and non-adulterated virgin olive oil samples was achieved by using 7.0 g of sample, whereas lower dispersion on a scores plot from SIMCA model was obtained when using 6.0 g of sample, and thus, it was selected as optimum. The addition of a chemical modifier to facilitate the enrichment of the headspace with the volatile compounds was also evaluated. Aliquots of 600 μl of ethyl acetate, isopropanol, ethanol and hexane were assayed, and the results were compared with those obtained in absence of the organic solvent. With the addition of organic solvent, distinction among classes was not improved; so, no chemical modifier was added to the oil samples.

The instrumental parameters that markedly affected the analytical performance of the method were the oven temperature and the equilibration time of the vial. Oven temperature was optimized between 70 and 120 °C; the highest degree of separation between classes (higher value of "interclass distance") was obtained at 120 °C; higher temperatures were not assayed to avoid oil degradation. Heating time was also optimized between 20 and 45 min; separation between non-

adulterated and adulterated oil samples (containing 20% of crude hazelnut oil) did not increased over 30 min. So, 120 °C and 30 min were selected as optimum values for oven temperature and heating time, respectively. The injection of the headspace generated in the vial, enriched with the volatile compounds of the sample, is a process composed by two steps: vial pressurization and headspace venting to fill the 3 ml loop of the injection valve. The time employed in both steps was also optimized (between 6 and 24 s) to obtain the maximum separation among each percentage of adulteration. Optimum values of pressurization and venting of 12 and 9 s were selected as they offered the highest values of "interclass distance".

3.2. Analytical performance of the method

Adequate regression models were created by using PLS and PCR techniques for both refined and virgin olive oil samples adulterated with variable amount of crude hazelnut oil (between 3 and 50% (w/w)), analyzed by using the procedure described under Section 2. 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 four multivariate parameters, namely prediction residual error sum of squares (PRESS), standard error of calibration (SEC), percentage of explained variance, and correlation coefficient (r). Mean-centering and autoscaling were assayed as pre-treatment techniques to improve the results obtained. 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).

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 mean-centering of the data. As can be seen, refined oil samples (Fig. 2A) of 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 of adulteration of 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.

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.

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.

3.3. 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 [28]. 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

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

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] Hazelnut oil found (%).
[b] Refined olive oil blended with 20% (w/w) of virgin olive oil.

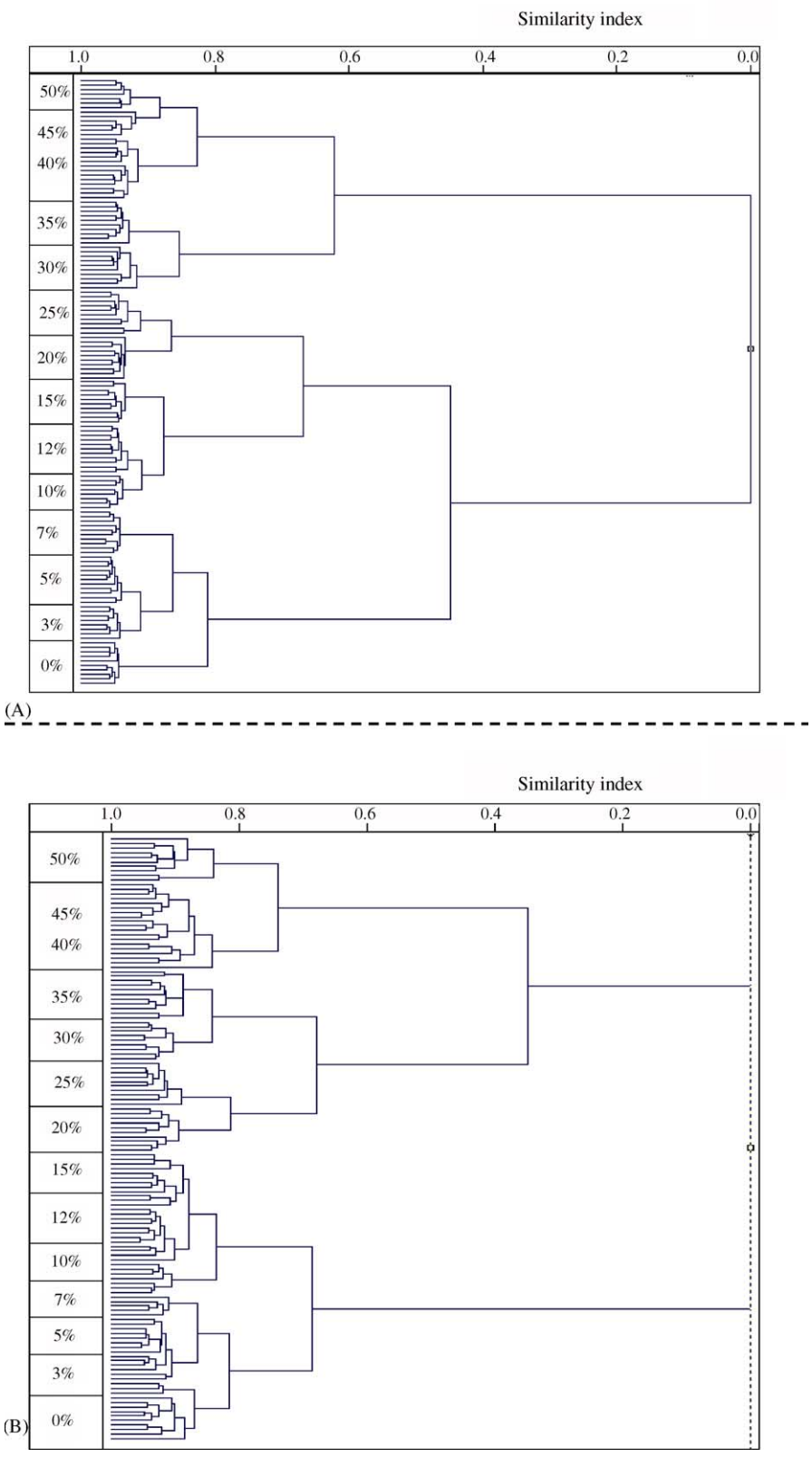

Fig. 2. Dendograms of the clusters analysis (CA) for both refined (A) and virgin (B) olive oil samples.

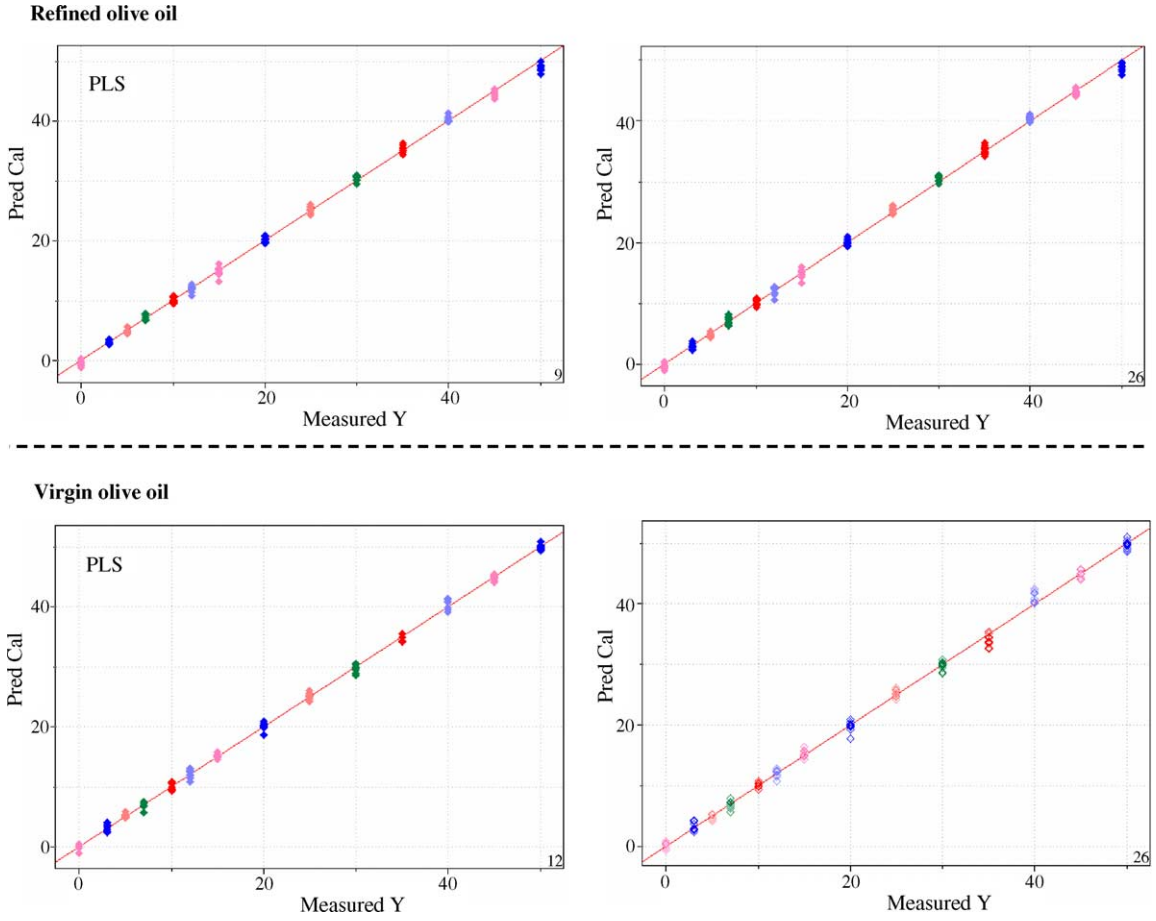

Fig. 3. Measured versus predicted adulteration plots for PLS and PCR models, by using both refined and virgin olive oil samples. At the right bottom corner of each plot, the number of factors employed is indicated.

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.

Finally, PLS model was applied for the detection of adulteration of commercial oil samples with hazelnut oil purchased at various local markets. Thirty 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).

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.

4. Conclusions

The great concern existing nowadays about oil authentication has lead to the need of the development of new methodologies capable to detect 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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