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Journal of Chromatography A, 945 (2002) 221–230

JOURNAL OF CHROMATOGRAPHY A

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Detection of adulterants in olive oil by headspace–mass spectrometry

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Received 3 August 2001; received in revised form 10 October 2001; accepted 9 November 2001

Abstract

In the present work, we propose the use of direct coupling of a headspace sampler to a mass spectrometer for the detection of adulterants in olive oil. Samples of olive oils were mixed with different proportions of sunflower oil and olive-pomace oil, respectively, and patterns of the volatile compounds in the original and mixed samples were generated. Application of the linear discriminant analysis technique to the data from the signals was sufficient to differentiate the adulterated from the non-adulterated oils and to discriminate the type of adulteration. The results obtained revealed 100% success in classification and close to 100% in prediction. The main advantages of the proposed methodology are the speed of analysis (since no prior sample preparation steps are required), low cost, and the simplicity of the measuring process.

Keywords: Olive oil; Linear discriminant analysis; Headspace analysis; Adulteration; Chemometrics

1. Introduction

The determination of food authenticity and the detection of adulterants are of increasing importance in the food industry, especially in products of high commercial value. Partial substitution of such products by cheaper ingredients may lead to significant economic benefits. An example is the case of olive oil, for which the International Olive Oil Council (IOOC) has established criteria [1] for its categorisation into various grades, namely virgin olive oil, refined olive oil and pure olive oil. The oil with the best quality is virgin olive oil, appreciated mainly for its taste and smell, since it maintains its ''fruity'' characteristics because it is free of artificial processing except for its mechanical extraction. Refined olive oil is obtained from virgin olive oil using refining methods that do not lead to alterations in the initial glyceridic structure, whereas pure olive oil is formed of a blend of the former two types. In addition, olive-pomace oil is defined as that obtained by extracting olive-pomace with authorised solvents.

Owing to its higher price, fraudulent practices have involved the adulteration of olive oil with small amounts of other seed oils (sunflower, maize, soy, etc.) or olive-pomace oil. However, besides the economic fraud, this may sometimes have severe health implications for consumers, such as happened

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0021-9673/02/$ – see front matter
PII: S0021-9673(01)01502-3

in the case of the Spanish toxic oil syndrome (TOS) [2–4]. Therefore, continuous vigilance is required to control the adulteration of olive oil products.

Although different techniques have been proposed for the characterisation of oils and for the detection of adulterants, none of them has been universally accepted for the determination of the authenticity of the different types of vegetable oils [5]. The technique most widely used is chromatography [6–12], with which it is possible to analyse the composition of the natural constituents of the oil and possible adulterants. Another approach employs spectroscopic data related to the composition of the oils, applying multivariate statistical techniques to interpret the data thus obtained [13–18]. Another reported technique for oil authentication is stable carbon isotope ratio analysis [19,20].

Most of these techniques require too much time to be used routinely in the food industry. Recent years have seen the advent of electronic olfactometry [21–23], which combines the responses of a set of chemical sensors, with partial specificity in the measurement of volatile components, with pattern recognition techniques for data interpretation. This method requires a much simpler sample preparation, leading to a decrease in the time and cost of analysis per sample [24,25]. One of the possible methodologies consists of coupling a headspace sampler to a mass spectrometer (HS–MS). These devices are able to recognise complex mixtures of volatiles and respond to all volatile compounds, without the problems associated with gas sensors [26]. This new methodology is cheaper and faster than chromatographic techniques as no prior sample preparation steps are required and it does not need organic solvents. However, very little information about the chemical volatiles species forming the generated headspace can be obtained.

Although with this approach it is not necessary to separate the individual components present in the sample, the basis of signal generation lies in two separation steps: the gas–liquid separation that takes place in the headspace sampler and the separation of the ionic fragments at the quadrupole. Here we propose the use of the HS–MS technique for the detection of adulterants in olive oil. Samples of pure oils and oils adulterated with different proportions of sunflower and olive-pomace oils were used.

2. Experimental

2.1. Samples

In the present work, 121 samples of olive oil, non-adulterated and adulterated with different proportions of sunflower and olive-pomace oils were analysed. The samples were divided as follows: 32 samples of commercial olive oil (virgin and refined), obtained from different suppliers, and 89 mixtures of these oils with sunflower and olive-pomace oils. The adulterated samples were prepared at the laboratory, using five different virgin olive oils, mixed with five commercial sunflower oils and five commercial olive-pomace oils at different levels of adulteration: 5, 10, 20, 40 and 60% by weight. The samples were stored in hermetically sealed topaz glass flasks.

2.2. Apparatus

The apparatus used to measure the patterns of volatiles of the oil samples was a Chemical Sensor HP4440 A system from Agilent Technology (Waldbronn, Germany). This comprises a headspace sampler (HP 7694) with a tray for 44 consecutive samples, an oven, where the headspace is generated, and a sampling system comprising a stainless steel needle, a 316-SS six-port valve with a nickel loop and two solenoid valves (for pressurisation and venting). The headspace sampler is coupled to a quadrupole mass spectrometer (HP 440, based on the HP 5973 MSD) by a transfer line. Data collection was performed with Pirouette 2.6 software from Infometrix on a Hewlett-Packard PC computer that also controlled the MS detector parameters.

2.3. Procedure

For the analysis of volatile compounds, aliquots of 5.0 ml of each oil sample were placed in 10-ml vials sealed hermetically with a silicone septum and a cap. The experimental conditions of the headspace sampler were as follows: oven temperature, 120 °C; loop temperature, 130 °C; transfer line temperature, 135 °C; headspace generation time, 30 min. In these conditions oxidation processes are likely to occur during the formation of the headspace. The mass range measured in the mass spectrometer was 35–

100. The carrier gas was helium, at an approximate flow-rate of 20 ml/min.

2.4. Mathematical treatment

Chemometric analysis of the data was accomplished using the PARVUS statistical package (Genova, Italy) [27], which was used to perform the linear discriminant analysis (LDA). Taking into account that the number of variables measured was relatively high, we first conducted a selection process to reduce the number of data and achieve the best set of discriminant variables. For this process we used the StepLDA program from PARVUS, using the selection criterion of the Mahalanobis distance.

The study was performed for three classification tasks: (1) non-adulterated olive oil/adulterated olive oil, (2) olive oil adulterated with sunflower oil/olive oil adulterated with olive-pomace oil and (3) non-adulterated olive oil/olive oil adulterated with sunflower oil/olive oil adulterated with olive-pomace oil. For each of these three studies, two steps were implemented. The first consisted of the generation of a classification model using all the samples as a known group; this was called the training set. The second step was cross-validation of the model to assess its use in the prediction of new samples. This consisted of dividing the total samples into groups, called cancellation groups, and using all the groups less one to generate the classification model and the remaining one for prediction. In this step we performed the study for three, four and five cancellation groups.

3. Results and discussion

The signals employed in the mathematical treatment of the data are the sum of the intensities of all the ions during the period of data acquisition from the mass spectrometer. Fig. 1 shows the signals corresponding to a sample of non-adulterated olive oil and to samples adulterated with sunflower oil and olive-pomace oil. The scale of the range of 50–100 mass/charge ratios has been amplified for better visualisation of the differences.

The matrix of the original data comprised 121 objects (rows), corresponding to the oil samples and

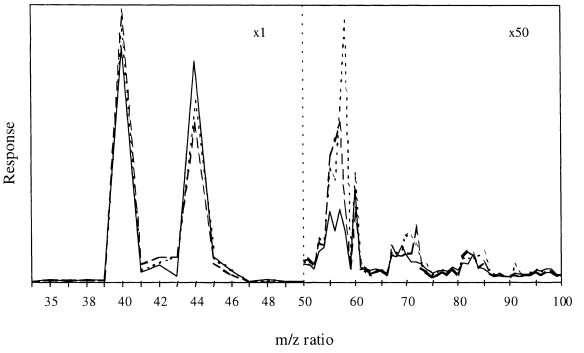

Fig. 1. Signals obtained for the three types of oil: non-adulterated olive oil (continuous line); olive oil adulterated with sunflower oil (dashed line); and olive oil adulterated with olive-pomace oil (dotted line).

65 variables (columns), corresponding to the 35–100 mass charge ratios obtained from the mass spectrometer.

Since no separation of the compounds is generated in the headspace, a single ion may arise from a large number of different compounds. However, with the methodology employed it is not necessary to establish relationships with the chemical composition of the samples since chemometric treatment of the signal profiles is carried out for those samples.

3.1. Classification of non-adulterated olive oil/adulterated olive oil

The 121 samples were assigned to each of the two categories (32 samples of non-adulterated olive oil and 89 samples of adulterated olive oil) and the process of variable selection was implemented. Table 1 shows the results obtained in the classification and prediction steps upon applying the LDA procedure, with cross-validation, for different numbers of variables. It may be seen that the model is consistent, since the hit rates both in classification and prediction were similar. It may also be seen that the percentages of success were improved upon increasing the number of variables selected, 100% being reached in classification from 30 variables in all cases. However, in prediction, the hit rate—which reached 99%—decreased slightly for a high number of variables, probably due to the introduction of

Table 1
Results of cross-validation for linear discriminant analysis applied to classification in two classes: non-adulterated olive oil/adulterated olive oil

Hit rate (%)						
Number of variables	Three cancellation groups		Four cancellation groups		Five cancellation groups	
	Classification	Prediction	Classification	Prediction	Classification	Prediction
5	91	89	90	88	91	89
10	98	95	97	93	96	97
15	99	98	99	96	99	98
20	100	97	99	96	100	97
25	99	99	99	98	99	97
30	100	96	100	98	100	96
35	100	98	100	98	100	97
40	100	95	100	99	100	97
45	100	95	100	98	100	96
50	100	94	100	97	100	96

non-useful information for the resolution of the problem in hand.

Fig. 2 shows the plot of the values of the discriminant scores obtained in the classification step and in one of the prediction stages, with four cancellation groups (the prediction set is represented by filled-in symbols). The results for a small number of variables (Fig. 2a) and for the optimum number (Fig. 2b) are shown. The optimum number of variables is the one for which the highest hit rate is obtained, both in classification and prediction. With four cancellation groups, this number was 40. When the number of variables was low (five variables) both in classification and in prediction, it was not possible to differentiate the two classes of samples. In contrast, for the optimum number it was possible to completely separate the samples of adulterated and non-adulterated olive oils.

The five most discriminant variables, according to the order in which they were selected by the feature selection step, were seen to be the intensities at m/z 85, 35, 99, 40 and 49. Fig. 3 shows the box plots for variables 85 and 35; some differences can be appreciated between the two classes. Thus, the class of adulterated olive oils (abscissa value 2) had higher values for variable 85 than the class of non-adulterated olive oils (abscissa value 1), whereas in the case of variable 35 the situation was the opposite, although with fewer differences between the classes.

3.2. Classification of olive oil adulterated with sunflower oil/olive oil adulterated with olive-pomace oil

In this part of the study only adulterated samples of olive oil were used and their differentiation into two classes was addressed. The 89 samples of adulterated olive oil were assigned to each of the two categories (51 samples adulterated with sunflower oil and 38 samples adulterated with olive-pomace oil) and the process of variable selection was implemented. Table 2 shows the results obtained in the classification and prediction steps upon applying the LDA procedure, carrying out the cross-validation, for the differentiation of the two classes of oil. As in the previous task, the hit rate in classification and in prediction was similar, indicating the consistency of the model. In this case the hit percentages, both in classification and prediction, were fairly high, even when the number of variables selected was low. In classification, 100% success was obtained as from the selection of only 20 variables. In prediction, the results, which achieved 99% success, were worse when the number of variables selected was 50, with which percentages of 81 and 90% were obtained for three and five cancellation groups, respectively.

Fig. 4 shows the plots of the values of the discriminant scores obtained in the classification and prediction steps, with four cancellation groups, the

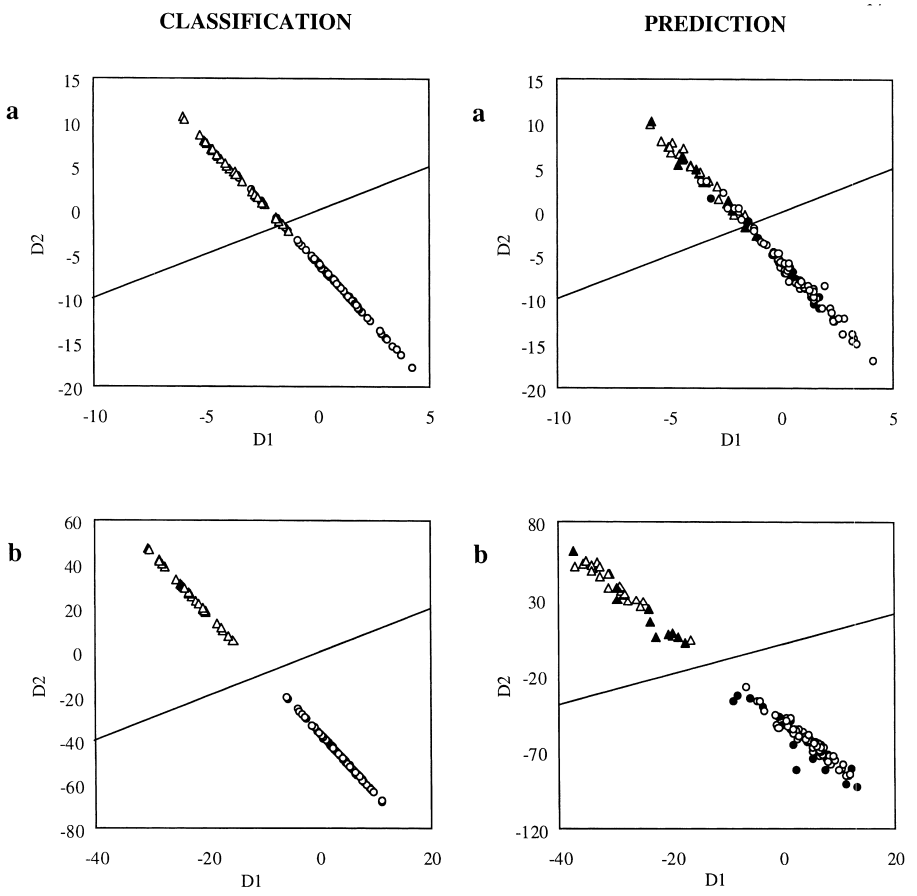

Fig. 2. Plots of the discriminant scores for the classification and prediction of oil samples belonging to the classes: (△) non-adulterated olive oil; (○) adulterated olive oil. (a) Five variables; (b) 40 variables.

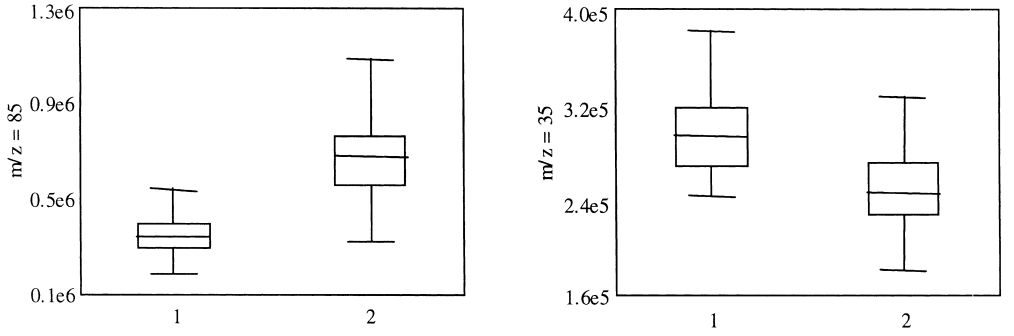

Fig. 3. Box plots for the discriminant variables 85 and 35. Abscissa values: (1) non-adulterated olive oil; (2) adulterated olive oil.

Table 2
Results of cross-validation for linear discriminant analysis applied to classification in two classes: olive oil adulterated with sunflower oil/olive oil adulterated with olive-pomace oil

Hit rate (%)						
Number of variables	Three cancellation groups		Four cancellation groups		Five cancellation groups	
	Classification	Prediction	Classification	Prediction	Classification	Prediction
5	97	97	97	98	97	97
10	99	96	99	94	98	94
15	99	97	99	96	99	96
20	100	97	100	97	100	96
25	100	97	100	98	100	97
30	100	98	100	99	100	98
35	100	98	100	98	100	96
40	100	97	100	98	100	96
45	100	96	100	99	100	96
50	100	81	100	96	100	90

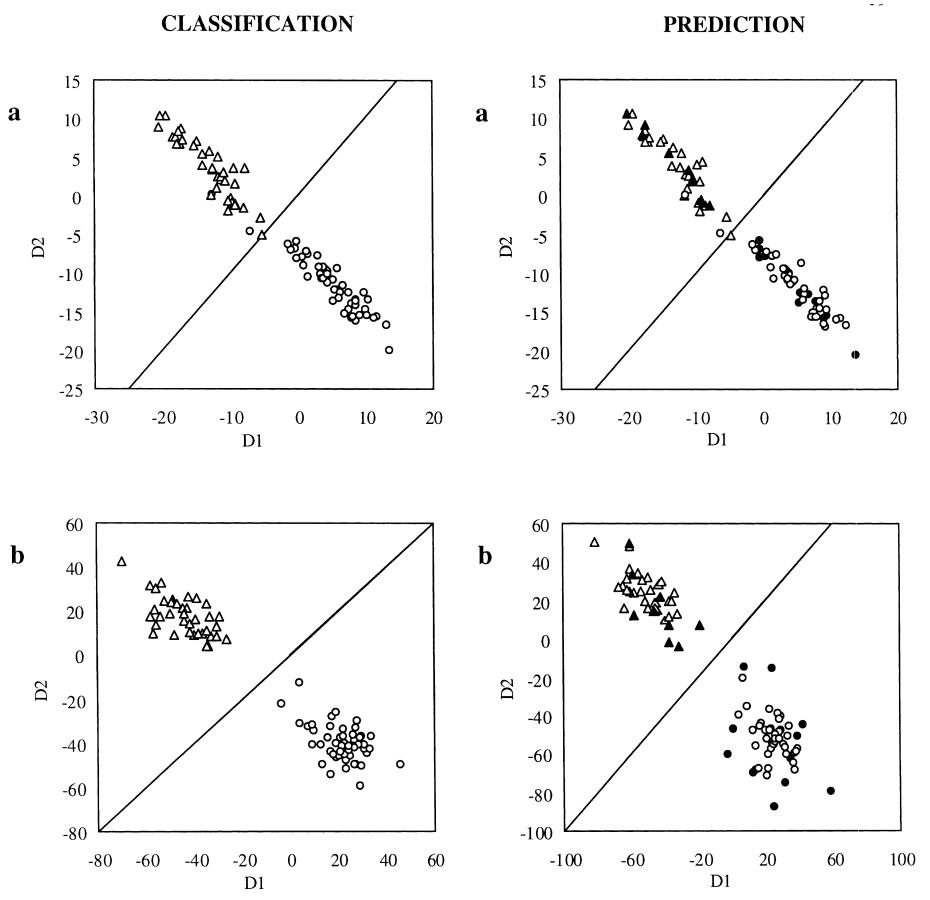

Fig. 4. Plots of the discriminant scores for the classification and prediction of oil samples belonging to the classes: (○) olive oil adulterated with sunflower oil; (△) olive oil adulterated with olive-pomace oil. (a) Five variables; (b) 30 variables.

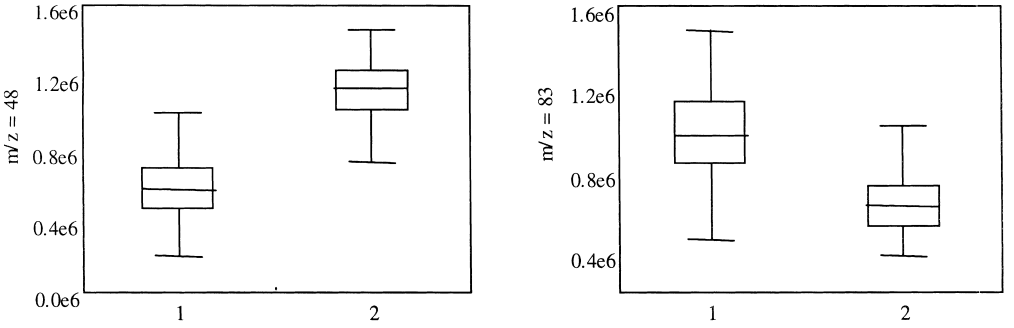

Fig. 5. Box plots for the discriminant variables 48 and 83. Abscissa values: (1) olive oil adulterated with sunflower oil; (2) olive oil adulterated with olive-pomace oil.

prediction group samples being represented by filled-in symbols. The plots show the results obtained with a small number of variables (Fig. 4a) and with the optimum number (Fig. 4b), in this case 30 variables. For a small number of variables, the samples corresponding to the two classes, although more separated than in the previous case, were not completely differentiated. By contrast, when the optimum number of variables was used it was observed that the spaces in which each of the classes are defined were completely separated. Fig. 5 shows the box plots for intensities at m/z 48 and 83, included in the first five most discriminant variables (48, 86, 83, 71 and 79) for this classification task. In this figure certain differences can be seen between the two classes.

3.3. Classification of non-adulterated olive oil/olive oil adulterated with sunflower oil/olive oil adulterated with olive-pomace oil

Finally, the classification task was addressed to the direct differentiation of all the samples in their three possible categories. The variable selection process was performed with the samples assigned to each of the three categories (32 samples of non-adulterated olive oil, 51 samples of olive oil adulterated with sunflower oil and 38 samples of olive oil adulterated with olive-pomace oil) and the LDA procedure was applied. Table 3 shows the results obtained in the classification and prediction steps, carrying out the cross-validation. As in the previous studies, it may

Table 3
Results of cross-validation for linear discriminant analysis classification in three classes: non-adulterated olive oil/olive oil adulterated with sunflower oil/olive oil adulterated with olive-pomace oil

Hit rate (%)						
Number of variables	Three cancellation groups		Four cancellation groups		Five cancellation groups	
	Classification	Prediction	Classification	Prediction	Classification	Prediction
5	92	90	90	88	93	90
10	96	94	97	93	96	93
15	98	93	98	95	97	93
20	99	94	99	93	98	93
25	99	93	99	95	99	95
30	100	95	99	97	99	96
35	100	96	100	98	100	98
40	100	98	100	98	100	98
45	100	94	100	96	100	96
50	100	92	100	94	100	93

be seen that the model is consistent, because the hit rate in classification and in prediction was similar. In classification, the percentage of hits increased as the number of variables selected was increased, reaching 100% as from 35 variables. In prediction, the hit rate increased with the increase in variables, up to the optimum number of variables, in this case 40, with which a success rate of 98% was obtained. For a higher number of variables, the prediction results became similar to those obtained with a small number of variables, in one case due to the lack of sufficient information to obtain a suitable differentiation of the samples and, in the other because of the introduction of irrelevant information.

Fig. 6 shows the plots of the values of the differences among discriminant scores obtained in the classification and prediction steps with four cancellation groups. In all cases, the samples not included in the training group are represented with filled-in symbols. Fig. 6a corresponds to a small number of variables selected (five variables), and it may be seen that it was not possible to differentiate the three types of oil and, on using some of the samples for prediction, some were assigned to classes to which they did not correspond. However, when 35 variables were used (Fig. 6b) the spaces in which each of the classes was defined were completely separated. In prediction, all the samples were correctly assigned to the class to which they belonged.

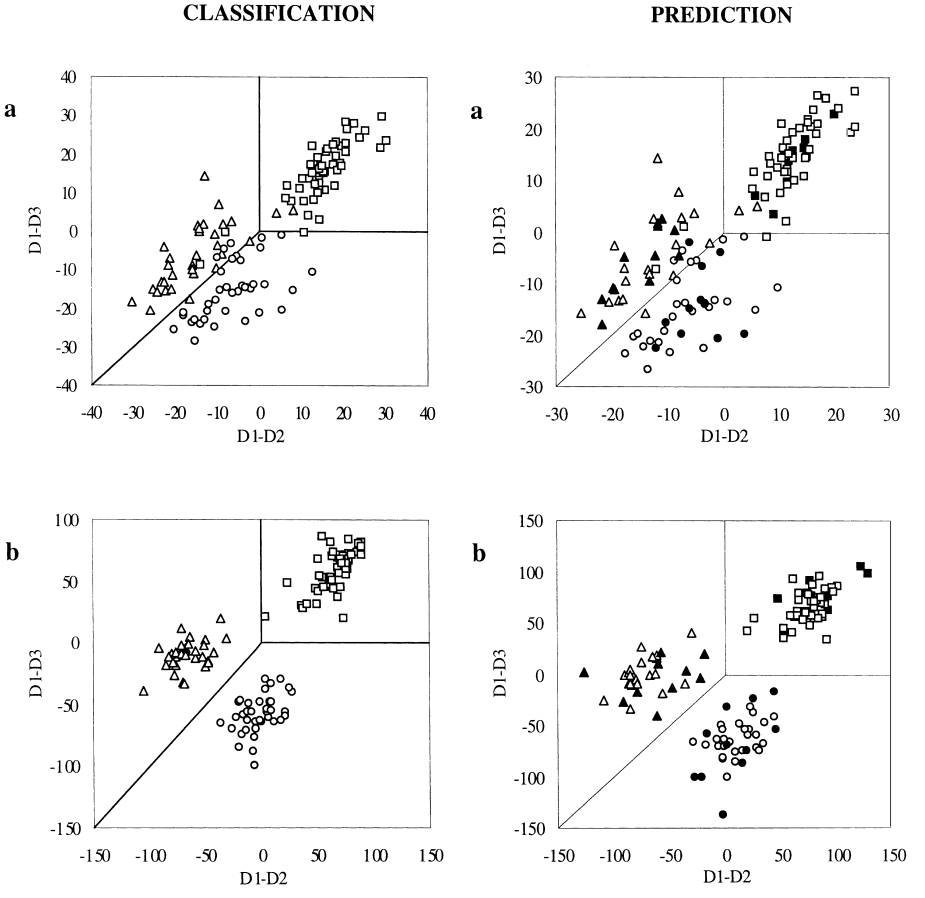

Fig. 6. Plots of differences among the discriminant scores for the classification and prediction of oil samples belonging to the classes: (□) non-adulterated olive oil; (△) olive oil adulterated with sunflower oil; (○) olive oil adulterated with olive-pomace oil. (a) Five variables; (b) 40 variables.

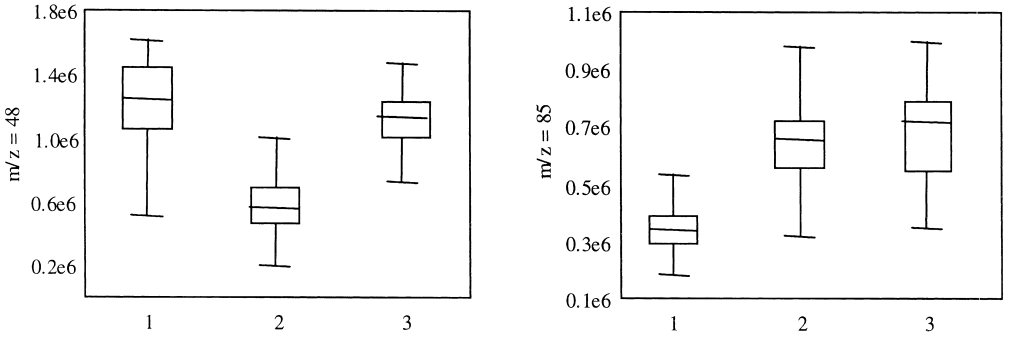

Fig. 7. Box plots for the discriminant variables 48 and 85. Abscissa values: (1) non-adulterated olive oil; (2) olive oil adulterated with sunflower olive oil; (3) olive oil adulterated with olive-pomace oil.

For this classification in three classes, the five most discriminant variables were the intensities at m/z 48, 85, 83, 37 and 71, four of which coincided with those selected (with the StepLDA program) when separation was conducted in two successive steps. The box plots for variables 48 and 85 and three classes are shown in Fig. 7. It is possible to observe a certain difference between class 2 (olive oil adulterated with sunflower oil) with respect to the other two classes as regards the values of variable 48. In contrast, variable 85 generated a certain separation of class 1 (non-adulterated olive oil) with respect to classes 2 and 3.

4. Conclusions

The use of a headspace sampler coupled to a mass spectrometer was successfully applied to the detection of adulterants in olive oil. Treatment of the signals generated by the LDA chemometric technique allowed discrimination between non-adulterated olive oil and adulterated olive oil samples, as well as distinguishing the type of adulteration. It is important to carry out a previous process of variable selection, using statistical criteria, in order to reduce the number of data and obtain the best set of discriminant variables. The use of all the data leads to a decline in the quality of the results in prediction.

The results obtained were very good for the three classification tasks addressed. However, they were slightly better (99% as compared with 98% in prediction) when the process was carried out in two steps, first dividing the non-adulterated olive oil samples from the adulterated ones and then differentiating the latter on the basis of the adulterant employed.

This new HS–MS methodology offers a number of advantages over other analytical techniques used in the authentication of edible oils, above all its simplicity and speed in the sample preparation step. Therefore, it might be used as a screening method. Besides the good results obtained, the method could be extended to other adulterants, such as hazelnut oil and lampante oil.

Acknowledgements

This work was supported by the DGICYT (Project PB97-1322) and the Consejería de Cultura y Turismo of the Junta de Castilla y León y la Unión Europea (Fondo Social Europeo. Project SA19/99). We also thank Professor Forina for kindly providing us with the Fortran version of PARVUS.

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