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Analytical Methods

Evaluation of the quality of olive oil using fatty acid profiles by direct infusion electrospray ionization mass spectrometry

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Abstract

Electrospray ionization mass spectrometry is used to predict the olive oil quality according to European Union marketing standards. Samples were 1:50 diluted in an alkaline 85:15 (v/v) propanol/methanol mixture and directly infused into the electrospray ionization source of an ion trap mass spectrometer. The establishment of ratios of the peak abundances of the free fatty acids followed by linear discriminant analysis was employed to predict the olive oil quality grade. In addition, using multiple linear regression and partial least-squares regression, the percentages of extra virgin and virgin olive oils in binary mixtures were predicted with 5–11% average prediction errors.

Keywords: Olive oil; Quality control; Mass spectrometry; Fatty acids; Linear discriminant analysis

1. Introduction

Olive oil is a fine product with high nutritional value and significant health benefits (Owen et al., 2000). Quality olive oils are expensive owing to the hard and time-consuming tasks involved in the cultivation of olive trees, the harvesting of the fruits, and the extraction of the oil. For this reason, adulteration of higher quality olive oils with either seed oils or olive oils of lower quality is a relatively common fraudulent practice. European Mediterranean countries, which are major suppliers of olive oils on the world market, have adopted common regulations to protect olive oil growers and consumers from fraud. According to the European Union Legislation (European Union Commission, 2003), there are several types of virgin olive and olive pomace oils. Thus, virgin olive oils are classified as extra virgin olive oil (EVOO), virgin olive oil (VOO) and lampante virgin olive oil (LVOO). Two further types of olive oils are distinguished: refined olive oil (ROO, obtained by refining virgin olive oils, and having a maximal free acidity of 0.5 g per 100 g), and olive oil (OO, a mixture of refined and virgin olive oils, excluding lampante oil, and having a maximal free acidity of 1.5 g per 100 g). Finally, three categories of olive pomace oil are recognized: crude olive pomace oil (COPO, obtained by treating olive pomace with solvents), refined olive pomace oil (ROPO, obtained by refining crude olive pomace oil, and having a maximal free acidity of 0.5 g per 100 g), and olive pomace oil (OPO, a mixture of refined olive pomace and virgin olive oils, excluding lampante oil, and having a maximal free acidity of 1.5 g per 100 g).

The authenticity of olive oils covers many aspects, including genetic variety, geographical origin and quality grade (Bianchi, 2002). Oil authentication can be carried out by a variety of methods, which have been recently reviewed (Aparicio & Aparicio-Ruíz, 2000; Aparicio & Luna, 2002). Many factors such as latitude, climatic conditions, irrigation regime, fruit ripening, harvesting and extraction technologies affect both the total fatty acid composition (particularly, the concentration of oleic acid), and the concentration profiles of many other oil components (Aparicio & Luna, 2002; Bruni, Cortesi, & Fiorino, 1994;

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Caponio, Alloggio, & Gomes, 1999; Di Giovacchino, Solinas, & Miccoli, 1994; Guimet, Boqué, & Ferré, 2004; Morello, Romero, & Motilva, 2004; Ranalli, Tombesi, De Mattia, Ferrante, & Giansante, 1997; Salvador, Aranda, Gómez-Alonso, & Fregapane, 2003; Torres & Maestri, 2006; Tura, Prenzler, Bedgood, Antolovich, & Robards, 2004; Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003).

Traditional methods, that employ organoleptic features to classify olive oils according to genetic variety and quality grade, are affected by assessor bias. For this reason, chemometric approaches constitute promising tools to classify olive oils. Fluorimetry at different excitation wavelengths, followed by cluster analysis, has been used to classify VOO, pure olive oil and olive pomace oil (Guimet et al., 2004). In order to distinguish between edible virgin olive oil from lampante olive oil, synchronous fluorescence and total luminiscence spectroscopy, followed by data analysis using principal component analysis and hierarchical cluster analysis, have been proposed (Poulli, Mousdis, & Georgiu, 2005). A 2% olive pomace oil has been estimated in EVOO by using Fourier transform-Raman spectroscopy, followed by partial least-squares regression (PLS) (Yang & Irudayaraj, 2001).

Olive oil adulterations have been also investigated using NMR (Fragaki, Spyros, Siragakis, Salivaras, & Dais, 2005; Fronimaki, Spyros, Christophoridou, & Dais, 2002; Zamora, Alba, & Hidalgo, 2001). The relationship between the ratio of 1,2-diglycerides with respect to the total amount of diglycerides, and the total amount of diglycerides determined by ^{31}P NMR spectroscopy has been used to classify commercial Cretan olive oils, ROO and pomace oils (Fronimaki et al., 2002). Also, ^{31}P NMR followed by multivariate supervised and non-supervised statistical techniques, has been used to classify Greek oils from different regions according to quality grade, and to detect EVOO adulteration with LVOO (Fragaki et al., 2005). The quality of edible oils has been also established by using sensor arrays (García-Gonzalez & Aparicio, 2002a, 2002b, 2003; Guadarrama, Rodríguez-Méndez, Sanz, Ríos, & De Saja, 2001). García-Gonzalez and Aparicio (2003) have used an array of seven metal oxide sensors and neural networks to detect LVOO in VOO with a 4.5% validation error. Guadarrama, Rodriguez-Mendez, De Saja, Rios, and Olias (2000) and Guadarrama et al. (2001) have described an array constructed with eight polymeric sensors to discriminate EVOO, VOO, LVOO and four deodorized oils. The adulteration of VOO with deodorized oils has also been studied using gas chromatography (CG) coupled to chemical ionization-mass spectrometry (CI-MS) (Saba, Mazzini, Raffaelli, Mattei, & Salvadori, 2005). Mixtures of high quality olive oils with lower quality grade olive oils, and with other vegetable oils, have also been studied by headspace-mass spectrometry (Marcos Lorenzo, Pérez Pavón, Fernández Laespada, García Pinto, & Moreno Cordero, 2002).

Direct infusion electrospray ionization mass spectrometry (ESI-MS) followed by linear discriminant analysis (LDA) of peak intensities and peak ratios has been successfully used to classify different types of samples into categories (Gama Melão, Simó-Alfonso, Ramis-Ramos, & Vicente, 2006; Peris-Vicente, Simó-Alfonso, Gimeno-Adelantado, & Domenech-Carbó, 2005). Direct infusion ESI-MS has been also used to classify vegetable oils according to biological origin, and to detect the adulteration of olive oil with soybean oil (Catharino et al., 2005).

In this work, the capability of ESI-MS to classify commercial olive oils of different quality grades (EVOO, VOO, LVOO and ROPO), and to evaluate mixtures of EVOO and VOO, and binary mixtures of these two oils with olive oils of lower quality grade has been studied. Infusion was performed with a simple dilution of the sample in a miscible alkaline solvent, and analyzed directly without any previous extraction step. Classification and evaluation studies were performed on the basis of fatty acid fingerprints obtained by direct infusion using ESI-MS in the negative-ion mode. Several chemometric techniques, including LDA, multiple linear regression (MLR) and PLS, were used to treat the data. The regression models provided fairly reliable predictions of the percentage of extra virgin and virgin olive oils in several oil mixtures.

2. Experimental

2.1. Instrumentation and working conditions

An HP 1100 series ion trap mass spectrometer (ITMS) provided with an ESI source (Agilent Technologies, Waldbronn, Germany) was used. A syringe pump (kd Scientific, Holliston, MA, USA) was used to infuse the samples at 0.3 ml h^{-1} (5 μl min^{-1}) through a 50 μm i.d. fused silica capillary. The MS working conditions were: nebulizer gas pressure, 25 psi; dry temperature, 200 °C; dry gas, 5 L min^{-1}; capillary voltage, 3.5 kV; voltages of skimmers 1 and 2, −26.8 V and −6.0 V, respectively. Nitrogen was used as nebulizer and dry gas (Gaslab NG LCMS 20 generator, Equcien, Madrid, Spain). The mass spectrometer was scanned within the *m/z* 100–800 range in the negative-ion mode. The target mass was set at *m/z* 281 ([M−H]$^-$ oleic acid peak). Maximum loading of the ion trap was 3×10^4 counts, and maximum collection time was 300 ms.

2.2. Reagents and samples

Analytical grade KOH (Probus, Barcelona, Spain), propanol (PrOH) and methanol (MeOH) (Scharlau, Barcelona, Spain) were used. Olive oil samples of the following quality grades were used: EVOO, VOO, LVOO and ROPO (European Union Commission, 2003). Samples of guaranteed quality, where the genetic variety was also known, were kindly donated by Coosur (Vilches, Jaén, Spain), Borges (Tàrrega, Lleida, Spain) and Grupo Hojiblanca (Antequera, Málaga, Spain). These samples were used to construct the models (see Table 1). Other samples, purchased at the local market (Table 1), were used to evaluate

Table 1
Olive oils used to construct LDA models

Grade	Brand	Genetic variety	Geographical origin	Set type
EVOO	Coosur	Hojiblanca[a]	Luque (Córdoba)	Training
		Arbequina[a]	Estepa (Sevilla) + La Roda de Andalucía (Sevilla)	Training
		Picual[a]	Villanueva del Arzobispo (Jaén) + Porcuna (Jaén)	Training
	Carbonell	Hojiblanca	Estepa (Sevilla)	Evaluation
		Arbequina	Aguadulce (Sevilla)	Evaluation
		Picual	Martos (Jaén)	Evaluation
	Borges	Hojiblanca[a]	Puente Genil (Córdoba)	Training
		Arbequina[a]	Huelva + Zaragoza + Palma del Río (Córdoba)	Training
		Picual[a]	Quesada (Jaén)	Training
	Torrereal	Arbequina	Vila Franca del Penedés (Barcelona)	Evaluation
	Duc	Arbequina	Vila Franca del Penedés (Barcelona)	Evaluation
	Oleastrum	Arbequina	Les Garrigues (Lleida)	Evaluation
	Hipercor	Hojiblanca	Antequera (Málaga)	Evaluation
	Grupo Hojiblanca	Hojiblanca[a]	Fuente de Piedra (Málaga)	Training
		Arbequina[a]	Antequera (Málaga)	Training
		Picual[a]	Montoro (Córdoba)	Training
VOO	Coosur	Mixture[b]	Vilches (Jaén)	Training
	Grupo Hojiblanca	Hojiblanca[a]	Archidona (Málaga)	Training
		Arbequina[a]	Antequera (Málaga)	Training
		Picual[a]	Lucena (Córdoba)	Training
LVOO	Coosur	Mixture[a]	Vilches (Jaén)	Training
	Borges	Mixture[a]	Jódar (Jaén)	Training
	Grupo Hojiblanca	Hojiblanca[a]	Archidona (Málaga)	Training
		Arbequina[a]	Hinojosa del Duque (Córdoba)	Training
		Picual[a]	La Rembla (Córdoba)	Training
ROPO	Coosur	Mixture[a]	Vilches (Jaén)	Training
	Borges	Mixture[a]	Palma del Río (Córdoba)	Training
OPO	*Confidential*	Mixture	Unknown	Evaluation[b]

[a] Guaranteed quality.
[b] Used exclusively to evaluate the MLR model.

the prediction capability of the models and to detect possible adulterations.

2.3. Procedures

A mixture of EVOO and VOO, and binary mixtures of these oils with lower quality grade oils, were prepared by weighing the appropriate amounts of the guaranteed oil samples provided by Coosur. An 85:15 (v/v) PrOH/MeOH mixture, containing 40 mM KOH, was used to dilute the oil samples and their mixtures in a 1:50 ratio (v/v). Lower dilutions led to a significant increase in background noise. MS experiments with saponified samples were also performed. However, the signal-to-noise ratios did not improve after saponification (data not shown). Thus, unsaponified samples were used. Between samples, the capillary was rinsed for 5 min with the alkaline PrOH/MeOH mixture. Before data acquisition, the diluted sample was infused until the signal remained constant. All samples were injected 4–5 times, and each time the data were averaged for 1 min. LDA and MLR models were constructed using the SPSS statistical package (v. 12.0.1, SPSS Inc., Chicago, IL, USA), and PLS1 models (for the prediction of a single response) were established with The Unscrambler (v. 7.6, CAMO Technologies Inc., Bergen, Norway).

3. Results and discussion

3.1. Normalization of the variables

In all cases, the MS spectra showed the $[M-H]^-$ peaks of the following fatty acids: myristic (C14:0, *m/z* 227), palmitoleic (C16:1, *m/z* 253), palmitic (C16:0, *m/z* 255), linolenic (C18:3, *m/z* 277), linoleic (C18:2, *m/z* 279), oleic (C18:1, *m/z* 281) and stearic (C18:0, *m/z* 283). The mass spectra were normalized by dividing each peak abundance by the abundance of the C16:0 peak (Fig. 1). As observed, oleic acid yielded the most intense signal, whereas palmitic, linoleic and stearic acids gave intermediate abundances. For each quality grade, closely similar peak profiles were obtained, independently of the genetic variety of the oils. The C14:0/C16:0 peak ratio was larger for VOO and LVOO than for the samples of other quality grades. Also, the C18:3/C16:0 peak ratio decreased according to LVOO > EVOO ≈ VOO > ROPO (Fig. 1). In agreement with these observations a chemometric study was carried out.

In order to reduce signal fluctuations between measurements, two normalization procedures were tried. First, the abundance of each fatty acid in each mass spectrum was divided by the total sum of the abundances of the seven

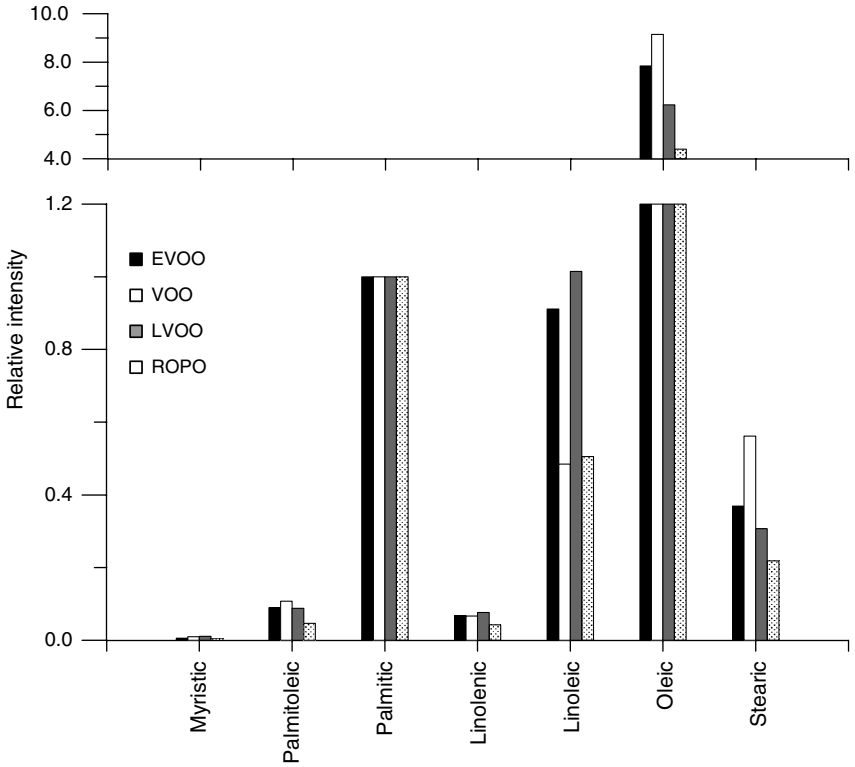

Fig. 1. Relative peak intensities of fatty acids observed in the mass spectra of different quality grade olive oils. The palmitic acid peak (*m*/*z* 255) was used as reference.

fatty acids (procedure A). Second, the abundance of each fatty acid was divided by each one of the abundances of the other six fatty acids; in this way, $(7 \times 6)/2 = 21$ non-redundant peak ratios to be used as predictors were obtained (procedure B).

3.2. Construction of the data matrix and quality grade prediction by LDA

As indicated above and in Table 1, the samples with guaranteed quality grade (9 EVOO, 4 VOO, 5 LVOO and 2 ROPO samples) were used to construct the training set for the LDA models. The other samples, also described in Table 1, were used as evaluation set. To improve the stability and prediction capability of the models, for each quality grade (EVOO, VOO and LVOO) samples of three genetic varieties (Hojiblanca, Arbequina and Picual), produced in different regions of Spain with rather dissimilar soils and climatic conditions, were used.

According to 4–5 injections of each sample, two matrices constituted by 123 cases, and by 7 and 21 predictors, after normalization by procedures A and B, respectively, were established. In order to classify the samples according to their quality grade, LDA models were constructed. In LDA, vectors minimizing Wilks' lambda (λ_w) are obtained (Vandeginste et al., 1998). To select the predictors to be included in the models, the SPSS stepwise algorithm was used. Using this algorithm, a predictor is selected when the reduction of λ_w produced by including the predictor in the model exceeds the entrance threshold of an *F*-test, F_{in}. However, the entrance of a new predictor modifies the significance of those predictors which are already present in the model. For this reason, after the inclusion of a new predictor, a rejection threshold, F_{out}, is used to decide if one of the other predictors should be removed from the model. The process terminates when there are no predictors entering or being eliminated from the model. The SPSS default values of F_{in} and F_{out}, 3.84 and 2.71, were respectively used.

Using the samples of the training set (EVOO, VOO, LVOO and ROPO), two LDA models, one for each normalization procedure, were constructed. The best results were obtained using normalization procedure B, which was selected. Using this procedure, the evaluation set samples were correctly classified with a probability higher than 95%. Then, both the training and evaluation sets were jointly used to construct a new model with an improved prediction capability. In this way, the geographical origin of the samples was also included in the statistical analysis. The λ_w for this model was 0.52. The predictors selected by the SPSS stepwise algorithm, and the corresponding model standardized coefficients, which show their discriminant capabilities, are given in Table 2. A score plot on the plane of the two first discriminant functions is shown in Fig. 2. EVOO category was very well resolved from the other three categories. To maximize resolution among the VOO, LVOO and ROPO categories, another LDA model was constructed without EVOO category. In this case, λ_w was

Table 2
Standardised coefficients of the discriminant functions obtained to predict the quality grade of olive oils

Predictors	Categories[a]				
	EVOO/VOO/LVOO/ROPO			VOO/LVOO/ROPO	
	f_1	f_2	f_3	f_1	f_2
C16:0/C14:0	–	–	–	3.4	2.5
C18:1/C14:0	0.042	−1.1	0.53	–	–
C18:0/C14:0	–	–	–	−2.3	−2.2
C16:0/C16:1	−8.8	−2.3	−1.2	4.5	−3.5
C18:3/C16:1	6.8	1.3	2.3	–	–
C18:2/C16:1	–	–	–	2.0	−3.3
C18:1/C16:1	15	2.8	4.1	–	–
C18:0/C16:1	−1.7	−0.18	−0.65	−6.1	10
C18:1/C16:0	0.79	−0.71	−1.5	2.5	−7.1
C18:0/C16:0	−7.7	−0.17	−3.7	–	–
C18:2/C18:3	–	–	–	0.17	2.4
C18:1/C18:3	−6.3	0.084	−1.9	–	–
C18:0/C18:3	3.1	0.69	1.7	−0.68	−0.85
C18:1/C18:2	0.71	1.1	1.9	−0.72	3.2
C18:0/C18:1	0.66	−0.38	0.53	2.9	−0.76

[a] Categories included in the training set.

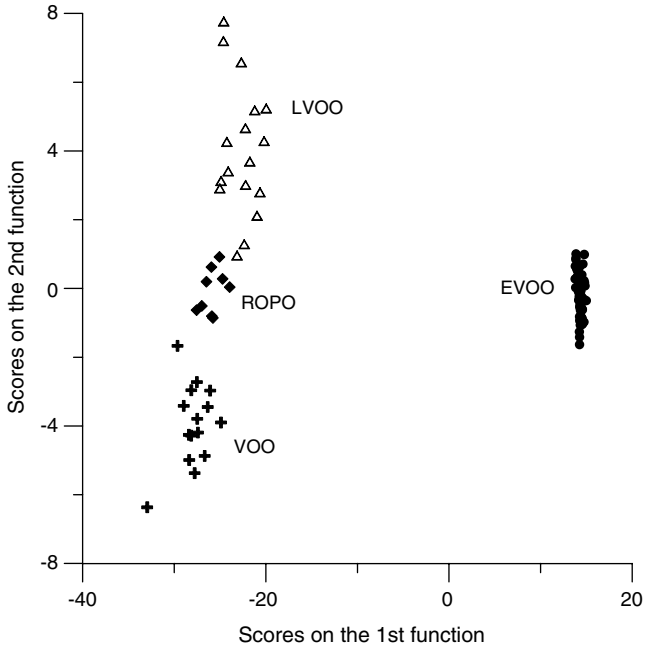

Fig. 2. Score plot on the plane of the two first discriminant functions of an LDA model constructed with four different quality grade olive oils using normalization procedure B.

0.19, which agrees with the excellent resolution between the three categories shown in the score plot of Fig. 3. The model standardized coefficients are also given in Table 2. Therefore, EVOO, VOO, LVOO and ROPO oil samples can be unequivocally classified by the sequential application of two LDA models, one constructed with and the other without EVOO category.

At the sight of Table 2 and Fig. 2, predictors C16:0/C16:1, C18:3/C16:1, C18:1/C16:1, C18:0/C16:0 and

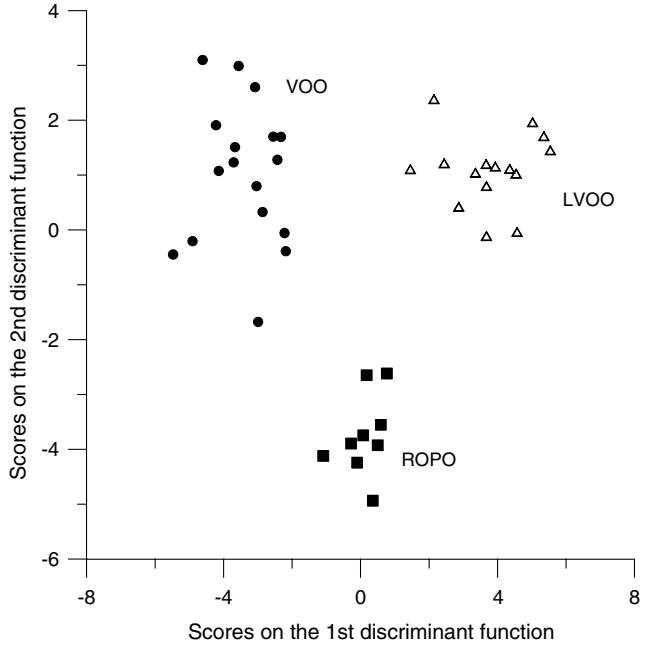

Fig. 3. Score plot on the plane of the two discriminant functions of an LDA model constructed with three different quality grade olive oils (EVOO excluded) using normalization procedure B.

C18:1/C18:3 were relevant to distinguish EVOO from the other three categories. Among these predictors, only C16:0/C16:1 was significant to distinguish VOO, LVOO and ROPO categories when EVOO was excluded from model construction (Fig. 3). The ratios C18:3/C16:1, C18:1/C16:1, C18:0/C16:0 and C18:1/C18:3 were characteristic to distinguish EVOO from the other categories. In addition to C16:0/C16:1, predictors C18:0/C16:1 and C18:1/C16:0 were also important to distinguish VOO, LVOO and ROPO categories.

3.3. Evaluation of binary mixtures of olive oils of different quality grade

Binary mixtures of EVOO and VOO, and either EVOO or VOO with another lower quality grade oil, were prepared. Mixtures with *ca.* 100%, 80%, 60%, 40%, 20% and 0% EVOO or VOO, were infused. For each binary combination of oils, the predictors obtained by using normalization procedures A and B were independently used to construct two matrices. Each mixture was injected in triplicate, thus, matrices with a total of 75 cases, and 7 and 21 predictors, were respectively obtained. A response vector containing the percentage of the higher quality grade oil in the mixture was added to the matrices.

To select the predictors used in the construction of MLR models, the SPSS backward algorithm was used. With this algorithm, all the predictors are initially introduced in the model, and then are sequentially eliminated according to an F-test. The SPSS default values, $F_{in} = 3.84$ and $F_{out} = 2.71$, were again used. The MLR models were constructed both without and with an

Table 3
Regression coefficients of the MLR and PLS1[a] models constructed to predict the composition of binary mixtures of oils of different quality grades

Predictor	EVOO/VOO		EVOO/LVOO		EVOO/ROPO		VOO/LVOO		VOO/ROPO	
	MLR	PLS1	MLR	PLS1	MLR	PLS1	MLR	PLS1	MLR	PLS1
C14:0	−0.71	−0.78	0.71	0.63	−0.75	−0.75	−0.32	−0.15	–	0.066
C16:1	−1.2	−0.54	−2.7	−1.3	1.9	0.48	0.45	0.098	–	−0.16
C16:0	1.9	0.31	2.3	0.72	−2.6	−0.48	–	–	–	−0.16
C18:3	2.2	0.67	1.4	0.42	–	0.10	–	0.16	–	–
C18:2	0.41	0.17	0.92	0.061	–	0.11	−1.5	−0.52	1.8	0.465
C18:1	–	−0.20	−1.6	−0.32	2.2	0.078	1.9	0.36	–	−0.23
C18:0	−1.9	−0.23	–	−0.14	–	0.096	–	0.25	−0.94	−0.33
Number of vectors[b]	6	4	6	5	4	4	4	3	2	3
Average prediction error (%)	10	10	9.7	10	9.6	9.0	5.1	5.3	3.4	4.4
C16:1/C14:0	3.0	0.58	–	−0.31	–	0.38	1.4	0.054	−1.1	–
C16:0/C14:0	−5.4	−0.89	–	−0.11	–	−1.2	–	–	–	–
C18:3/C14:0	–	1.8	–	0.15	–	–	2.3	–	–	–
C18:2/C14:0	−0.64	−1.1	−0.35	−0.14	1.4	0.054	−1.7	–	–	–
C18:1/C14:0	3.0	1.1	–	–	–	0.24	–	–	1.3	–
C18:0/C14:0	–	−1.6	–	–	−0.92	−0.85	−1.9	–	–	–
C16:0/C16:1	–	–	–	0.22	−0.44	−0.30	0.82	–	0.54	–
C18:3/C16:1	1.6	0.39	−1.1	−0.20	–	–	−2.9	–	–	0.059
C18:2/C16:1	–	−0.77	2.0	0.39	–	−0.25	1.2	−0.10	–	0.14
C18:1/C16:1	–	0.99	0.77	−0.17	–	−1.2	1.2	0.071	–	–
C18:0/C16:1	−0.69	−0.44	−0.77	−0.14	–	–	–	–	−0.59	–
C18:3/C16:0	−0.69	−0.066	–	−0.17	–	–	0.71	–	0.42	–
C18:2/C16:0	−1.2	−0.80	−0.71	0.28	–	–	–	−0.169	–	0.168
C18:1/C16:0	–	0.82	–	−0.17	–	0.080	–	0.095	–	–
C18:0/C16:0	–	−1.1	–	−0.097	–	–	–	–	–	−0.051
C18:2/C18:3	2.2	2.0	–	0.20	–	–	−1.2	–0.236	0.95	0.165
C18:1/C18:3	−0.40	−0.41	−1.2	−0.41	–	1.1	–	0.064	−0.48	–
C18:0/C18:3	–	0.091	–	−0.38	0.30	–	−0.40	–	0.71	–
C18:1/C18:2	–	−0.44	–	0.28	0.68	0.19	1.8	0.24	–	−0.14
C18:0/C18:2	–	−0.20	0.68	0.35	–	–	−2.3	0.18	–	–
C18:0/C18:1	–	0.71	–	–	–	–	0.98	–	–	−0.11
Number of vectors[b]	10	10	8	7	5	2	14	4	8	3
Average prediction error (%)	5.3	4.8	4.5	5.8	11	15	2.2	7.1	3.0	5.5

[a] PLS1 coefficients smaller than 0.05 in absolute values are not given.
[b] Number of vectors selected by the forward algorithm of SPSS (MLR), or recommended by The Unscrambler (PLS1 *k*-values).

independent term (a constant). The use of a constant improved the quality of the models constructed with the peak intensity ratios. For all the PLS1 models, the number of vectors recommended by The Unscrambler after the PLS1 rotation (*k*-value) was adopted.

The regression coefficients of the MLR and PLS1 models are given in Table 3. In most cases, predictors with large regression coefficients were common to both models. An average prediction error, calculated as the average absolute difference between the expected and predicted oil percentages, divided by the number of predictions, was used to evaluate model quality. As can be seen in Table 3, in most cases MLR showed average prediction errors slightly better than PLS1. Using MLR, normalization procedure B gave better values of the average prediction errors than procedure A. The MLR regression model for VOO/ROPO mixtures was applied to quantify a guaranteed OPO sample (a commercial mixture of ROPO and VOO). The declared and found percentages in ROPO were $95 \pm 3\%$ and $92 \pm 5\%$, respectively.

4. Conclusions

A quick ESI-MS method, capable of predicting the olive oil quality grade has been developed. After a simple 1:1 dilution, the oil samples were infused in a mass spectrometer, and the peak abundances of the fatty acids were measured. Using LDA, the oils were unequivocally classified according to European Union marketing standards. Using MLR, binary mixtures of different quality grade oils can be evaluated with average prediction errors within the 3–5% range; however, errors of the order of 11% should be expected for EVOO/ ROPO mixtures. The present procedure can be easily applied to the quality control of legal mixtures and in fraud detection.

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