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Original article

Detection of low-quality extra virgin olive oils by fatty acid alkyl esters evaluation: a preliminary and fast mid-infrared spectroscopy discrimination by a chemometric approach

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Summary A set of eighty-one extra virgin olive oils (EVOOs) was analysed according to the new quality parameters relative to the total amount of methyl and ethyl esters of fatty acids [Σ (FAMEs + FAEEs)] and the ratio between ethyl and methyl esters [ratio of FAEEs/FAMEs (RFF)]. Acquisition of the mid-infrared spectra was also performed by Fourier Transform Infrared Spectroscopy (FT-IR). Chemical and spectroscopy data were chemometrically elaborated, and FT-IR coupled by Partial Least Square (PLS) methodology was developed. Results were statistically similar to official procedure in terms of analytical performance for Σ (FAMEs + FAEEs) and RFF in EVOOs: a good agreement between predicted and actual values on calibration data sets was found (0.98 and 0.83, respectively) and the limit of quantification was low enough (29.3 mg kg^{-1}) considering the actual limits for Σ (FAMEs + FAEEs). This new approach, time-saving and environmentally friendly, can be considered as a useful tool for screening procedures.

Keywords Extra virgin olive oil, fatty acid alkyl esters, FT-IR spectroscopy, low-quality oils., mildly deodorized olive oils.

Introduction

Extra virgin olive oil (EVOO) is characterised by one of the highest economic value in comparison with other vegetable oils, thanks to its well-known nutritional and sensory qualities (Velasco & Dobarganes, 2002). Unfortunately, EVOO is also easy to falsify: because of its prestige, it has always been illegally mixed with cheaper and low-quality oils (Harwood & Aparicio, 2000), especially to obtain EVOO sold in supermarkets and discount stores at low cost (Bendini *et al.*, 2009a). The so-called lampante low-quality olive oils cannot be used as raw foodstuff for direct human consumption, as they have an acidity level that is too high, and their volatile profile is characterised by 'soft' off-flavours, derived from low-quality olives or from inappropriate procedures during oil extraction or storage. Deodorization applying a 'mild' technology, developed under vacuum and at low temperature, is able to remove unacceptable defects (mainly winey-vinegary, fusty-muddy sediment and musty), avoiding the formation of chemical traces in the oils exploited as forensic proof of fraud (Cerretani *et al.*, 2008). In the last decades, several analytical methods have been proposed to detect such low-quality EVOOs and their admixtures, such as the determination of diacylglycerols and pheophytin (Serani & Piacenti, 2001a; Serani *et al.*, 2001b), the amount of water present in the micro-emulsion of the oil and study of the volatile profile, especially taking into account of the ratio between ethanol and *E*-2-hexenal (Cerretani *et al.*, 2008; Bendini *et al.*, 2009a). In addition to the proposed analytical methods, one of the most reliable techniques seems to be determination of fatty acid methyl and ethyl esters (fatty acid alkyl esters, FAAEs) as methyl esters of fatty acid

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(FAMEs) and the ethyl esters of fatty acids (FAEEs) which are present in the waxy fraction of olive oils (Mariani & Fedeli, 1986; Mariani *et al*., 1991, 1992; Mariani & Bellan, 2008). In good quality EVOOs, FAMEs and FAEEs are present in very small amounts, while they are present in higher amounts in virgin, lampant olive oils (Mariani & Bellan, 2011) and in second-olive processing oil (the so-called repaso) (Cerretani *et al*., 2011). Actually, these compounds are formed as a consequence of degradation and fermentation processes of low-quality olives, which can be overripe, damaged or simply poorly preserved before they are processed (Biedermann *et al*., 2008). These alterations lead to a production of short chain alcohols by the degradation of the pectins by endogenous pectin-methyl-esterases (methanol) and the aerobic metabolism of microorganisms (ethanol) (Biedermann *et al*., 2008). At the same time, lipolysis of triacylglycerols with liberation of free fatty acids may occur. In these conditions, the formation of FAMEs and FAEEs by esterification can take place: this reaction is catalysed by the temperature reached during the 'mild' deodorization step (Perez-Camino *et al*., 2008), while it does not seem to occur during the storage of high-quality EVOOs (Mariani & Bellan, 2011). In reality, these molecules have two different but complementary meanings: first they are related to the olive (and consequently the olive oil) quality (Biedermann *et al*., 2008), and FAAEs can be considered as 'virtual' markers of possible 'mild-deodorization', as they resist and are not removed by this illegal treatment. In fact, the contemporary presence of a high level of FAAEs, without a clearly perceivable sensory defect, can be reasonably explained by the application of 'mild-deodorization'. Different analytical methods have been performed for the determination of FAMEs and FAEEs in VOO in recent years: the older ones are done by solid phase extraction (SPE) (Perez-Camino *et al*., 2008), while some modifications have been added (Bendini *et al*., 2009b and Cerretani *et al*., 2011). As of April 2011, the determination of alkyl esters, first proposed by the IOC (COI/T.20/DOC. NO. 28, 2009), became an official method adopted by the European Community law (EC Reg. 61/2011 and corrigendum). This method is based on solid–liquid chromatography (LC) by traditional glass column for isolating the fraction containing alkyl esters and waxes, with the aim to assign the evaluated sample to the commercial category of EVOO. Indeed, for EVOO, the concentration of the sum of FAMEs and FAEEs [Σ (FAMEs + FAEEs)] cannot exceed 75 mg kg^{-1} (EC Reg. 61/2011 and corrigendum). If Σ (FAMEs + FAEEs) is between 75 and 150 mg kg^{-1}, the oil can be considered as EVOO only if the ratio of FAEEs/FAMEs (RFF) is ≤ 1.5 (EC Reg. 61/2011 and corrigendum). It is interesting to underline that the European law permits a higher amount of alkyl esters for EVOO (between 75 and 150 mg kg^{-1}) only if RFF is lower or equal than 1.5, as the FAMEs are typically formed with the technological transformation of overripe olive fruits (Biedermann *et al*., 2008).

The determination of food authenticity and the detection of adulteration are current problems of increasing importance in the food industry. EVOO adulteration was extensively studied because it is a high added value product and adulteration employs more sophisticated methods nowadays (Arvanitoyannis & Vlachos, 2007). Traditionally, the chemical treatments of the samples required for determining authenticity of EVOOs are complex, expensive, time-consuming and tedious. On the contrary, FT-IR is a highly useful molecular spectroscopy technique because it is rapid, non-destructive, simple to perform and does not require sample pre-treatment. The employment of several multivariate methods [like principal component analysis, canonical analysis, linear discriminant analysis, cluster analysis, partial least squares (PLS), and surface response methodology] has become a prerequisite for several applications related primarily to food quality control in terms of authentication/adulteration, thanks to a substantial simplification of the classification/grouping task (Tzouros & Arvanitoyannis, 2001). Among the possible analytical approaches, the Fourier transform mid-infrared (FTIR) spectroscopy combined with multivariate chemometric procedures has been used by several authors for predicting the level of authentication/adulteration in EVOO samples based on chemical composition. This approach has been apply to correctly discriminate among genuine and adulterated olive oils containing soybean, corn, olive pomace oils or between pure EVOOs and the same oils adulterated with sunflower oil or with refined oils or with walnut or hazelnut oil (Arvanitoyannis & Vlachos, 2007; Özdemir & Öztürk, 2007; Gurdeniz & Ozen, 2009; Lerma-García *et al*., 2010; Maggio *et al*., 2010). Several chemometric approaches were used to treat variables of olive oil samples to classify extra virgin and ordinary olive oil samples and partial least-squares regression (PLS) resulted in higher prediction success rates (Tzouros & Arvanitoyannis, 2001). Nevertheless, to the best of our knowledge, there are no investigations regarding the application of this technique for a quick check of quality limits for EVOOs introduced by the new regulation. For this aim, the total amount of methyl and ethyl esters [Σ (FAMEs + FAEEs)], and the ratio between ethyl esters and methyl esters (RFF) of a set of eighty-one EVOO samples, sold in Italian supermarkets, were evaluated and compared with the limits proposed by the EC Reg. 61/2011 and corrigendum. Next, correlation between these chemical parameters and spectroscopy data of the oils was performed to detect low-quality EVOOs with a statistical model.

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Materials and methods

Samples

A set of eighty-one EVOO samples was purchased from local supermarkets, where they were sold (and labelled) as EVOOs at low cost. The samples were bought in two different years, and the method used to evaluate the alkyl esters followed the historical evolution of the method, first reported in the literatures and then adopted by the European Community law (EC Reg. 61/2011 and corrigendum).

Apparatus

Gas chromatography analyses of alkyl esters were performed using a Carlo Erba MFC 500 (Carlo Erba, Milan, Italy) instrument equipped with a flame ionization detector (FID).

The FTIR spectra were acquired on a Tensor 27™ FTIR spectrometer system (Bruker Optics, Milan, Italy), using a Rocksolid™ interferometer and a DigiTect™ detector system. The coupled attenuated total reflectance (ATR) accessory (Specac Inc., Woodstock, GA, USA) was equipped with a ZnSe 11 reflection crystal.

Materials, reagents and standards

The SPE cartridges (6 mL) STRATA Si-1 Silica (55 μm, 70 Å) packed with silica gel phase (1000 mg) were obtained from Phenomenex (Torrence, CA, USA). The silica gel stationary phase (60–200 mesh) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The standard used for FAAEs quantification [heptadecanoic acid methyl ester (C17:0ME)] was acquired from Sigma-Aldrich, as were methyl pentadecanoate, ethyl pentadecanoate, methyl palmitate, ethyl palmitate, methyl oleate, ethyl oleate, methyl linoleate and ethyl linoleate, which were used to identify the alkyl esters. All solvents used were analytical grade (Merck & Co. Inc., Darmstadt, Germany).

Determination of FAMEs and FAEEs by gas chromatographic analyses

Methyl esters of fatty acids and FAEEs were extracted from oil samples by three different methods (Bendini *et al*., 2009b; Cerretani *et al*., 2011; EC Reg. 61/2011) and quantified by gas chromatographic analyses following the analytical procedure reported in EC Reg. 61/2011. For standardising and harmonisation of the results, FAAEs were referred to the same analytical standard (C17:0 ME) for all samples analysed; moreover, all the response factors related to the GC-FID were set to 1.000.

Extraction of the alkyl esters by traditional liquid chromatography (samples 1–46)

This extraction follows the method reported in COI/T.20/DOC. NO. 28 (2009) 'Determination Of The Content Of Waxes, Fatty Acid Methyl Esters And Fatty Acid Ethyl Esters By Capillary Gas Chromatography' and recently adopted as official law by the European community (EC Reg. 61/2011 and corrigendum). A 0.5 ± 0.0001 g of the sample was mixed with 0.250 mL of standard solution of the internal standard (methyl heptadecanoate, C17:0 ME, 0.02% m/v). Next, 15 g of silica gel were suspended in *n*-hexane and settled spontaneously into a glass column for LC (internal diameter 15 mm, length 30–40 cm). The settling was complete with the aid of an electric shaker to make the chromatographic bed more homogeneous. Then, 30 mL of *n*-hexane were percolated to remove any impurities. The samples were transferred to the chromatography column with the aid of two 2-mL portions of *n*-hexane. The solvent was allowed to flow to 1 mm above the upper level of the absorbent. The alkyl esters were then collected eluting 220 mL of a freshly prepared mixture of *n*-hexane/ethyl ether (99:1, v/v) at a flow of about fifteen drops every 10 s. The resultant fraction was evaporated in a rotary evaporator until the solvent was almost removed, drying the last 2 mL under a weak flow of nitrogen. The fraction containing the methyl and ethyl esters was diluted with 2 mL of *n*-heptane, and 1 μL of this solution was injected.

Extraction of the alkyl esters by SPE (SPE 1) (samples 47–75)

For these twenty-nine samples, the extraction method followed the conditions described by Bendini *et al*. (2009b). A 0.2 ± 0.0001 g of oil sample was mixed with 250 μL of standard solutions of C15:0 EE and C17:0ME (both 50 μg g^{-1}), respectively, for the quantification of ethyl esters and methyl esters, and *n*-hexane was added to obtain a volume of 2 mL. This oil solution was split in two fractions of 1 mL and eluted separately. Silica SPE cartridges (1000 mg) were placed in an automatic vacuum elution apparatus and conditioned by passing 12 mL of *n*-hexane. Next, 1 mL of the oil solution was charged, and the solvent was pulled through at 0.5 mL min^{-1}, leaving the samples and the standards on the cartridge. The elution was made with 7 mL of the solvent mixture *n*-hexane:toluene (85:15, v/v), and this fraction was rejected. Next, the alkyl esters were collected by elution with 10 mL of the same mixture at a flow rate of 1 mL min^{-1}. The eluate was evaporated in a rotary evaporator at room temperature under vacuum until dry. The residue was dissolved in 200 μL of heptane, and a 1 μL of this solution was injected.

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Extraction of the alkyl esters by SPE, with a different method (SPE2) (samples 76–81)

For these six samples, the extraction method followed the conditions described by Cerretani *et al.* (2011). A 1 ± 0.0001 g of oil sample was mixed with 500 μL of standard solutions of C17:0 ME, (200 μg g^{-1}) and 500 μL of standard solutions of lauryl arachidate (400 μg g^{-1}). Next, *n*-hexane was added to reach the volume of 5 mL. Silica SPE cartridges (1000 mg) were placed in an automatic vacuum elution apparatus and conditioned by passing 8 mL of toluene. Then, 0.5 mL of the oil solution was charged, and the solvent was pulled through at 0.5 mL min^{-1}, leaving the samples and the standards on the cartridge. The elution was made with 4 mL of the solvent mixture *n*-hexane/toluene (85:15, v/v), and this fraction was rejected. The alkyl esters were then collected by elution with 13 mL of the same mixture at a flow rate of 1 mL min^{-1}. The eluate was evaporated in a rotary evaporator at room temperature under vacuum until dry. The residue was dissolved with 200 μL of *n*-heptane, and 1 μL of this solution was injected.

GC condition of the analyses, followed for both extractions, by SPE and by traditional liquid chromatography

Compared to the official method, a slight modification in the programmed temperature of the oven was introduced to not exceed the maximum temperature of the capillary column. The official method employed a cold injector for direct on-column injection, while we set the temperature of the injector at 325 °C, with a split ratio fixed at 1:30. The capillary column was a ZB–5MS (Phenomenex) (30 m length × 0.25 mm i.d. × 0.25-μm-film thickness). Helium, at a flow rate of 1.2 mL min^{-1}, was the carrier gas. The oven temperature was programmed from 80 °C (kept for 1 min) to 140 °C at a rate of 15 °C min^{-1}, then raised to 325 °C at a rate of 4.5 °C min^{-1} and kept for 20 min. The FID detector was set at 325 °C. The amount of alkyl esters was expressed as mg of C17:0 ME kg^{-1} of oil. The average, for each sample, was calculated from three replicates.

Acquisition of FTIR spectra

Analyses were carried out in triplicate at room temperature. Spectra were acquired (thirty-two scans/sample or background) in the range of 4000–700 cm^{-1} at a resolution of 4 cm^{-1}, using OPUS r. 6.0 (Bruker Optics) software. The absorbance spectrum was collected against a background obtained with a dry and empty ATR cell. Each sample was uniformly spread throughout the crystal surface. Before acquiring each spectrum, the ATR crystal was cleaned with a cellulose tissue soaked in *n*-hexane and then rinsed with acetone.

Data analysis

Data were exported in an ASCII compatible OPUS 6.0 software format (using a OPUS MACRO). PLS models were computed on respective training set samples for each parameter. The calculations were executed by MVC1 routines (Olivieri *et al.*, 2004) written for Matlab (Mathworks Inc., Natick, MA, USA). The moving window of variable size strategy (Ferraro *et al.*, 2001) was also implemented using MVC1. For each parameter, samples were treated independently. The selection of samples for calibration and validation groups was made using the Kennard & Stone algorithm, in both cases.

Results

PLS models for determination of FAMEs and FAEEs

As previously stated, the determination of the Σ (FAMEs + FAEEs) was carried out by previous extraction using three different pre-treatments, namely traditional LC (samples 1–46) and extraction by SPE with two different methods (SPE1, samples 47–75 and SPE2, samples 76–81). Table 1 shows the three groups of samples and their concentrations for Σ (FAMEs + FAEEs) and RFF. To develop and validate a robust PLS, only samples analysed by the official extraction method (EC Reg 61/2011) (LC) were taken into account. The obtained PLS parameters were applied on other groups of samples (SPE1 and SPE2). The content of Σ (FAMEs + FAEEs) for the first group EVOOs, determined by capillary GC, was in the range 13–116 (mg kg^{-1}): seven of the forty-six samples were close to the limits fixed for EVOOs. Figure 1a shows the full IR spectral range 4000–650 cm^{-1} for EVOO

Table 1 Sample groups and related contents for Σ (FAMEs + FAEEs) and RFF

	LC samples	SPE1 samples	SPE2 samples
Number of samples	46	29	6
Mean of Σ (FAMEs + FAEEs) content (mg kg^{-1})	47.1	54.0	437.5
Σ (FAMEs + FAEEs) range (mg kg^{-1})	13–116	9–159	32–749
Σ (FAMEs + FAEEs) SD (mg kg^{-1})	24	41	286
Mean of RFF	1.1	1.4	3.0
RFF range	0.4–1.8	0.3–4.1	1.9–4.1
RFF SD	0.40	1.0	1.1

FAEEs, ethyl esters of fatty acids; FAMEs, methyl esters of fatty acid; LC, liquid chromatography; RFF, ratio of FAEEs/FAMEs; SPE, solid phase extraction.

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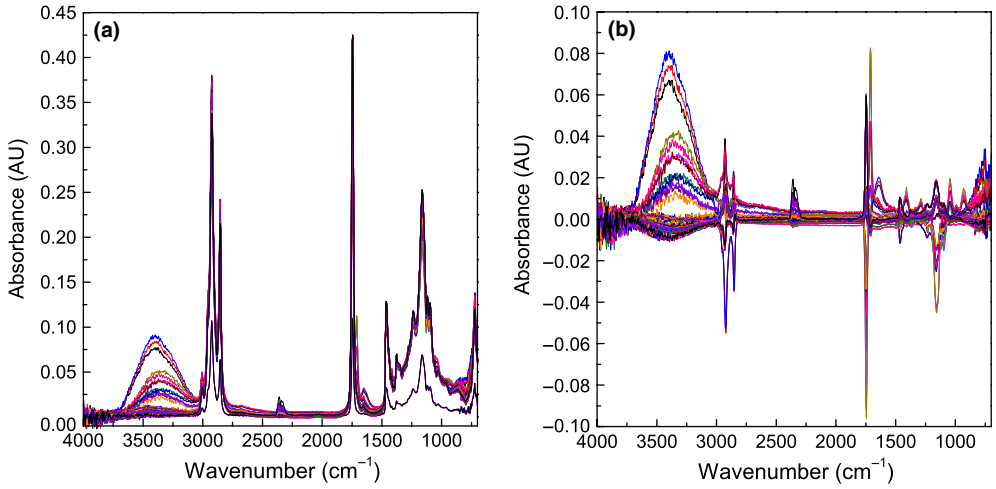

Figure 1 Full spectral range of Fourier transform mid-infrared (FTIR) for extra virgin olive oil (EVOOs) samples (a). Multiplicative Scatter Correction (MSC) and MC pre-treated FTIR spectra of EVOO samples (b).

samples. Different PLS calibration models were initially built by employing full spectra and reduced spectral ranges, obtained by a moving window of variable size strategy. However, none of these models provided an acceptable calibration, and predictions were unsatisfactory. Next, data were Mean-Centred (MC) and Multiplicative Scatter Correction (MSC) was employed to improve the performance of the method during calibration (pre-treated spectra are shown in Fig. 1b). Additionally, the spectral range was shortened to 2839.0–912.3 cm^{-1} to leave out regions where signal-to-noise ratio was very poor. By applying the Haaland and Thomas statistical criterion ($\alpha = 0.75$), the appropriate number of model dimensions was 6 (Haaland & Thomas, 1988). The results regarding calibration models between reduced pre-treated spectra and Σ (FAMEs + FAEEs) contents are reported in Table 2.

Table 2 Method parameters, statistical summary and figures of merits for Σ (FAMEs + FAEEs) PLS models

	LC	SPE1
Method parameters		
PLS factors	6	
Pre-treatment	MC – MSC	
Spectral range (cm^{-1})	2839.0–912.3	
Statistical summary: calibration		
Root Mean Square Deviation (RMSD, mg kg^{-1})	2.60	9.91
Percentage Relative Error In Calibration (REC, %)	5.98	17.35
R^2	0.98	0.95
Statistical summary: validation		
Mean recovery (%)	123	94
Relative Standard Deviation (RSD, %)	27	26
Figures of merit		
Sensitivity	0.00010	0.000026
Analytical sensitivity	0.36	0.19
Minimum detectable difference of concentration		
Σ (FAMEs + FAEEs), mg kg^{-1}	2.78	5.26
LOD Σ (FAMEs + FAEEs), mg kg^{-1}	8.8	7.0
LOQ Σ (FAMEs + FAEEs), mg kg^{-1}	29.3	20.9
Mean spectral residue (AU)	0.00029	0.000080

FAEEs, ethyl esters of fatty acids; FAMEs, methyl esters of fatty acid; LC, liquid chromatography; LOQ, Limit of Quantification; MSC, Multiplicative Scatter Correction; PLS, Partial Least Square; SPE, solid phase extraction.

PLS models for RFF determination

Following the same line of the previous developed models to estimate Σ (FAMEs + FAEEs), a PLS model, using LC samples, was built for detecting RFF. As in the PLS for Σ (FAMEs + FAEEs), MSC and MC spectral data pre-treatments were employed (Fig. 1b) to improve the performance of the model. Table 3 lists the calibration and prediction parameters used. Subsequently, the parameters of the calibration model obtained with LC samples were applied to SPE1 and SPE2 samples. As observed, the PLS model for RFF yields very good correlation coefficients and low Root Mean Square Deviation (RMSD) values; predictions were also very satisfactory, in terms of Relative Error in Calibration (REC) and recovery rate values (Table 3). The validation of the model was also carried out using the independent LC-validation mentioned above.

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Table 3 Method parameters, statistical summary and figures of merits for RFF-PLS models

	LC	SPE1
Method parameters		
PLS factors	9	
Pre-treatment	MC – MSC	
Spectral Range (cm^{-1})	2839.0–912.3	
Statistical summary: Calibration		
Root Mean Square Deviation (RMSD, %)	0.15	0.17
Percentage Relative Error in Calibration (REC, %)	14	12
R^2	0.83	0.97
Statistical summary: validation		
Mean recovery (%)	103	107
Relative Standard Deviation (RSD, %)	17	36
Figures of merit		
Sensitivity	0.01	0.00
Analytical sensitivity	20.00	8.80
Minimum detectable difference of concentration	0.05	0.11

LC, liquid chromatography; MSC, Multiplicative Scatter Correction; PLS, Partial Least Square; RFF, ratio of FAEEs/FAMEs; SPE, solid phase extraction.

Discussion

PLS models for determination of FAMEs and FAEEs

The suitability of the proposed method for the objective was evaluated by analysing the model figures of merit and the results of validation samples. The values obtained for both RMSD and REC% were acceptable (Table 2). The Limit of Quantification (LOQ) was low enough considering the limits set by the EU for EVOO. Calibration R^2, which describes the goodness-of-fit of the predicted concentrations to their actual values was 0.98. The validation set exhibited almost quantitative recoveries that contain 100% in its confidence range (123 ± 27%). A Relative Standard Deviation (RSD) value of 27% shows the natural dispersion of the LC extraction method. The results (Fig. 2a) show good agreement between predicted and actual values on calibration and validation data sets. The slopes and intercepts of the curves depicted in this plot were close to unity and zero, respectively, indicating low bias and absence of systematic regression errors.

To demonstrate the inter-changeability and the transferability of the method (possibility of transference to another laboratory), a calibration model using a set of SPE1 samples and previously obtained PLS parameters (pre-treatments, PLS factors and spectral range obtained for PLS-LC model) was built, with satisfactory predictions. Almost quantitative recoveries (mean recovery = 94.38%, Table 2) were found, and high sample dispersion could be attributed to extraction procedure (Table 2 and Fig. 1b). The slope and intercept of the curves were also within required limits

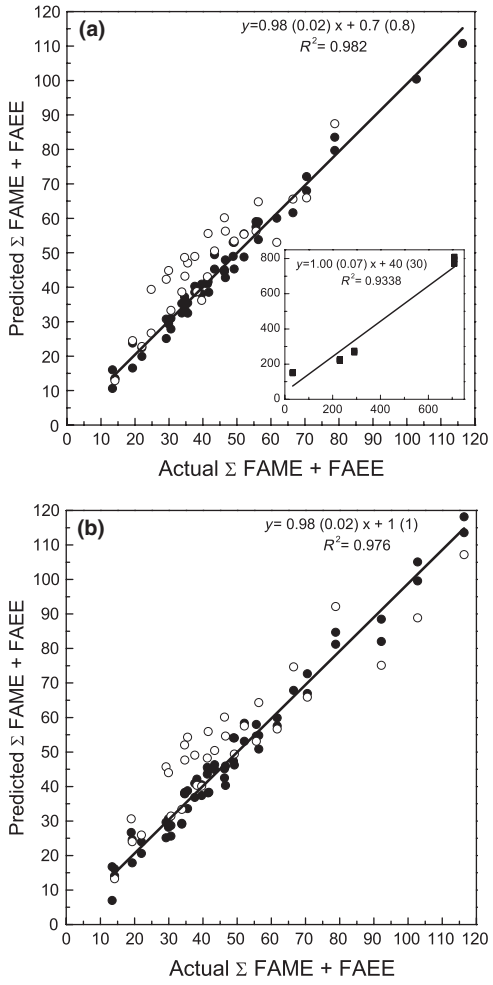

Figure 2 Actual vs. predicted Σ (FAMEs + FAEEs) for liquid chromatography (LC) calibration (●) and validation samples (○) (a). Prediction for SPE2 samples (a, subplot). Actual vs predicted Σ (FAMEs + FAEEs) content for SPE1 calibration (●) and validation samples (○) (b). Equations curves: Predicted = slope (SD_{slope})* Actual + intercept ($SD_{intercept}$). FAMEs, methyl esters of fatty acid; SPE, solid phase extraction.

(1 and 0). On the other hand, very few samples were analysed using the SPE2 method, making the development of a PLS model impossible. Nevertheless, SPE2 samples were analysed using PLS model developed with LC samples obtaining only an estimative prediction (Fig. 2a). As expected, almost all samples in the SPE2 group were out of the calibration range, making both figures of merit and statistical analyses meaningless.

PLS models for RFF determination

Precision and accuracy of RFF-PLS models were accessed by the evaluation of prediction errors of

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(17%) and mean recovery (103%, mean value of sample recovery rates), and the latter were close to quantitative. Low bias and absence of systematic errors were demonstrated by the slopes and intercepts of the actual vs. predicted regression lines (Fig. 3a), which had values of unity and zero in their 90% joint confidence interval, respectively.

The same PLS parameters (pre-treatments, PLS factors and spectral range) were used to construct PLS models with SPE1 samples, and satisfactory results were found (Fig. 3b), which demonstrate the interchangeability of the method. Recoveries were similar to that obtained for LC samples (107 ± 37%, Table 2), but dispersion was higher. Consistent with the above, RFF values for SPE2 samples were out of the calibration range, being inappropriate to carry out predictions about this group. In addition, this group was too small to make a division in the validation and calibration groups: for this reason, SPE2 samples were not analysed.

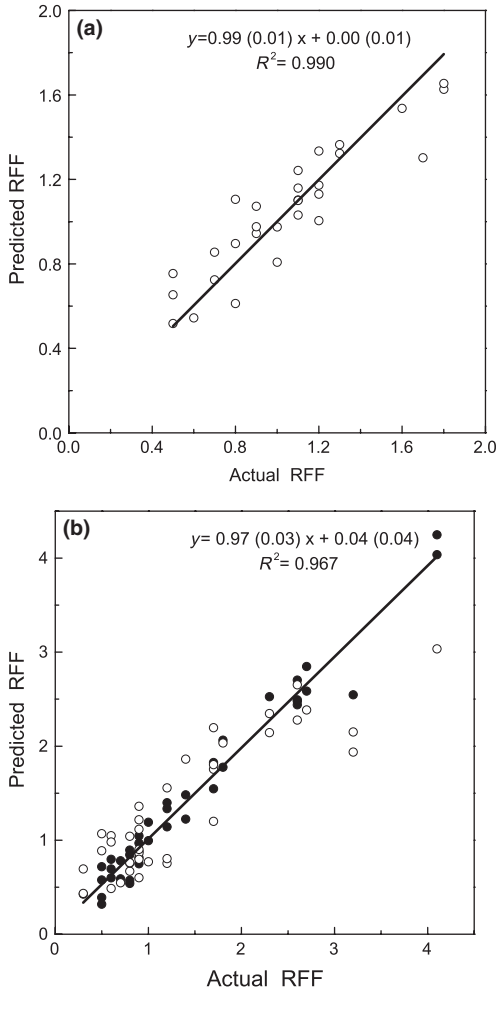

Figure 3 Actual vs predicted ratio of FAEEs/FAMEs (RFF) for liquid chromatography (LC) calibration (●) and validation samples (○) (a). Actual vs Predicted RFF content for solid phase extraction (SPE)1 calibration (●) and validation samples (○) (b). Equations curves: Predicted = slope (SD_{slope})* Actual + intercept ($SD_{intercept}$).

Conclusions

Fourier transform mid-infrared-PLS methodology was developed and demonstrated to be useful for analytical predictions of the Σ (FAMEs + FAEEs) content and RFF in EVOOs. The FTIR-PLS models provided results that were statistically similar to official procedures (LC), in terms of analytical performance, and are thus a useful tool for screening procedures. Moreover, the procedure permits high sample throughput, with significant time-saving, and is more environmentally friendly because no pre-treatment of samples was required. The results obtained here need to be confirmed through the acquisition of a larger set of olive oils, in terms of sample number and Σ (FAMEs + FAEEs) content, to increase the robustness of the model.

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