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Analytical Methods

Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares

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ABSTRACT

A rapid Fourier transformed infrared (FTIR) attenuated total reflectance (ATR) spectroscopic method was applied to the determination of fatty acid (FA) profile and peroxide value (PV) of virgin olive oil. Calibration models were constructed using partial least squares (PLS) regression. A FA calibration model was constructed in the spectral range from 3033 to 700 cm^{-1}. Oleic acid (62.0–80.0%), linoleic acid (5.3–15.0%), saturated fatty acids (SFA, 12.6–19.7%), mono-unsaturated fatty acids (MUFA, 64.4–81.0%) and poly-unsaturated fatty acids (PUFA, 6.0–15.9%) were considered for chemometric analysis. PV (5.7–15.7 meq O_2 kg^{-1}) was calibrated using the signal of the full spectral range 4000–700 cm^{-1} with first derivative pre-treatment. The LODs of the FTIR-chemometric methods were: 3.0% for oleic acid, 0.5% for linoleic acid, 1.3% for SFA, 3.0% for MUFA, 0.3% for PUFA and 1.0 meq O_2 kg^{-1} for PV. Analytical methods were evaluated by use of validation samples ($n = 25$ for all the FA related parameters and $n = 10$ for PV) with nearly quantitative recovery rates (98–103%). The proposed method provided results comparable to official procedures, with the advantages of being less expensive and more rapid.

1. Introduction

Virgin olive oil (VOO) is one of the most used dressings and cooking fats in Mediterranean countries, and is a central component of the diet in this region (Bendini et al., 2007a). One of the most important characteristics of olive oils is the presence of a high content of oleic acid, which accounts for 60–80% of the total fatty acids (FA) and for approximately 90% of the mono-unsaturated fatty acids (MUFA) (Uceda & Hermoso, 1998). Poly-unsaturated fatty acids (PUFA), namely linoleic and linolenic acids, account for 5–8% of total fatty acids, and together with MUFA are considered to be nutritionally favourable (FAO, 1978). VOO is an excellent oil for high temperature cooking being rich in MUFA, low in PUFA, particularly in linolenic acid, and in addition it is free of *trans* fatty acids, thus fulfilling all major criteria of the stable frying fats (Gomez-Alonso, Fregapane, Salvador, & Gordon, 2003).

During storage and cooking triacylglycerols may undergo a series of reactions such as hydrolysis, oxidation, isomerisation, and polymerisation (Kamal-Eldin & Appelqvist, 1996; Takeoka, Perrino, & Buttery, 1996). The hydrolized MUFA and PUFA may undergo oxidative reactions leading to the generation of peroxides, aldehydes and ketones, with an overall reduction in quality in terms of nutritional and sensory properties (Takeoka, Full, & Dao, 1997).

Determination of SFA, MUFA and PUFA is usually carried out according to the official method by capillary gas chromatography with flame ionisation detection (cGC-FID), which is time consuming and involves sample pre-treatment; peroxide value determination also relies on a titration involving large amounts of solvents and reagents (EEC Reg. No. 2568/91). Fourier transformed infrared spectroscopy-based methods (FTIR) are fast, simple to perform and do not require sample pre-treatment. FTIR has been successfully used in the last 15 years to quantitate a number of olive oil parameters such as acidity (Al-Alawi, van de Voort, & Sedman, 2004; Bertran et al., 1999; Iñón, Garrigues, Garrigues, Molina, & de la Guardia, 2003a; Iñón, Garrigues, Garrigues, Molina, & De la

Abbreviations: ATR, attenuated total reflectance; FA, fatty acid; FTIR, Fourier transformed infrared spectroscopy; GC, gas chromatography; LOD, limit of detection; LOQ, limit of quantification; LV, latent variables; MUFA, mono-unsaturated fatty acids; PLS, partial least squares; PUFA, poly-unsaturated fatty acids; PV, peroxide value; REC%, percentage relative error in calibration; RMSD, root mean square deviation; SFA, saturated fatty acids; VOO, virgin olive oils.

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Guardia, 2003b), peroxide (Bendini et al., 2007b; Li, van de Voort, Ismail, & Cox, 2000a) and iodine values (Li et al., 2000b), in addition to olive oil authenticity (Lerma-García, Ramis-Ramos, Herrero-Martínez, & Simó-Alfonso, 2009; Marigheto, Kemsley, Defernez, & Wilson, 1998; Yang & Irudayaraj, 2001) and freshness (Sinelli, Cosio, Gigliotti, & Casiraghi, 2007). ATR-FTIR has been interestingly used in combination with multivariate analysis to discriminate samples at different periods of storage in dark or light conditions. More recently (Koca, Rodriguez-Saona, Harper, & Alvarez, 2007; Yoshida et al., 2008), FTIR has been used for the monitoring and quantitation of free fatty acids; in the first application it was used to measure short-chain free fatty acids, after an extraction procedure, from cheese (Koca et al., 2007), whilst long chain free fatty acids, saturated, mono-unsaturated, and poly-unsaturated (Yoshida et al., 2008) have been monitored in samples from oral mucosa. Both applications were related to the determination of free fatty acids, whereas with our approach the total composition of fatty acids, both free and esterified, is achieved. Spectroscopic methods are also well suited for on-line or at-line process monitoring, allowing fast and precise control of various production steps. For these reasons, FTIR-based methodology is a valuable tool for the rapid determination of SFA, MUFA and PUFA and other quality indexes.

Multivariate calibration is a useful chemometric method for analysis of complex mixtures as it enables the rapid and simultaneous determination of each component in the mixture without time-consuming separations and with minimum sample preparation. Partial least squares (PLS) is a factor-based multivariate calibration method that decomposes data into spectral loadings and scores and, assuming compliance with Beer's Law, builds the corresponding calibration models from these new variables. The background of PLS has been extensively discussed (Geladi & Kowalski, 1986; Martens & Naes, 1989; Thomas, 1994). Due to their potential ability to increase minor spectral features, derivative techniques have also been used in conjunction with spectrophotometric methods, especially when improved sensitivity and selectivity were required (Geladi & Kowalski, 1986; Rossi & Pardue, 1985).

The aim of this work was to develop and validate an analytical method based on ATR-FTIR spectroscopy, in combination with multivariate calibration methodologies, for the simultaneous evaluation of important quality parameters of virgin olive oil, including the composition of total fatty acids and peroxide value. A series of virgin olive oil samples coming from different Italian regions and produced during two harvest seasons were analysed by official methods, taken as a reference, and by the proposed ATR-FTIR method. This approach represents an easy and convenient means for monitoring olive oil quality with the advantage of ease of operation, rapidity and no sample pre-treatment.

2. Experimental

2.1. Samples

A series of 86 virgin olive oils (VOOs) were sampled from different Italian regions (Abruzzo, Marche and Puglia) during the harvest seasons 2006 and 2007. The olives differed in terms of cultivar, ripening degree, area of growth and extraction system (type, productive capacity and manufacturer). Samples produced during the harvest seasons 2007 have been analysed in a range from 1 week and 2 months after production.

A set of 25 of the 86 samples was selected at random as an external validation set for FA determination, whilst the rest were used to build up the calibration model. The PV determination was carried out with the samples from the harvest season 2006. Ten of the 34 samples were used as an external validation set, whilst the remaining were used to build the calibration model.

2.2. Instrumentation and spectral acquisition

All spectra were acquired on a Tensor 27™ FTIR spectrometer system (Bruker Optics, Milan, Italy), fitted with a Rocksolid™ interferometer and a DigiTect™ detector system coupled to an attenuated total reflectance (ATR) accessory. The ATR accessory (Specac Inc., Woodstock, GA, USA) was equipped with a ZnSe 11 reflection crystal. Analyses were carried out at room temperature. Spectra were acquired (32 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.

For each sample (1–1.5 mL uniformly spread throughout the crystal surface), the absorbance spectrum was collected against a background obtained with a dry and empty ATR cell. One spectrum per sample was recorded. Before acquiring each spectrum, the ATR crystal was cleaned with a cellulose tissue soaked in *n*-hexane and then rinsed with acetone.

2.3. Data processing and calibration models

Data were exported in an ASCII compatible OPUS 6.0 software format and processed employing PLS routines written for Matlab (Mathworks Inc., Natick, MA, USA) with 'ad hoc' PLS routines. Partial least squares models were computed on respective training set samples for each parameter. A moving-windows strategy was also executed by 'ad hoc' Matlab routines.

2.4. Reference analyses

Peroxide value: This parameter (PV, expressed as meq O_2 kg^{-1} of oil) was determined according to the official method described in

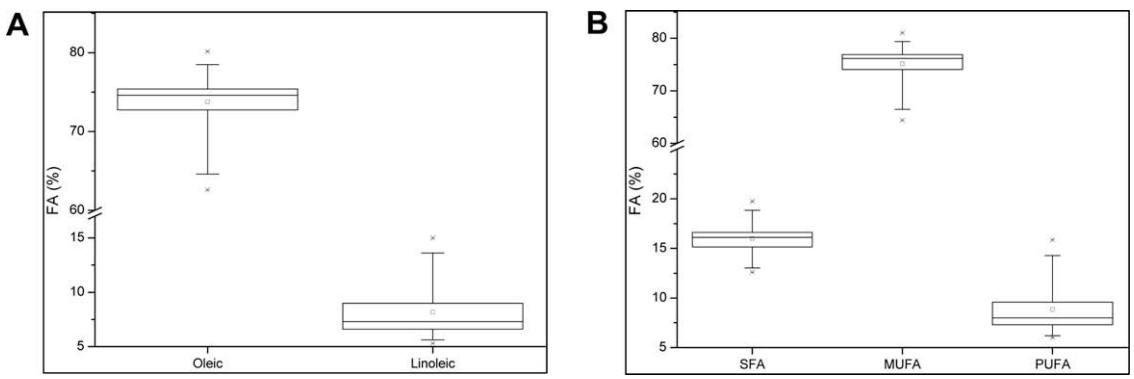

Fig. 1. Box and whiskers plot showing the distribution of (A) oleic and linoleic acid, and (B) SFA, MUFA and PUFA carried out by GC analysis in the 86 olive oil samples.

the EEC Reg. No. 2568/91 and subsequent amendments (European Community, 1991). The analyses were carried out on samples stored for 3 months in the dark at room temperature.

Fatty acid composition: The fatty acid composition of oil samples was determined as the corresponding methyl esters by capillary gas chromatography (GC) (Clarus 500 GC Perkin Elmer Inc., Shelton, CT, USA) analysis according to Bendini, Cerretani, Vecchi, Carrasco-Pancorbo and Lercker (2006). The esters were prepared by alkaline treatment carried out by mixing 0.05 g of oil dissolved in 2 mL of *n*-hexane with 1 mL of 2 N potassium hydroxide in methanol, according to Christie (1998).

3. Results and discussion

The fatty acid profiles of VOOs were obtained by capillary GC. As reported in Fig. 1A, oleic acid and linoleic acid accounted for 62.0–80.0% and 5.3–15.0%. The sum of SFA (C16:0, C17:0, C18:0, C20:0, C22:0 and C24:0), MUFA (C16:1, C17:1, C18:1 and C20:1) and PUFA (C18:2 and C18:3) were 12.6–19.7%, 64.4–81.0%; and 6.0–15.9% range, Fig. 1B. These wide intervals were particularly suited to build a robust calibration model for the FTIR method and for a challenging validation set. PV were in the interval 5.7–15.7 meq O_2 kg^{-1} of oil.

ATR-FTIR spectra of the virgin olive oil samples are reported in Fig. 2A. The oils have different substitution patterns, also differing in the chain length of the acyl moieties, as well as in their unsaturation degree and position. Guillén and Cabo (1999) reported a list of IR bands and shoulders of some edibles oils and made a tentative assignment to functional groups. The IR region of interest for FA profile was between 3033 and 700 cm^{-1} (Fig. 2B). Moreover they attributed to the very weak O–H stretching of the hydroperoxide group the band located at 3444 cm^{-1}.

Starting from these considerations, the initial calibration ranges of spectra were 3033–700 cm^{-1} for FA (Fig. 2B) and 4000–700 cm^{-1} for PV (Fig. 2A).

Spectral differences in these regions are not easily detectable by univariate analysis. Hence for quantitative analysis a multivariate approach was used to access the useful information.

3.1. FTIR data correction and pre-treatment

For the FA profile quantification, the region between 2400 and 2260 cm^{-1} was deleted prior to calculations because of its low signal to noise ratio and the presence of fluctuations independent from sample composition (Iñón et al., 2003a, 2003b). The region between 4000 and 3033 cm^{-1} was also removed because it contained no useful chemical information and contributed to instrumental noise (Fig. 2B). Therefore, 1422 data points were considered, in the wavenumber ranges from 3033 to 2400 and from 2260 to 700 cm^{-1}. Data were mean-centred before calculation (Fig. 2B).

On the other hand, full spectra were taken for the determination of PV, and their first derivative (Fig. 2C) was employed in order to

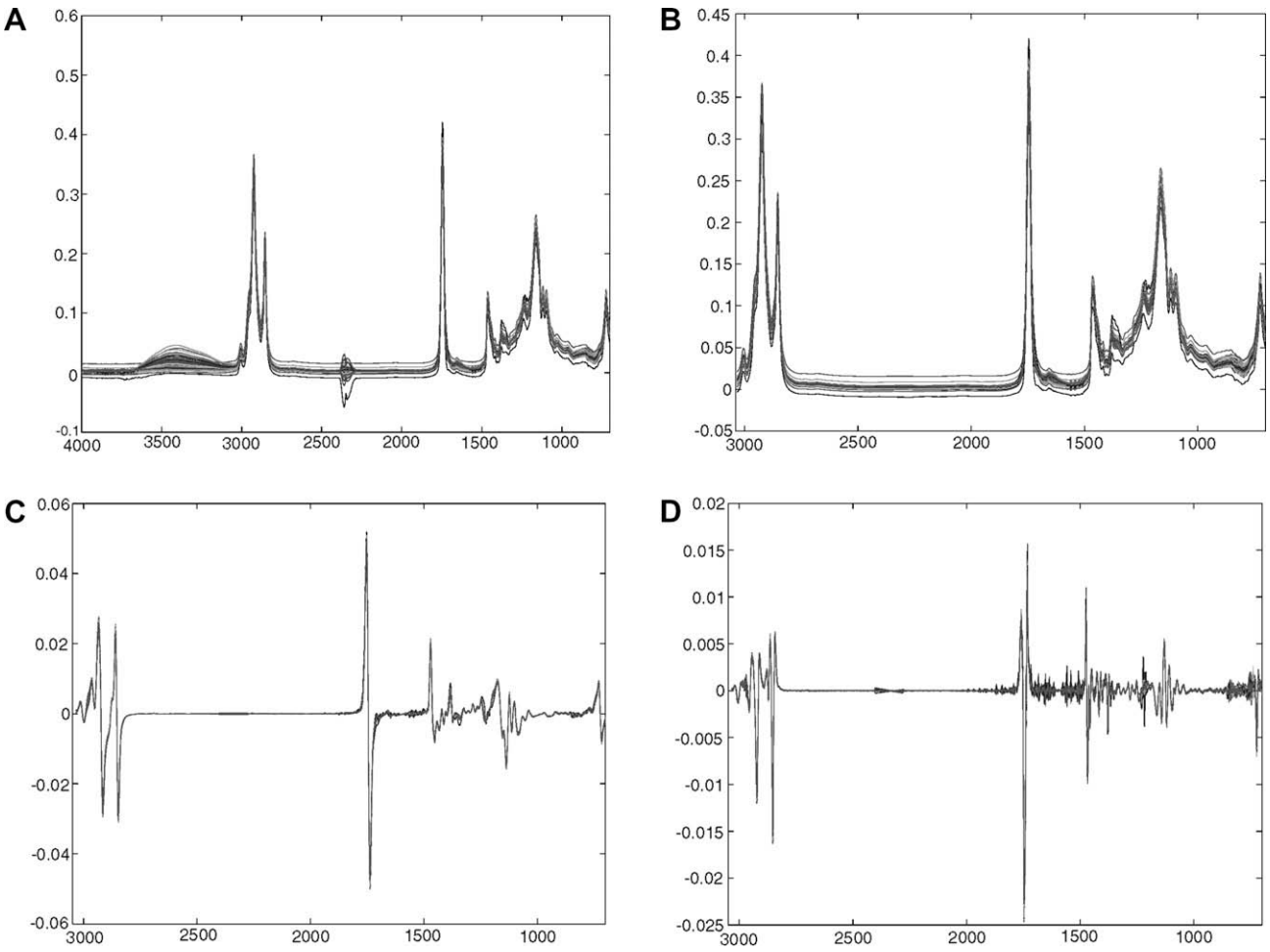

Fig. 2. (A) Full FTIR spectra (4000–700 cm^{-1}); (B) polished spectra in the selected spectral range (3033–700 cm^{-1}); (C) first derivative and (D) second derivative of full spectra.

Table 1
Calibration and validation of oleic acid and linoleic acid.

Property	Oleic acid	Linoleic acid
Calibration		
Spectral range (cm^{-1})	3033–2400 plus 2260–700	
Linear range (% in VOO)	62.0–80.0	5.0–15.0
Number of factors (LV)	14	13
Number of training samples (N)	61	61
PRESS[a]	10.88	9.33
Root mean square deviation (RMSD)	0.42	0.39
Relative error in calibration (REC%)	0.51	4.64
r^2	0.99	0.98
Selectivity	0.18	0.20
Sensitivity (SEN)	0.0009	0.0016
Analytical sensitivity, $[\gamma = (SEN/\sigma_o)]$	0.18	1.17
Minimum difference (% in VOO)	5.6	0.9
Limit of detection (LOD, % in VOO)	3.0	0.5
Limit of quantification (LOQ, % in VOO)	10.0	1.7
Validation		
Number of validation samples	25	25
Recovery rates (%)	100	98
Relative error in prediction (REP%)	1	7
r^2	0.92	0.94
y_0	4 ± 4	0.1 ± 0.4
Slope	0.94 ± 0.06	0.96 ± 0.05

[a] $PRESS = \sum_{i=1}^{N}(y_i - \hat{y}_i)^2$; $RMSD = (PRESS/N)^{0.5}$; $REP\ (\%) = 100 * RMSD/\bar{y}$, and $r^2 = [\sum_{i=1}^{N}(y_i - \hat{y}_i)^2]/[\sum_{i=1}^{N}(y_i - \bar{y}_i)^2]$, where $y, \hat{y}, \bar{y}$ represent the true, predicted and mean concentration of the analyte, in the N training samples, respectively.

Table 2
Calibration and validation of the FTIR-PLS determination of MUFA, PUFA and SFA.

Property	MUFA	PUFA	SFA
Calibration			
Spectral range (cm^{-1})	3033–2400 plus 2260–700		
Linear range (% in VOO)	64.0–81.0	6.0–15.9	12.6–19.7
Number of factors (LV)	14	15	13
Number of training samples (N)	61	61	61
PRESS[a]	10.6	2.5	6.1
Root mean square deviation (RMSD, %)	0.42	0.20	0.32
Relative error in calibration (REC%)	0.56	2.23	1.95
r^2	0.99	0.99	0.96
Selectivity	0.17	0.20	0.14
Sensitivity (SEN)	0.0009	0.0015	0.0020
Analytical sensitivity, $[\gamma = (SEN/\sigma_o)]$	0.17	2.07	0.32
Minimum concentration difference % in VOO	6.0	0.48	3.17
Limit of detection (LOD, % in VOO)	3.0	0.3	1.3
Limit of quantification (LOQ, % in VOO)	10.0	0.9	4.5
Validation			
Number of validation samples	25	25	25
Recovery rates (%)	100	103	98
Relative error in prediction, (REP, %)	1	4	6
r^2	0.89	0.98	0.71
y_0	5 ± 5	0.4 ± 0.2	5 ± 1
Slope	0.93 ± 0.07	0.98 ± 0.03	0.7 ± 0.1

[a] $PRESS = \sum_{i=1}^{N}(y_i - \hat{y}_i)^2$; $RMSD = (PRESS/N)^{0.5}$; $REP(\%) = 100^{*}\ RMSD/\bar{y}$, and $r^2 = [\sum_{i=1}^{N}(y_i - \hat{y}_i)^2]/[\sum_{i=1}^{N}(y_i - \bar{y}_i)^2]$, where y, $\hat{y}$and $\bar{y}$ represent the true, predicted and mean concentration of the analyte, in the N training samples, respectively.

improve the performance of the method. Derivative data were mean-centred before analysis (Fig. 2C and D).

3.2. PLS models for the FA profile

In order to predict the FA profile (oleic acid, linoleic acid, MUFA, PUFA and SFA) in VOO, three multivariate calibration models were built by the partial least squares regression (PLS) algorithm, using the pre-processed spectral data. The appropriate number of model dimensions, which was individually found out to be two for each quality parameter, was determined applying the Haaland and Thomas statistical criterion ($\alpha = 0.75$) (Haaland & Thomas, 1988). In Table 1, the results regarding oleic and linoleic acid are reported. Low values were obtained for both RMSD (root mean square deviation) and REC% (percentage relative error in calibration), which measure the average error in the analysis and evaluate the goodness of fit of the calibration data to the models developed during calibration. The low LOQs (limit of quantification) and r^2 which describes the goodness of fit of the predicted concentrations to their actual values, and the figures of merit, demonstrated the quality of the models and the suitability of the method for the proposed determinations.

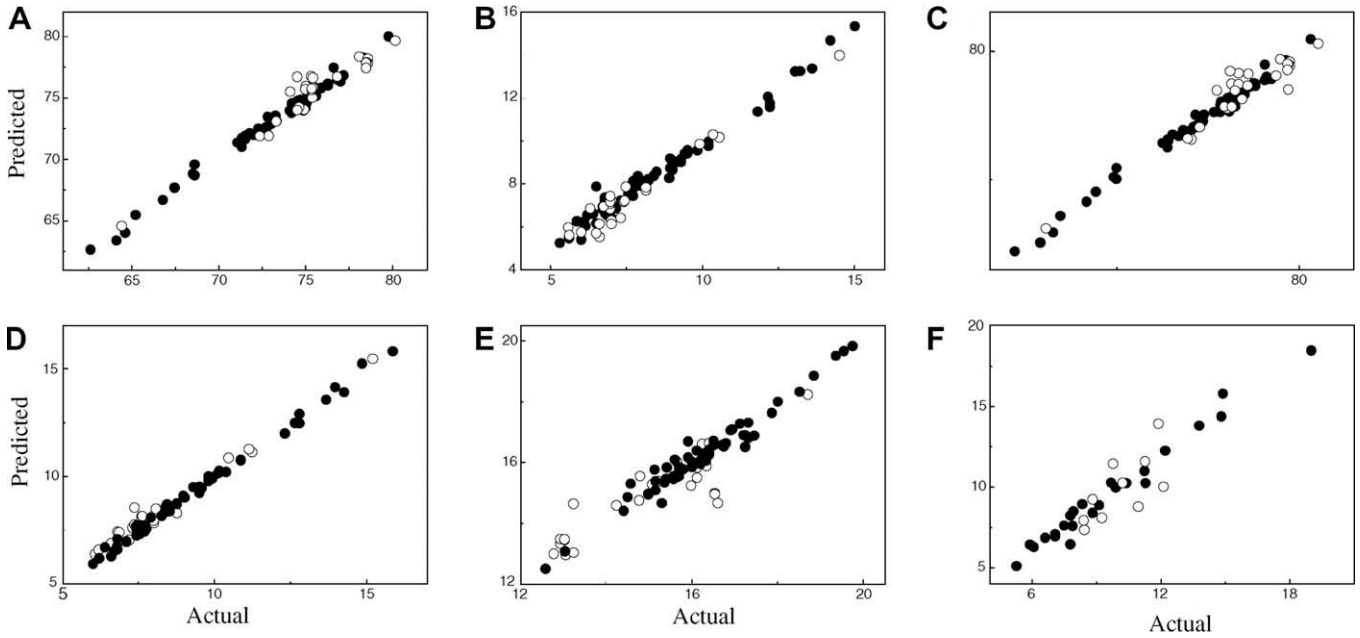

Fig. 3. GC-FID vs. FTIR-PLS predicted values in the calibration (•) and validation (○) sets for (A) OA, (B) LA, (C) MUFA, (D) PUFA and (E) SFA. (F) Peroxide value (meq O_2 kg^{-1}, tritrimetric method) vs. FTIR-PLS predicted concentration.

The validation set exhibited nearly quantitative recoveries (100% and 98% for oleic and linoleic acid, respectively) and relative standard deviations below 7%. The yields obtained are illustrated in Fig. 3A and B, respectively, showing a good agreement between predicted and actual levels of oleic and linoleic acid on validation data sets. The slopes and intercepts of the curves depicted in this plot are close to unity and zero, respectively, indicating low bias and absence of systematic regression errors (Table 1).

A similar procedure was carried out for the determinations of the content of MUFA, PUFA and SFA (Table 2). Calibration models were built, using the pre-processed spectral data, by partial least squares regression (PLS); their critical parameters, including the RMSD, REC and number of retained latent variables (LVs) are listed in Table 2.

Validation of the MUFA, PUFA and SFA PLS models was carried out using the same independent validation set, and the results obtained exhibited prediction errors below 6% when 14, 15 and 13 latent variables were used, respectively. Low bias and absence of systematic errors were demonstrated by the slopes and intercepts of the actual vs. predicted regression lines, which were close to unity and zero, respectively, as depicted in Fig. 3C–E.

3.3. PLS models for determination of PV

In order to predict the content of PV in VOO, different PLS calibration models were initially built employing full spectra and reduced spectral ranges, obtained by using the moving window of variable size strategy (Ferraro, Castellano, & Kaufman, 2001). However, none of these models provided an acceptable calibration and predictions were unsatisfactory. Therefore, use of first (D') and second (D") spectral derivatives as sample data pre-treatment were attempted (Fig. 2C and D). Table 3 lists the calibration and prediction parameters for the model using normal and derivatives spectra. As observed, D' and D" models performed properly for calibration, yielding very good correlation coefficients and low RMSD values; predictions were also very satisfactory, in terms REP and recovery rate values.

Table 3
Calibration and validation of the FTIR-PLS determination of PV, D0: normal spectrum, D': first derivative, D": second derivative.

Property	D^0	D'	D"
Calibration			
Spectral range (cm^{-1})	4000–700	4000–700	4000–700
Calibration range (meq O_2 kg^{-1}oil)	5.7–15.7	5.7–15.7	5.7–15.7
Number of factors (LV)	5	10	7
Number of training samples	24	24	24
PRESS[a]	175	152	191
Root mean square deviation, RMSD (RMSD, %)	1.43	0.69	0.95
Relative error in calibration, REC (%)	15.6	7.2	9.9
r^2	0.80	0.98	0.95
Selectivity	1.0	0.35	0.55
Sensitivity (SEN)	0.0044	0.0001	0.0001
Analytical sensitivity, $[\gamma = (SEN/\sigma_o)]$	1.2	1.1	1.1
Minimum concentration difference (meq O_2 kg^{-1}oil)	0.8	0.9	0.9
Limit of detection (LOD) (meq O_2 kg^{-1}oil)	3.1	1.0	1.6
Limit of quantification (LOQ) (meq O_2 kg^{-1}oil)	10.3	3.4	5.2
Validation			
Number of validation samples	10	10	10
Recovery rates (%)	70	100[b]	100
Relative error in prediction, REP (%)	20	10	10

[a] $PRESS = \sum_{i=1}^{N}(y_i - \hat{y}_i)^2$; $RMSD = (PRESS/N)^{0.5}$; $REP\ (\%) = 100 * RMSD/\bar{y}$, and $r^2 = [\sum_{i=1}^{N}(y_i - \hat{y}_i)^2]/[\sum_{i=1}^{N}(y_i - \bar{y}_i)^2]$, where y, $\hat{y}$ and $\bar{y}$ represent the true, predicted and mean concentration of the analyte, in the N training samples, respectively.

[b] $PV_{actual} = (0.7 \pm 0.3) * PV_{predicted} + (3 \pm 2)$, $r^2 = 0.54$, $n = 10$. With forced y_0 adjustment: $PV_{actual} = (1.07 \pm 0.04)^{*} PV_{predicted}$, $r^2 = 0.99$, $n = 10$.

In view that similar results were obtained from the D' and D" models, the first one was selected for sample analysis, because it displayed a better signal to noise ratio, represented a simpler model and exhibited some time saving in the computer time involved in the derivation process.

The validation of the PLS-D' model for PV was carried out using an independent validation set, and the results obtained exhibited prediction errors of 10%. Low bias and absence of systematic errors were demonstrated by the slopes and intercepts of the actual vs. predicted regression lines (Fig. 3F), which enclosed values of unity and zero in their 90% confidence interval, respectively. However, a more appropriate regression was obtained when the line was forced to pass through the origin (Table 3), yielding a r^2 value over 0.99.

The results of our method (Fig. 3F) improve those obtained by Bendini et al. (2007b) in terms of the measurable range (3.4–15.7 vs. 11.1–49.7 meq O_2 kg^{-1}). This could be due to the use of the entire spectra rather than the 3600 and 3400 cm^{-1} region. The results (Fig. 3F) also improved the measurable range obtained by NIR spectroscopy where the PV value was measured in the interval 0–10 meq O_2 kg^{-1} (Li et al., 2000b). Moreover, in that method, the addition of triphenylphosphine was needed to obtain a measurable signal whereas our method is reagent-less.

4. Conclusions

We have developed an ATR-FTIR-PLS based strategy for the determination of quality parameters of VOO. For the determination of the FA profiles, very good results of PUFA, MUFA and were obtained. Moreover, SFA content shows a very good correlation coefficient and a low RMSD value in calibration whilst the correlation coefficient was less satisfactory in prediction. The proposed approach was more appropriate than selecting a spectral region by the moving window of variable size strategy and furnished a satisfactory determination of linoleic acid, whilst providing excellent results for the quantification of oleic acid (recovery rates = 100 ± 1%).

In addition, the best prediction ability for PV was achieved when the PLS calibration was carried out on the mean-centred first derivative of the spectral data. No systematic and bias errors were detected in the prediction of calibration samples, and the calibration models exhibited satisfactory figures of merit.

The proposed chemometrically-assisted FTIR analysis provided results statistically similar to official procedures of traditional use in terms of analytical performance. Nevertheless, the ATR-FTIR-PLS method developed here is very rapid; the complete determination takes only a few minutes instead of approximately 30 min for the peroxide value (PV) determination by titrimetric analysis and 1 h for the gas-chromatographic analysis of fatty acids as their methyl esters (FAME), considering preparative, analytical and calculation steps. Therefore, the proposed spectroscopic method furnishes a highly convenient alternative in terms of time and solvent savings for routine analysis of a high number of virgin olive oil samples, specially for high throughput determinations along the industrial process without sacrificing accuracy or reproducibility. The procedures also permit high sample throughput and were more environmentally friendly than previously reported alternatives, since no sample pre-treatment was required and virtually no solvent waste was produced.

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References

Al-Alawi, A., Van de Voort, F. R., & Sedman, J. (2004). New FTIR method for the determination of FFA in oils. *Journal of the American Oil Chemists' Society, 81*, 441–446.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A., et al. (2007a). Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules, 12*, 1679–1719.

Bendini, A., Cerretani, L., Di Virgilio, F., Belloni, P., Bonoli-Carbognin, M., & Lercker, G. (2007b). Preliminary evaluation of the application of the FTIR spectroscopy to control the geographic origin and quality of virgin olive oils. *Journal of Food Quality, 30*, 424–437.

Bendini, A., Cerretani, L., Vecchi, S., Carrasco-Pancorbo, A., & Lercker, G. (2006). Protective effects of extra virgin olive oil phenolics on oxidative stability in the presence or absence of copper ions. *Journal of Agricultural and Food Chemistry, 54*, 4880–4887.

Bertran, E., Blanco, M., Coello, J., Iturriaga, H., Maspoch, S., & Montoliu, I. (1999). Determination of olive oil free fatty acid by Fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society, 76*, 611–616.

Christie, W. W. (1998). The preparation of derivatives of fatty acids. In *Gas Chromatography and Lipids* (pp. 64–84). Ayr, Scotland: The Oily Press.

European Community, Commission Regulation (EEC, 1991) No. 2568/91, July 11. *Official Journal of European Communities*, *L248*, 1–83.

Ferraro, M. C. F., Castellano, P. M., & Kaufman, T. S. (2001). A spectrophotometric-partial least squares (PLS-1) method for the simultaneous determination of furosemide and amiloride hydrochloride in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis, 26*, 443–451.

Food and Agriculture Organization of the United Nations (FAO). (1978). Dietary fats and oils in human nutrition. Food and Nutrition Paper, Rome.

Geladi, P., & Kowalski, B. R. (1986). Partial least-squares regression: A tutorial. *Analytica Chimica Acta, 185*, 1–17.

Gomez-Alonso, S., Fregapane, G., Salvador, M. D., & Gordon, M. H. (2003). Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. *Journal of Agricultural and Food Chemistry, 44*, 667–672.

Guillén, M. D., & Cabo, N. (1999). Usefulness of the frequencies of some Fourier transform infrared spectroscopic bands for evaluating the composition of edible oil mixtures. *Fett/Lipid, 101*, 71–76.

Haaland, D. M., & Thomas, E. V. (1988). Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Analytical Chemistry, 60*, 1193–1198.

Iñón, F. A., Garrigues, J. M., Garrigues, S., Molina, A., & de la Guardia, M. (2003a). Selection of calibration set samples in determination of olive oil acidity by partial least squares-attenuated total reflectance-Fourier transform infrared spectroscopy. *Analytica Chimica Acta, 489*, 59–75.

Iñón, F. A., Garrigues, J. M., Garrigues, S., Molina, A., & De la Guardia, M. (2003b). Selection of calibration set samples in determination of olive oil acidity by partial least squares-attenuated total reflectance-Fourier transform infrared spectroscopy. *Analytica Chimica Acta, 489*, 59–75.

Kamal-Eldin, A., & Appelqvist, L. A. (1996). Aldehydic acids in frying oils: Formation, toxicological significance and analysis. *Grasas y Aceites, 47*, 342–348.

Koca, N., Rodriguez-Saona, L. E., Harper, W. J., & Alvarez, V. B. (2007). Application of Fourier transform infrared spectroscopy for monitoring short-chain free fatty acids in Swiss cheese. *Journal of Dairy Science, 90*, 3596–3603.

Lerma-García, M. J., Ramis-Ramos, G., Herrero-Martínez, J. M., & Simó-Alfonso, E. F. (2009). Authentication of extra virgin olive oils by Fourier-transform infrared spectroscopy. *Food Chemistry*, in press.

Li, H., van de Voort, F. R., Ismail, A. A., & Cox, R. (2000a). Determination of peroxide value by Fourier transform near-infrared spectroscopy. *Journal of the American Oil Chemists' Society, 77*, 137–142.

Li, H., van de Voort, F. R., Ismail, A. A., Sedman, J., Cox, R., Simard, C., et al. (2000b). Discrimination of edible oil products and quantitative determination of their iodine value by Fourier transform near-infrared spectroscopy. *Journal of the American Oil Chemists' Society, 77*, 29–36.

Marigheto, N. A., Kemsley, E. K., Defernez, M., & Wilson, R. H. (1998). A comparison of mid-infrared and Raman spectroscopies for the authentication of edible oils. *Journal of the American Oil Chemists' Society, 75*, 987–992.

Martens, H., & Naes, T. (1989). *Multivariate calibration*. Chichester, UK: Wiley.

Rossi, D. T., & Pardue, H. L. (1985). Effects of wavelength range on the simultaneous quantitation of polynuclear aromatic hydrocarbons with absorption spectra. *Analytica Chimica Acta, 175*, 153–161.

Sinelli, N., Cosio, M. S., Gigliotti, C., & Casiraghi, E. (2007). Preliminary study on application of mid infrared spectroscopy for the evaluation of the virgin olive oil "freshness". *Analytica Chimica Acta, 598*, 128–134.

Takeoka, G. R., Full, G. H., & Dao, L. T. (1997). Effect of heating on the characteristics and chemical of selected frying oils and fats. *Journal of Agricultural and Food Chemistry, 45*, 3244–3249.

Takeoka, G. R., Perrino, C., & Buttery, R. (1996). Volatile constituents of used frying oils. *Journal of Agricultural and Food Chemistry, 44*, 654–660.

Thomas, E. V. (1994). A primer on multivariate calibration. *Analytical Chemistry, 66*, 795A–804A.

Uceda, M., & Hermoso, M. (1998). La calidad del aceite de oliva. In D. Barranco, R. Fernandez-Escobar, L. Rallo (Eds.), *El Cultivo del Olivo* (pp. 547–572). Madrid, Spain: Junta de Andalucıa Ediciones Mundi-Prensa.

Yang, H., & Irudayaraj, J. (2001). Comparison of near-infrared, Fourier transform-infrared, and fourier transform-Raman methods for determining olive pomace oil adulteration in extra virgin olive oil. *Journal of the American Oil Chemists' Society, 78*, 889–895.

Yoshida, S., Okazaki, Y., Yamashita, T., Ueda, H., Ghadimi, R., Hosono, A., et al. (2008). Analysis of human oral mucosa ex vivo for fatty acid compositions using Fourier-transform infrared spectroscopy. *Lipids, 43*, 361–372.