Research Article

Rapid FTIR determination of water, phenolics and antioxidant activity of olive oil

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A rapid Fourier transformed infrared (FTIR) attenuated total reflectance (ATR) spectroscopic method was applied to the determination of water content (WC), total phenol amount (TP) and antioxidant activity ($ABTS^{\cdot+}$) of virgin olive oils (VOO) and olive oils. Calibration models were constructed using partial least squares regression. Oil samples with WC ranging from 289 to 1402 mg water/kg oil, with TP from 46 to 877 mg gallic acid/kg oil and with $ABTS^{\cdot+}$ from 0 to 5.7 mmol Trolox/kg oil were considered for chemometric analysis. Better results were obtained when selecting suitable spectral ranges; in particular, from 2260 to 1008 cm^{-1} for WC, from 3610 to 816 cm^{-1} for TP and from 3707 to 1105 cm^{-1} for $ABTS^{\cdot+}$. Satisfactory LOD values by the FTIR-chemometric methods were achieved: 9.4 (mg/kg oil) for WC; 12.5 (mg gallic acid/kg oil) for TP, and 0.76 (mmol Trolox/kg oil) for $ABTS^{\cdot+}$. The evaluation of the applicability of these analytical approaches was tested by use of validation sample sets ($n = 16$ for WC, $n = 11$ for TP and $n = 14$ for ABTS) with nearly quantitative recovery rates (99–114%). The FTIR–ATR method provided results that were comparable to conventional procedures.

Practical applications: The presented method is based on ATR–FTIR in combination with multivariate calibration methodologies and permits a simultaneous evaluation of important quality parameters of VOO (WC, TP and $ABTS^{\cdot+}$). This approach represents an easy and convenient means for monitoring olive oil quality with the advantage of ease of operation, speed, no sample pretreatment and no consumption of solvents. The data obtained with this method are comparable to those obtained using the official reference method. Therefore, the technique is highly plausible as an alternative to the standard procedure for routine analysis or control at-line of production processes.

Keywords: Antioxidant / FTIR / Olive oil / Partial least squares model / Water

Received: May 21, 2010 / Revised: June 23, 2010 / Accepted: July 23, 2010

DOI: 10.1002/ejlt.201000356

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Abbreviations: **ABTS$^{\cdot+}$**, antioxidant activity; **ATR,** attenuated total reflectance; **DEO,** mildly deodorized olive oils; **EJRC,** ellipse of joint region confidence; **FTIR,** Fourier transformed infrared; **OO,** olive oil; **PLS,** partial least squares; **REC,** relative error in calibration; **RMSD,** root-mean-square deviation; **TP,** total phenol content; **VOO,** virgin olive oil; **WC,** water content

1 Introduction

Among edible oils, virgin olive oil (VOO) has nutritional, storage and sensory characteristics that make it a unique and basic ingredient of the Mediterranean diet. These properties are attributed not only to the fatty acid composition (high level of oleic acid), which is not significantly different from refined olive oil, but especially to the high content of phenolic compounds, which are reduced by the refining process (in particular during the bleaching and deodorization phases). Unfortunately, a low phenolic content is also seen in many

VOO currently sold a low prices in supermarkets and discount stores, which are most likely illegal blends of VOO and mildly deodorized olive oils (DEO) [1, 2].

Phenolic compounds, acting as natural antioxidants, increase the resistance of oil to storage and heating [3, 4]. Moreover, phenols are the main contributors to the typical tastes of VOO (bitter and pungent attributes), and may also contribute to the prevention of several human diseases [3].

It is well known that phenolic compounds can act as radical scavengers or chain breakers decreasing the total rate of lipid oxidation and in particular the phenols with a catechol moiety, known as *o*-diphenols, are particularly effective antioxidants [5]. The antioxidant activity ($ABTS^{\cdot+}$) of phenolic fraction is measured by different tests, and the $ABTS^{\cdot+}$ radical scavenging test has been largely utilized [6–12].

Among the minor compounds of olive oil, water has been recently taken into consideration. In fact, olive oil also contains microdrops of water that are dispersed in the lipid phase and stabilized by the aggregation–dissolution of a group of polar, water-soluble and/or water-compatible substances such as mineral salts, free acids, diglycerides, phospholipids, alcoholic and phenolic substances. The water content (WC) in edible olive oils varies from 300 to 2000 mg/kg of oil, and is closely affected by several factors: extraction technologies (continuous *versus* traditional systems), technological variables used to process olives [13], addition of extraction coadjuvants, filtration procedures and storage conditions [14]. Therefore, because of the capacity of water to dissolve phenolic compounds and other small or medium-sized molecules having a low affinity to oily phase, water contributes directly to the taste perception of oil bitterness and pungency as well as indirectly to its oxidation stability [14].

In particular, VOO is characterized by an average WC between 1000 and 1200 mg/kg of oil, whereas due to the refining processes which decrease the WC [13], olive oils and olive pomace oils generally have $<$500 mg/kg of oil. The amount of water has also been proposed [13] as an analytical parameter to detect 'mild deodorization'. In fact, this practice is employed to reduce or eliminate small molecules such as volatile and polar compounds, which are responsible for off-flavours of olive oil. This effect is due to a sort of molecular distillation under vacuum or in a stream of nitrogen at a low temperature or by absorption mechanisms on high polarity powders or filtering membranes. As these treatments alter the compositional balance of polar minor compounds in the oil, they affect also the stability of microemulsified water, which inevitably decreases. Hence, samples of mildly deodorized oil or blends of VOO and mildly deodorized oil are expected to have $<$700 mg/kg of oil [13].

The determination of the WC in edible oils has been commonly carried out by the Karl Fischer titration method [13, 15]. In addition, spectroscopic Fourier transformed infrared (FTIR) coupled with chemometric methods have been successfully used to detect olive oil adulteration [16–18] and freshness [19]. The chemometric algorithm partial least squares (PLS) has been repeatedly and extensively used to obtain different quality parameters of edible oils [20–25]. In particular, FTIR–PLS has been recently applied to the evaluation of the fatty acid composition and other quality parameters of VOO [26] and also to predict the total antioxidant capacity of the red wine samples [27].

PLS is a factorial multivariate calibration method that decomposes spectral data into loadings and scores, building the corresponding calibration models from these new variables [28]. This method requires that the analytes comply with Beer's Law in order for the property to be measured.

The aim of the present work was to develop a new application of the FTIR–ATR–PLS association as a rapid, inexpensive and nondestructive tool able to determine very important parameters linked to the quality of olive oils as the water and phenol content and antioxidant activities. This approach represents an easy and convenient method for monitoring olive oil quality with the advantages of ease of operation, high sample turnover and no sample pretreatment.

2 Materials and methods

2.1 Samples

The 47 VOO and 7 olive oil (OO) samples used in this study were purchased in farms and markets in Italy. Samples were stored in dark bottles without headspace at room temperature before analysis.

All experiments and calculations were done in triplicate.

2.2 FTIR spectra

The FTIR spectra were acquired on a Tensor 27^{TM} FTIR spectrometer system (Bruker Optics, Milan, Italy), fitted with a Rocksolid^{TM} interferometer and a DigiTect^{TM} 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 (2 mL uniformly spread throughout the crystal surface), the absorbance spectrum was collected against a background, obtained with a dry and empty ATR cell. Three spectra per sample were 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 Determination of water content in virgin olive oil

The WC was analyzed with a TitroMatic 1S instrument (Crison Instruments, S. A.; Alella, Barcelona, Spain). This

measurement uses a Karl-Fischer titration based on a bivoltametric indication (2-electrode potentiometry). A solution of chloroform/hydranal solvent oil (a methanolic solvent) 2:1 v/v was used to dissolve the sample and hydranal-titran 2 was used as a titrating reagent (hydranal-titran 2 and hydranal-solvent oil were from Riedel-deHaën, Seelze, Germany). Each sample was introduced three times and the quantity of the sample was measured using the back weighting technique. The sample was dissolved in a solution of chloroform:hydranal-solvent oil, and the titrating reagent was added until the equivalence point was reached. The quantity of water was expressed as mg water/kg of oil (mean of $n = 3$).

2.4 Extraction of polar phenolic fraction

Phenolic compounds were extracted from VOO and OO by a liquid–liquid extraction method according to Pirisi *et al.* [29]. The dry extracts were dissolved in 0.5 mL of a methanol/water (50:50, v/v) solution and filtered through a 0.2 μm syringe filter (Whatman Inc., Clinton, NJ, USA). Extracts were frozen and stored at −43 °C.

2.5 Spectrophotometric determination of total phenol content

The total phenol (TP) content of the extracts was measured using the Singleton and Rossi [30] method with slight modifications. Phenolic extracts were analyzed spectrophotometrically with Folin–Ciocalteu reagent and absorbance was determined at 750 nm. Total phenols were quantified using a gallic acid calibration curve ($r^2 = 0.994$). The results were expressed as mg gallic acid/kg of oil.

2.6 The $ABTS^{\cdot+}$ assay

A stable stock solution of $ABTS^{\cdot+}$ was produced by reacting a 7 mmol/L aqueous solution of ABTS (Sigma, ST. Louis, MO, USA) with 2.45 mmol/L potassium persulphate (Sigma) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Before use, the $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm at 30°C. Next, 1 mL of this $ABTS^{\cdot+}$ solution was added to 0.01 mL of extract and the decrease in absorbance was recorded for 10 min. Absorbance values were corrected for radical decay using a blank solution (0.01 mL of methanol/water 50:50, v/v). Measurements were made in triplicate and the $ABTS^{\cdot+}$ was calculated as mmol Trolox equivalent for kg of oil ($r^2 = 0.9830$) [31].

2.7 Statistical analysis

Data were exported in ASCII compatible OPUS 6.0 format with the assistance of an OPUS macro script and processed employing MVC1 routines [32] written for Matlab (Mathworks Inc., Natick, MA, USA).

PLS models were computed for each analytical parameter with the respective training set samples. A moving-window strategy was also executed with the MVC1 program, setting the minimum window width to 50 sensors.

3 Results and discussion

3.1 Water content, total phenol and antioxidant activity of virgin olive oil and olive oil samples

Forty-seven samples of both VOO and OO (this category of oils is obtained by blending VOO with refined olive oil so that OO are normally characterized by a lower phenolic compound and WC) were analyzed with the aim of obtaining a wide range of variability in the selected parameters. In fact, as shown in Fig. 1, WC ranged from 289 to 1402 mg water/kg oil, TP from 46 to 877 mg gallic acid/kg oil and $ABTS^{\cdot+}$ from 0 to 5.7 mmol Trolox/kg oil. These wide intervals were particularly suited to build a robust calibration model for the FTIR method and thus a challenging validation set.

The TP of VOO, evaluated by the Folin–Ciocalteu test, may range between 40 and 900 mg gallic acid/kg of oil. Nevertheless, a higher concentration (up to 1000 mg gallic acid/kg) has also been reported in several olive oils [33, 34]. As reported by many authors [6–10, 35], the $ABTS^{\cdot+}$ of olive oil, measured by the ABTS radical scavenging test, is highly linear correlated to the TP determined by spectrophotometry, which confirms the important role of phenolic compounds (in particular *o*-diphenols) in protection from autooxidation.

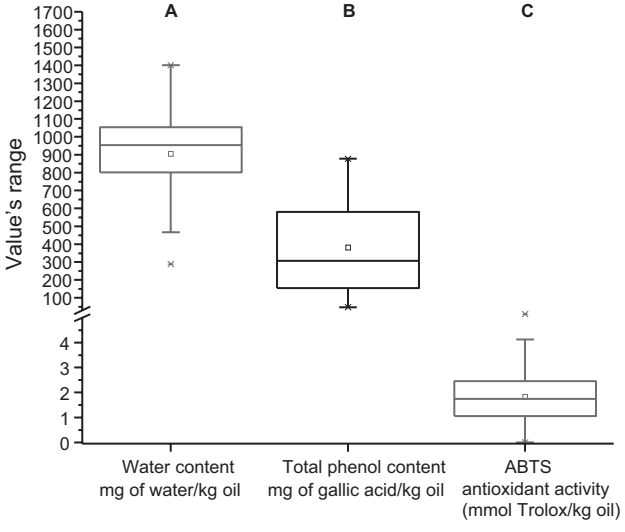

Figure 1. Box and whiskers plot showing the distribution of (A) water content, (B) total phenol content and (C) $ABTS^{\cdot+}$ antioxidant activity in VOO and OO samples.

Both agronomic factors (olive cultivar, place of origin, olive ripening stage, agronomic techniques adopted) and the technological parameters used to process olives (extraction methods and storage conditions) significantly affect the TP as well as the $ABTS^{\cdot+}$ and WC in olive oil so that they vary from very low amounts to high values [6, 13]. For example, VOOs obtained by different extraction techniques from healthy and medium ripe olives coming from different Sardinian cultivars and grown in different geographical areas have a TP amount ranging from 108 to 441 mg gallic acid/kg oil, and an $ABTS^{\cdot+}$ ranging from 1.35 to 2.45 mmol Trolox/kg oil [7]. Moreover, some olive cultivars as Coratina or Nostrana di Brisighella may contain a very high content of phenols (>500 mg gallic acid/kg oil), and many other VOOs produced by unripe olives are characterized by elevated TP and $ABTS^{\cdot+}$ values [3, 13]. In contrast, VOOs obtained from ripe olives or from specific olive cultivars may generally be characterized by a moderate phenolic content (*e.g.* <100–200 mg gallic acid/kg oil), and consequently by poor antioxidant capacity [3, 7, 36].

Technological choices, such as the kind of extraction and filtration system, may have a more direct influence on the content of water remaining in the VOO and, therefore, the antioxidant power in terms of the TP and the $ABTS^{\cdot+}$. Generally, VOO produced by two-phase plants and filtered contain a lower amount of water than those obtained by a traditional system and veiled, *e.g.* 800–900 mg compared to 1100–1300 mg water/kg oil [13, 37, 38].

Additionally, the refining process applied to produce refined oils in OO causes a severe depletion of water and phenolic molecules, and thus a decrease of the antioxidant power, reaching values less than 500 mg water/kg of oil (WC) and 100 mg gallic acid/kg of oil (TP). As for the $ABTS^{\cdot+}$, it can be less than 0.70 mmol Trolox/kg oil [11, 13]. For example, Pellegrini and coworkers [11, 35] studied the TP and the $ABTS^{\cdot+}$ of several commercial olive oils: commercial VOO showed TP and $ABTS^{\cdot+}$ values ranging from 73 to 265 mg gallic acid/kg oil and from 1.53 to 2.69 mmol Trolox/kg oil, respectively. However, the antioxidant power was lower in samples of OO. In fact, the TP values ranged from 14 to 30 mg gallic acid/kg oil, and the $ABTS^{\cdot+}$ of OO ranged from 0.72 to 1.06 mmol Trolox/kg oil.

Mildly deodorized olive oils commonly show physicochemical characteristics similar to those of genuine VOO, although their antioxidant power is highly decreased. In fact, some studies have reported that the WC of blends of VOO and DEO may be less than 700 mg/kg oil, while the TP content may range from 100 to 250 mg gallic acid/kg oil with a $ABTS^{\cdot+}$ less than 0.70 mmol Trolox/kg oil [2]. Domestic heating treatments such as frying, boiling and conventional and microwave heating can also affect the antioxidant properties of commercial olive oils, strongly inducing a high depletion of the TP and, therefore, a decrease of the $ABTS^{\cdot+}$ as a result of the different extent of radical formation and different thermal stability of phenolic compounds [2, 11]. Finally, the storage conditions (storage time and temperature) can significantly decrease the antioxidant power of VOO. A reduction of the TP to values less than 100–200 mg gallic acid/kg oil, and therefore a loss of the antioxidant power was observed after production up to 1 year with more remarkable losses in the last 6 months of storage [8]. With regard to the storage temperature, sudden changes in the temperature may have a significant effect both on the TP and the $ABTS^{\cdot+}$. Although a low storage temperature may slow radical formation, it can still destabilize microdrops of water in which phenols are dissolved, so that the antioxidant power will decrease [6, 39, 40].

3.2 PLS models construction

In order to predict the WC, TP and $ABTS^{\cdot+}$ in VOO, three multivariate calibration models were built by the PLS regression algorithm, using digitalized spectral data. As reported in Fig. 2, the optimal number was obtained by Haaland and Thomas statistical criterion ($a = 0.75$) [41] using Predicted REsidual Sums of Squares (PRESS) (Eq. (1)). PRESS is the sum of squares of the differences between predicted ($\hat{C}_i$) and real values of analyte concentrations (C_i); thus, it is a measure of the predicting ability of the calibration model

$$\mathrm{PRESS} = \left[\sum_{i=1}^{N} (\hat{C}_i - C_i)^2\right]^{0.5} \tag{1}$$

The optimal spectral intervals were obtained by a minimum PRESS search employing the moving window strategy with the 'leave one out' cross-validation procedure (Fig. 3). This procedure is known to enhance the performance of the method [42]. The 'leave one out' cross-validation technique systematically generates PLS validation models by excluding one by one each sample from the dataset and then predicting the value for the omitted sample. After this is accomplished for every sample in the dataset, a PRESS for the PLS cross-validated model is calculated. Additionally, good values of statistical parameters, such as the root-mean-square deviation of calibration (RMSD, Eq. (2)) and the relative error in calibration (REC (%), Eq. (3)) were found (Table 1)

$$\mathrm{RMSD} = \left[\frac{\mathrm{PRESS}}{N}\right]^{0.5} \tag{2}$$

$$\mathrm{REC}(\%) = 100\frac{\mathrm{RMSD}}{C_{\mathrm{mean}}} \tag{3}$$

As shown in Table 1, the spectral range selection permitted an increase of the R^2 value for WC and TP, while no increment in the $ABTS^{\cdot+}$ evaluation was observed for R^2. The lower value of R^2 for $ABTS^{\cdot+}$ prediction could be explained considering that this test is based on a kinetic measure having a higher intrinsic variation. In the same

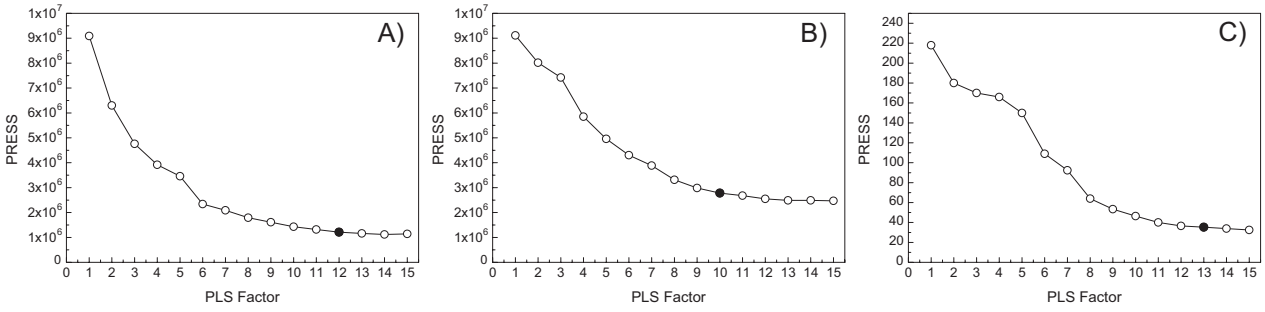

Figure 2. PRESS value *versus* PLS factors for PLS models of (A) water content [(mg/kg oil)^2], (B) total phenol content [(mg gallic acid/kg oil)^2] and (C) ABTS$^{\cdot+}$ antioxidant activity [(mmol Trolox/kg oil)^2], optimization of calibration parameters.

way, the selection of spectral range caused an improvement in mean recoveries, all between 99 and 114% (Table 1). Finally, marked reduction of standard deviation values for TP (81–29%) and ABTS$^{\cdot+}$ (77–33%) models was obtained.

For WC, the optimized model (Fig. 4A) exhibited very good performance in terms of actual *versus* FTIR–PLS predicted values in both the calibration and validation sets of samples. In addition, for total phenol evaluation, the optimized model (Fig. 4B) showed good results in terms of actual *versus* predicted values in both the calibration and validation sets of samples but with an acceptable dispersion in the prediction. On the other hand, ABTS$^{\cdot+}$ estimation model (Fig. 4C) exhibited good performance for the calibration samples but a high dispersion was evident for validation set. Figure 4D–F reports the ellipse of joint region confidence (EJRC) test for the three models. The three EJRC tests evidence the absence of systematic errors because the intercept is closed to 0 and in addition the absence of bias errors in the prediction due to the slope is near to 1.

Moreover, satisfactory LOD values for the three analytical parameters were achieved by the FTIR-chemometric methods: 9.4 (mg/kg oil) for WC; 12.5 (mg gallic acid/kg oil) for TP, and 0.76 (mmol Trolox/kg oil) for ABTS$^{\cdot+}$. In fact, considering the data intervals (Fig. 1) registered for WC (from 289 to 1402 mg water/kg oil), TP (from 46 to 877 mg gallic acid/kg oil) and ABTS$^{\cdot+}$ (from 0 to 5.7 mmol Trolox/kg oil), the calculated LOD values permit to analyze both VOO and OO samples.

4 Conclusions

The results of chemometrically assisted FTIR analysis were statistically similar to those obtained by official and traditional procedures in terms of analytical performance. Moreover, the ATR–FTIR–PLS method developed herein was faster; the complete determination takes only a few minutes compared to the long time required for both the WC analysis (approximately 30 min) by titrimetric analysis and TP and ABTS$^{\cdot+}$ spectrophotometric determinations (several hours taking into consideration phenol extraction, reactive preparation and the long wait for the complete reaction). Therefore, the proposed spectroscopic method can be a highly convenient alternative in terms of time and solvent savings for routine analysis of a large number of VOO samples, especially for high throughput determinations

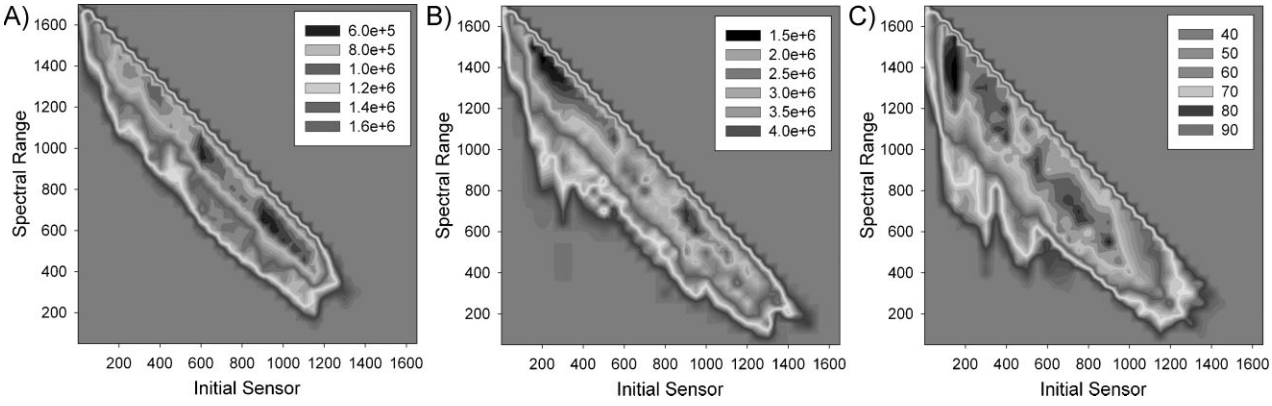

Figure 3. PRESS surface in the spectral range of calibration (sensors) for PLS models of (A) water content [(mg/kg oil)^2], (B) total phenol content [(mg gallic acid/kg oil)^2] and (C) ABTS$^{\cdot+}$ antioxidant activity [(mmol Trolox/kg oil)^2], optimization of calibration parameters.

Table 1. Calibration and validation parameters of the FTIR–ATR–PLS determination for water content (WC, expressed as mg water/kg oil), total phenol content (TP, expressed as mg gallic acid/kg oil) and $ABTS^{\cdot+}$ antioxidant activity ($ABTS^{\cdot+}$, expressed as mmol Trolox/kg oil)

Parameter	WC		TP		$ABTS^{\cdot+}$	
Statistical summary						
Linear range	289–1402		46–877		0.021–5.660	
Number of spectra used in calibration	146		116		145	
Number of spectra used in validation	16		11		14	
Spectral range (cm^{-1})	2260–1008	Full	3610–816	Full	3707–1105	Full
PLS factors	12	12	10	10	13	13
RMSD (conc. units)	78	93	85	120	0.49	0.58
REC (%)	8.6	10.3	22.4	31.5	26.5	31.7
R^2	0.89	0.84	0.87	0.74	0.63	0.67
Mean recovery of validation set (%)	99	103	114	113	98	131
Standard deviation of validation set (%)	8	11	29	81	33	77
Figures of merit						
Sensitivity (10^{-6}, conc units)	7.00	28.0	12.0	22.0	190	360
Analytical sensitivity	0.03	0.024	0.026	0.019	4.1	3.1
Selectivity based on total signal	0.003	0.011	0.003	0.005	0.002	0.004
Mean spectral residue	0.00019	0.001	0.00045	0.001	0.00046	0.001

Parameter improvement by spectral range selection.
RMSD, root mean square deviation; REC, relative error of calibration.

during industrial processing with slight sacrifice of accuracy. This method can also allow in-process optimization of technological parameters (*i.e.* malaxation time and temperature control) of VOO production based on water and phenol content. Furthermore, the procedures permit high sample throughput and are eco-friendly compared to previously reported alternatives, since no sample pretreatment is required and virtually no solvent waste is produced.

The authors have declared no conflict of interest.

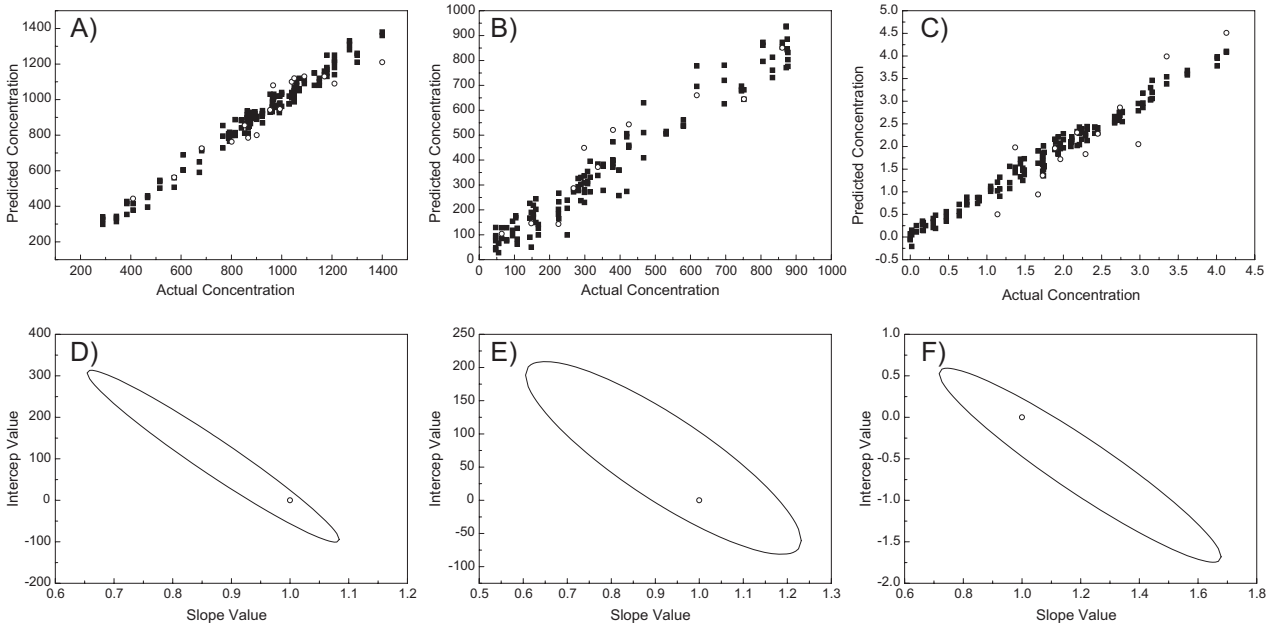

Figure 4. Actual *versus* FTIR–PLS predicted values in the calibration (■) and validation (○) sets for (A) water content (mg/kg oil), (B) total phenol content (mg gallic acid/kg oil) and (C) $ABTS^{\cdot+}$ antioxidant activity (mmol Trolox/kg oil). Ellipse of joint region confidence (EJRC) for slope and intercept of actual *versus* predicted values curve in the validation set (statistical level of significance used is 95%), for (D) water content, (E) total phenol content and (F) $ABTS^{\cdot+}$antioxidant activity.

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