LWT - Food Science and Technology 50 (2013) 153–159

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Fourier transform infrared spectroscopy–Partial Least Squares (FTIR–PLS) coupled procedure application for the evaluation of fly attack on olive oil quality

Ana Maria Gómez-Caravaca [a,b,*], Rubén M. Maggio [c], Vito Verardo [b], Angelo Cichelli [d], Lorenzo Cerretani [b]

[a] Departamento de Química Analítica, Universidad de Granada, C/Fuentenueva s/n, E-18071 Granada, Spain
[b] Dipartimento di Scienze degli Alimenti, Alma Mater Studiorum-Università di Bologna, piazza Goidanich, 60, I-47521 Cesena (FC), Italy
[c] Área Análisis de Medicamentos, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Instituto de Química de Rosario (IQUIR-CONICET), Suipacha 531, Rosario S2002LRK, Argentina
[d] DEC, Laboratorio Merceologico, Università G. d'Annunzio Chieti-Pescara, Viale Pindaro 42, I-65127 Pescara (PE), Italy

ARTICLE INFO

Article history:
Received 4 October 2011
Received in revised form
4 June 2012
Accepted 8 June 2012

Keywords:
Virgin olive oil
Bactrocera oleae
Fly attack
FTIR
Quality parameters
Chemometrics

ABSTRACT

A Fourier transform infrared spectroscopy–Partial Least Squares (FTIR–PLS) strategy for the determination of the quality of olive fruits and the respective virgin olive oil (VOO) has been developed. This methodology has been demonstrated as able to correlate the level of fly attack in olive oils with their FTIR spectra. A multivariate calibration model was built by the PLS algorithm using the 4000–700 cm^{-1} spectral range on pretreated data and an evaluation of some of the usual quality parameters of VOO (free acidity, fatty acids composition, oxidative stability by OSI time and phenolic compounds obtained by capillary electrophoresis) and was performed to corroborate the real influence of fly attack on the quality of the oils. Furthermore, the evaluation of the FTIR data showed some differences in the regions of the fatty acids and phenolic compounds depending on the percentage of fly attack on the olives. This non-destructive method easily allows a non-destructive measure and, at the same time, establishes the level of *Bactrocera oleae* attack in olive fruit thus analysing directly the olive oil obtained without any previous treatment.

1. Introduction

Olive tree (*Olea europaea* L.) is one of the most important fruit trees in the Mediterranean area; covering more than 8 million ha, accounting for almost 98% of the world crop which demonstrates the great economic and social importance of this crop. Virgin olive oil (VOO), as it is widely known, is obtained only by mechanical or physical procedures (Commission Regulation 1513/01) and because of this, its quality reflects the health status of the olives from which it was extracted. In fact, the quality of the VOO depends on its chemical composition that changes qualitatively and quantitatively in relation to a number of parameters including the health status of the fruits (Gallina-Toschi, Cerretani, Bendini, Bonoli-Carbognin, & Lercker, 2005). The action of parasites before harvest or fungal attack between harvesting and extraction are the main external agents responsible for unwanted metabolic processes in olives that lead to subsequent reduction in oil quality (Kiritsakis, 1998).

Bactrocera oleae or olive fruit fly is one of the most detrimental enemies of the quality of olive oil and it has been considered the most devastating insect pest in the Mediterranean region for 2000 years (Mraicha et al., 2010). There is evidence in literature about the reduction of oil yield, the increment of the acidity, the decrease of oxidative stability, the negative alteration of chemical composition and the changes in the flavour characteristics as a consequence of microorganisms' activity (Angerosa, Di Giacinto, & Solinas, 1992; Bendini, Cerretani, Cichelli, & Lercker, 2008; Gómez-Caravaca et al., 2008; Tamendjari, Angerosa, Mettouchi, & Bellal, 2009). For this reason, upon arrival at the mill, an estimation of the fly attack on olive fruit is conducted to assess the economic value of the olive fruits.

The severity of the negative effects depends on different variables such as the stage of the development of the olive fly, the intensity of the attack and the olive variety.

In the last few years, Fourier transform infrared (FTIR) spectroscopy has been used for the study of edible oils and fats. FTIR methods have demonstrated themselves to be rapid and non-destructive powerful analytical tools and in most cases they require minimal or no sample preparation. FTIR is also an excellent tool for quantitative analysis, since the intensities of the spectral bands are proportional to concentration. Several applications have

* Corresponding author. Departamento de Química Analítica, Universidad de Granada, C/Fuentenueva s/n, E-18071 Granada, Spain. Tel.: +39 0547338117; fax: +39 0547382348.
E-mail address: anagomez@ugr.es (A.M. Gómez-Caravaca).

0023-6438/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2012.06.007

been performed using this analytical approach together with chemometric methods: to detect olive oil adulteration (De la Mata et al., 2012; Lerma-García, Simó-Alfonso, Bendini, & Cerretani, 2011; Maggio, Cerretani, Chiavaro, Kaufman, & Bendini, 2010; Ozen & Mauer, 2002), to evaluate olive oil freshness (Sinelli, Cosio, Gigliotti, & Casiraghi, 2007), to assess oil oxidation (Guillén & Cabo, 2002; Muik, Lendl, Molina-Diaz, Valcarcel, & Ayora-Canada, 2007; Vlachos et al., 2006) and to study thermal stress (Maggio et al., 2011).

Thus, the aim of this research has been to evaluate the percentage of olive fly attack by a rapid FTIR methodology. Besides, other chemical parameters that have been described as directly related to fly attack, such as fatty acids composition and oxidative stability; free acidity and phenolic compounds have also been analysed.

2. Materials and methods

2.1. Samples

Thirty-two virgin olive oils from the Abruzzo region (Italy) that differed in the percentage of fly attack, variety of olive cultivars, technological system used (pressure or centrifugation, with or without a destoning phase) and produced during the 2009–2010 season were studied. The degree of infestation was calculated as the number of damaged olives per 100 fruits, considering both the presence of exit-holes and grubs (See Table 1).

2.2. Free acidity

Free acidity was determined according to the official method described in European Regulation EEC 2568/91 and amendments (Commission Regulation 2568/91).

Table 1
Variety and percentage of fly attack of the different olive oils studied.

% fly attack	Olive varieties
Outliers	
2.5%	Intosso
5%	Dritta
5%	Dritta
5%	Dritta
5%	Gentile
5%	Dritta, Leccino
7.5%	Mix
10%	Gentile
35%	Mix
60%	Gentile, Leccino
60%	Mix
Calibration	
2%	Dritta, Leccino
2.5%	Dritta, Intosso
2.5%	Leccino
5%	Leccino
5%	Gentile
7.5%	Tortiglione
7.5%	Leccino
15%	Gentile
15%	Intosso
25%	Mix
25%	Dritta
30%	Leccino
35%	Dritta
35%	Mix
45%	Mix
Validation	
4%	Dritta, Leccino
5%	Dritta
5%	Carpinetina
10%	Mix
10%	Mix
85%	Mix

Table 2
Fatty acid composition, free acidity at zero time (FA) and before 3 months of shelf life (FA3) and oxidative stability of virgin olive oils.

Samples	% fly attack	C 16:0	C 16:1	C 17:0	C 17:1	C 18:0	C 18:1	C 18:2	C 20:0	C 18:3	C 20:1	C 22:0	C 24:0	FA	FA 3	OSI
VOO1	2	12.80	1.00	0.05	0.08	2.70	74.60	7.40	0.40	0.60	0.20	0.10	0.07	0.43	0.38	27.90
VOO2	2.5	13.00	0.90	0.06	0.10	2.30	75.30	7.00	0.30	0.70	0.20	0.10	0.04	0.23	0.23	41.01
VOO3	2.5	13.00	0.90	0.06	0.20	2.20	74.50	7.70	0.30	0.70	0.30	0.10	0.04	0.20	0.23	35.03
VOO4	2.5	13.20	1.10	0.06	0.10	1.90	76.80	5.60	0.30	0.60	0.20	0.10	0.04	0.24	0.23	41.05
VOO5	4	13.00	0.90	0.04	0.06	2.70	74.90	7.00	0.40	0.60	0.20	0.10	0.10	0.37	0.37	27.94
VOO6	5	13.20	1.00	0.05	0.07	2.70	74.10	7.40	0.40	0.60	0.30	0.10	0.08	0.44	0.37	23.00
VOO7	5	12.80	1.00	0.05	0.08	2.70	75.00	7.00	0.40	0.60	0.20	0.10	0.07	0.52	0.42	24.15
VOO8	5	12.80	1.00	0.04	0.06	2.70	75.20	6.60	0.40	0.60	0.30	0.10	0.20	0.57	0.46	17.64
VOO9	5	13.00	1.00	0.06	0.07	2.60	74.90	7.00	0.40	0.60	0.20	0.10	0.07	0.64	0.52	18.01
VOO10	5	12.80	1.20	0.07	0.20	2.10	75.10	7.30	0.30	0.60	0.20	0.10	0.03	0.36	0.34	28.84
VOO11	5	14.40	1.30	0.06	0.10	1.80	74.60	6.40	0.30	0.70	0.20	0.10	0.04	0.28	0.25	24.11
VOO12	5	16.40	1.30	0.10	0.20	2.30	64.10	14.20	0.35	0.70	0.20	0.10	0.05	0.35	0.31	14.35
VOO13	5	16.00	1.30	0.10	0.20	2.30	65.20	13.60	0.26	0.70	0.20	0.10	0.04	0.53	0.46	13.51
VOO14	5	14.00	1.10	0.06	0.10	1.80	74.50	7.00	0.30	0.70	0.30	0.10	0.04	0.71	0.62	19.05
VOO15	7.5	14.20	1.30	0.06	0.10	1.80	75.90	5.30	0.30	0.70	0.20	0.10	0.04	0.26	0.30	37.59
VOO16	7.5	13.60	1.00	0.07	0.20	2.30	72.00	9.40	0.40	0.70	0.20	0.10	0.03	0.40	0.37	21.45
VOO17	7.5	16.80	1.40	0.10	0.20	2.40	62.60	15.20	0.36	0.60	0.20	0.10	0.04	0.26	0.25	21.04
VOO18	10	14.00	1.10	0.06	0.10	1.80	75.60	6.20	0.20	0.60	0.20	0.10	0.04	0.18	0.22	26.55
VOO19	10	14.00	1.30	0.06	0.10	1.90	75.30	6.10	0.30	0.60	0.20	0.10	0.04	0.58	0.52	19.89
VOO20	10	13.50	1.10	0.05	0.10	2.00	72.80	9.00	0.30	0.70	0.30	0.10	0.05	0.30	0.30	22.33
VOO21	15	16.00	1.30	0.10	0.20	2.20	64.40	14.50	0.30	0.70	0.20	0.06	0.04	0.57	0.49	9.84
VOO22	15	13.50	0.90	0.10	0.20	2.80	74.30	6.50	0.40	0.80	0.30	0.10	0.10	0.85	0.77	23.04
VOO23	25	12.80	1.20	0.07	0.10	2.00	76.60	6.00	0.30	0.60	0.20	0.10	0.03	0.30	0.28	27.67
VOO24	25	14.30	1.20	0.06	0.10	2.40	74.20	6.50	0.30	0.60	0.20	0.10	0.04	0.72	0.66	32.14
VOO25	30	14.40	1.40	0.03	0.10	1.80	74.50	6.50	0.30	0.70	0.20	0.05	0.02	0.93	0.75	18.04
VOO26	35	13.90	1.40	0.06	0.20	1.80	75.40	6.00	0.30	0.60	0.20	0.10	0.04	0.91	0.82	17.54
VOO27	35	13.80	1.10	0.05	0.10	2.00	74.70	6.80	0.30	0.70	0.30	0.10	0.05	1.12	0.83	13.12
VOO28	35	14.00	1.30	0.06	0.10	1.90	75.10	6.10	0.30	0.70	0.30	0.10	0.04	0.67	0.52	26.06
VOO29	45	14.20	1.20	0.07	0.30	2.20	73.00	7.70	0.30	0.70	0.20	0.10	0.03	0.50	0.48	20.02
VOO30	60	13.90	1.30	0.06	0.20	2.00	74.50	6.60	0.30	0.70	0.30	0.10	0.04	2.27	1.83	13.38
VOO31	60	16.70	1.50	0.10	0.20	2.20	64.60	13.20	0.36	0.80	0.20	0.10	0.04	3.78	3.01	8.01
VOO32	85	13.40	1.10	0.06	0.10	2.20	72.80	8.90	0.40	0.60	0.30	0.10	0.04	1.84	1.65	8.86

All fatty acids were expressed as g/100 g of oil, FA and FA3 were expressed as g of oleic acid/100 g of oil; OSI was expressed in hours.

2.3. Fatty acids determination

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

2.4. Oxidation stability index (OSI) time

These analyses were carried out according to the method of Gómez-Caravaca et al. (2008). Briefly, the analyses were carried out in an eight-channel OSI instrument (Omnion, Decatur, IL, USA). Virgin olive oil samples (5.0 ± 0.1 g) were heated at 110 °C under atmospheric pressure, and air (150 mL min^{-1} of flow rate) was allowed to bubble through the oil. Under these conditions, the oxidative process reaches its final steps, and the short-chain volatile acids produced are recovered and measured conductimetrically in distilled water. The time required to produce a sudden increase in conductivity (due to volatile acid formation) determines an induction period (OSI time), expressed in hours and hundredths of hours, which can be used to measure the stability of oil.

2.5. Determination of polar phenolic fraction

To collect phenolic compounds from olive oil a liquid–liquid extraction method optimized by Pirisi, Cabras, Falqui Cao, Migliorini, and Mugelli (2000) was used. 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.

A capillary zone electrophoresis method optimized by Carrasco-Pancorbo et al. (2006) was used to perform the capillary electrophoretic analyses. This method uses a capillary with 50 μm i.d. and a total length of 47 cm (40 cm to the detector) with a detection window of 100 × 200 μm, and a buffer solution containing 45 mmol l^{-1} sodium tetraborate pH 9.3. The capillary electrophoresis instrument was a P/ACE 5500 (Beckman Instruments, Inc., Fullerton, CA, USA) connected to a diode array detector.

2.6. FTIR analysis

All spectra were acquired using a Tensor 27™ FTIR spectrometer system (Bruker Optics, Milan, Italy), fitted with a Rocksolid™ interferometer and a DigiTect™ detector system coupled with an attenuated total reflectance (ATR) accessory. The ATR accessory (Specac Inc., Woodstock, GA, USA) was equipped with a ZnSe 11 reflection crystal. A small amount of the oil samples (about 1 g) was uniformly deposited on the crystal surface of ATR accessory and all 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 the absorbance spectrum was collected against a background obtained with a dry and empty ATR cell. Three spectra were recorded for each sample ($n = 3$). Before acquiring each spectrum, the ATR crystal was cleaned with a cellulose tissue soaked in *n*-hexane and then rinsed with acetone. Data was exported in ASCII compatible format of OPUS 6.0 software for further analysis.

2.7. Statistical analysis

PLS data analysis (Martens & Naes, 1989; Thomas, 1994), was performed in Matlab R2008a (Mathworks, Inc., Natwick, MA, USA) using MCV1 Toolbox written by Olivieri, Goicoechea, and Iñón (2004). PLS was run on mean-centred data. Statistical analyses and data plots were performed with OrigingPro 8 SR0 (OriginLab, Northampton, MA, USA).

The values reported are the averages of at least three repetitions ($n = 3$), unless otherwise stated. Tukey's honest significant difference (HSD) multiple comparison (oneway ANOVA) and Pearson's linear correlations are both at $p < 0.05$.

3. Results and discussion

3.1. Evaluation of tendencies among fly attacks and some quality parameters

An evaluation of some of the usual quality parameters of olive oil was performed to corroborate the real influence of fly attack on the quality of the oils.

The fatty acids detected in the olive oil samples analysed in this study are reported in Table 2. As expected, experiments revealed the oils tested contained large amounts of unsaturated fatty acids that were in the range of 64.4–78.2% of total fatty acids (Table 2). The most abundant fatty acid was the mono-unsaturated oleic acid (C18:1) that was determined in the range of 62.6–76.8%. Palmitic (C16:0) and linoleic acids (C18:2) were the second and third fatty acids ranging from 12.8 to 16.8% and from 5.3 to 15.2%, respectively. The oleic acid/linoleic acid ratio was higher than 7 except for five samples. This data was in agreement with literature (Gómez-Caravaca et al., 2008) and respected the characteristics established in the Commission Regulation 61/2011 for extra-virgin olive oils. No statistical differences ($p < 0.05$) were

Table 3
Phenolic content of olive oil samples quantified by capillary electrophoresis.

Samples	% fly attack	% simple phenols	% lignans	% secoiridoids	% EA	Total phenols mg/kg
VOO1	2	2.7	7.5	87.6	2.2	159.19 (c)
VOO2	2.5	0.7	4.3	93.4	1.7	286.06 (a)
VOO3	2.5	1.9	1.5	95.2	1.4	247.11 (a)
VOO4	2.5	2.3	4.3	91.3	2.2	200.96 (b)
VOO5	4	2.6	8.5	86.6	2.2	159.26 (c)
VOO6	5	4.4	10.2	83.7	1.7	120.41 (c,d)
VOO7	5	4.3	9.5	83.7	2.5	96.08 (d)
VOO8	5	2.7	12.3	82.9	2.1	87.29 (d)
VOO9	5	5.2	15.6	76.8	2.4	71.46 (d)
VOO10	5	3.0	4.1	92.9	0.0	154.43 (c)
VOO11	5	5.6	11.9	81.2	1.3	49.52 (f)
VOO12	5	3.5	28.1	64.9	3.5	100.14 (d)
VOO13	5	4.7	31.8	60.4	3.2	66.81 (e)
VOO14	5	6.8	22.6	70.7	0.0	36.42 (g)
VOO15	7.5	1.2	2.1	94.8	2.0	153.24 (c)
VOO16	7.5	4.8	13.5	80.6	1.1	140.87 (c)
VOO17	7.5	3.1	17.8	75.6	3.4	161.28 (c)
VOO18	10	4.4	3.1	91.2	1.3	85.71 (d)
VOO19	10	8.4	9.5	81.2	0.9	26.18 (h)
VOO20	10	3.0	5.8	91.2	0.0	116.32 (d)
VOO21	15	5.4	22.6	72.0	0.0	35.60 (g)
VOO22	15	1.4	8.1	89.0	1.5	130.77 (c,d)
VOO23	25	4.0	6.8	87.3	1.8	100.55 (d)
VOO24	25	3.1	5.6	89.7	1.6	169.26 (b,c)
VOO25	30	7.0	17.5	75.4	0.0	18.68 (i)
VOO26	35	4.9	12.0	83.0	0.0	20.92 (i)
VOO27	35	4.0	28.5	64.9	2.7	61.18 (e)
VOO28	35	5.6	7.1	86.2	1.1	49.09 (f)
VOO29	45	3.7	11.0	84.2	1.0	76.28 (e)
VOO30	60	12.7	8.3	78.9	0.1	15.36 (i)
VOO31	60	5.9	15.1	79.0	0.0	26.59 (h)
VOO32	85	5.2	19.7	75.1	0.0	16.24 (i)

Simple phenols, lignans and elenolic acid (EA) were quantified with a calibration curve of 3,4-dihydroxyphenilacetic acid at $\lambda = 214$ nm secoridoids were quantified with a calibration curve of oleuropein glucoside at $\lambda = 214$ nm.
Different letters in the same column means significantly different values ($p < 0.05$).

reported among single and total fatty acid composition and percentage of fly attack.

Furthermore, according to Gómez-Caravaca et al. (2008), *B. oleae* attack was correlated to free acidity ($r = 0.79$, $p < 0.05$) and free acidity after three months of oil storage ($r = 0.81$, $p < 0.05$). The evaluation after three months was performed because this period is long enough to observe the beginning of the oxidative reactions and to see differences in the free acidity.

The fly attack did not influence the oxidative stability determined by OSI (Table 2).

Table 3 shows the phenolic composition of the samples. It was observed that the phenolic content changed from 16.3 to 286.1 mg/kg of oil. As reported in literature (Bendini, Cerretani, et al., 2007), the secoiridoids were the most representative family of phenolic compounds (71–95.2%). Lignans were the second family with an average content of 9.4%; simple phenols were in third position representing 3.2% and elenolic acid (obtained by cleavage of secoiridoids) was in a concentration of 1.7% of total phenolic content. The lowest phenolic contents were found for olives with the highest influence of fly attack (Tamendjari et al., 2009). A decrease of secoiridoid derivatives and elenolic acid in cases of strong fly attack may be related to the increase of the polyphenol oxidase activity due to the entrance of oxygen from the exit hole made by fly larvae, which enhances phenolic oxidation (El Riachy, Priego-Capote, León, Rallo, & Luque de Castro, 2011; Gómez-Caravaca et al., 2008).

The trend graphics between the different parameters analysed (free acidity, oleic/linoleic acid ratio, oxidative stability by OSI time and phenolic compounds obtained by capillary electrophoresis) and the percentage of fly attack were also defined (Fig. 1, parts A–D).

Moreover, the free acidity at initial time and after three months increased with fly attack following grade 2 polynomial behaviour (Fig. 1, parts A and B). This fact is in agreement with previous results found in bibliography (Mraicha et al., 2010; Pereira, Alves, Casal, & Oliveira, 2004) where free acidity has been described as one of the parameters that better correlates to fly infestation. Thus, it is possible to attribute this increase in acidity to hydrolytic processes occurring in damaged olives.

The analysis of the oxidative stability showed a lineal decrease at higher percentages of *B. oleae* attack (Fig. 1, part C). Despite this, some healthy oils presented low oxidative stability values because this parameter is affected by several factors (Aparicio, Roda, Albi, & Gutiérrez, 1999; Bonoli, Bendini, Cerretani, Lercker, & Gallina-Toschi, 2004). Finally the behaviour of the phenolic compounds was evaluated. The three main families of phenolic compounds of olive oil (simple phenols, lignans and secoiridoids) and total phenolic compounds had a similar tendency. An exponential decrease of each of them was observed when fly attack increased. Fig. 1 part D shows the graphic of total phenolic content vs. fly attack. These results are in agreement with numerous authors that have reported a high negative linear correlation between the infestation degree and total phenols content (Bendini, Gómez-Caravaca, et al., 2007; Koprivnjak et al., 2010).

3.2. Evaluation of FTIR data

FTIR olive oil spectra showed the typical absorption bands. Fig. 3A shows the untreated FTIR spectra of two oils from the olives with the lowest (continue line spectra) and the highest (dotted line

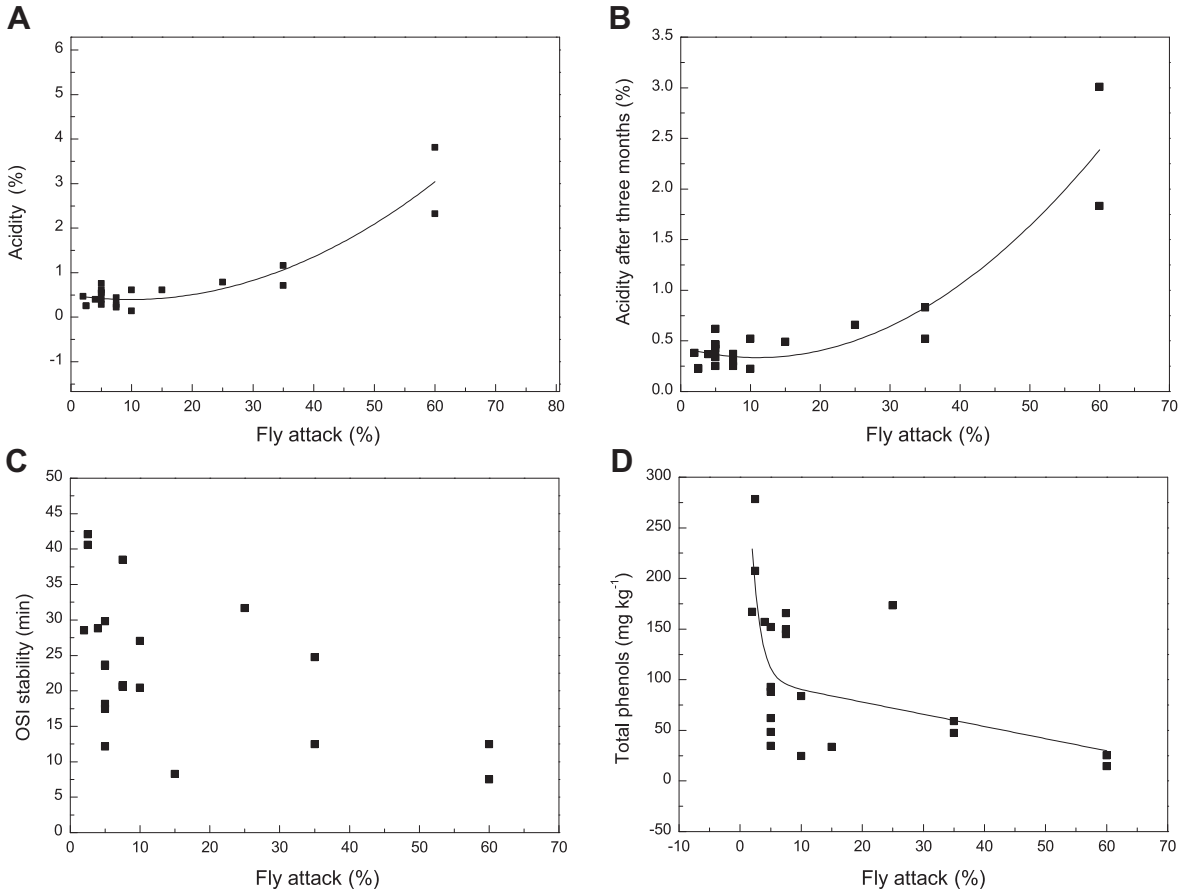

Fig. 1. Evaluations of tendencies among fly attack and quality parameters: A) Acidity, B) Acidity after three months, C) OSI, oxidative stability index and D) Total phenol content.

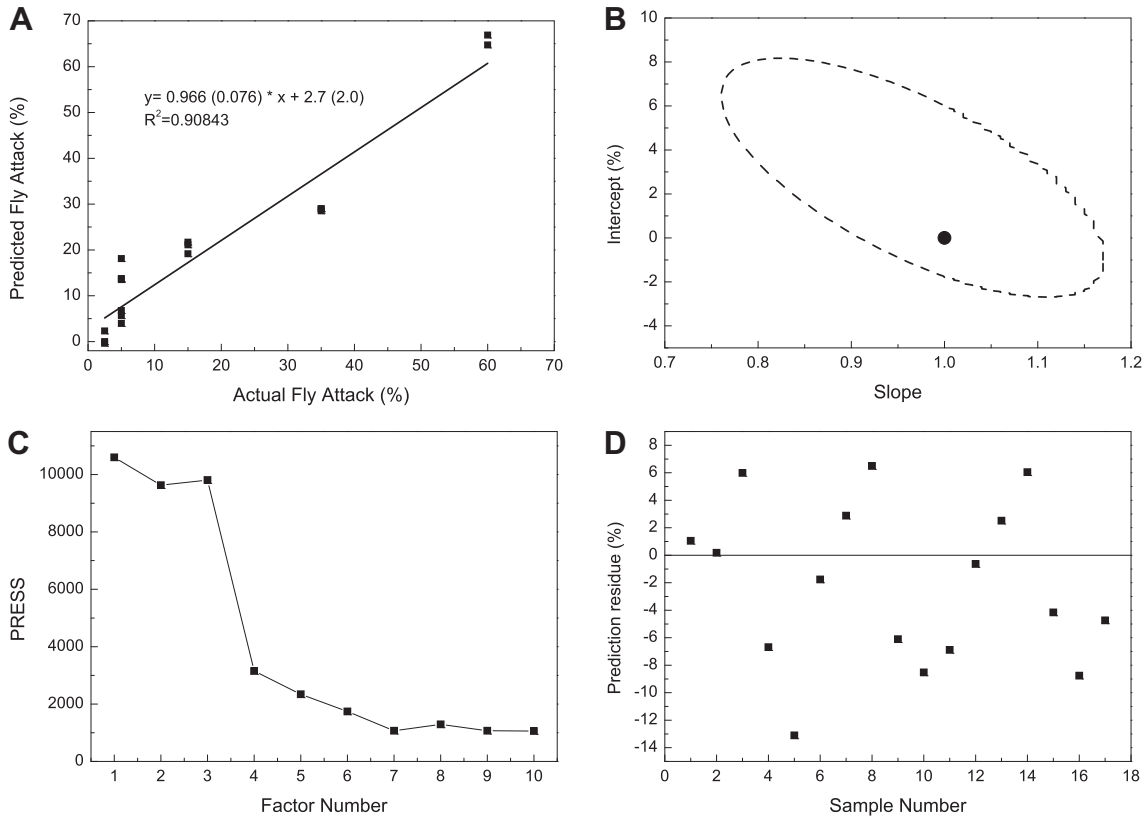

Fig. 2. Evaluation of validation set samples: A) Curve actual vs. predicted fly attack values, B) Joint confidence ellipse test, C) Cross-validation of calibration set, PRESS value vs. PLS factors, optimal number of factors 7, and D) Concentration residues plot.

spectra) percentages of fly attack. As the fly attack increases, several changes occurred in the FTIR spectra of the respective olive oils.

The main changes in the spectra appeared in the following regions:

a) 1800–1630 cm^{-1}; according to Beltrán Sanahuja, Prats Moya, Maestre Pérez, Grané Teruel, and Martín Carratala (2009), this region is related to the vibration of the carbonyl group of triacylglycerol esters. It is normally observed in the infrared spectrum of olive oil that both aliphatic and carbonyl groups give a very strong absorption in the corresponding regions. The more oxidized samples demonstrated a high absorption in this zone. Nunes, Martins, Barros, Galvis-Sánchez, and Delgadillo (2009) describe an indirect determination of olive acidity that allows a rapid evaluation of olive oil quality using FTIR methodology. As reported in the Fig. 3B a strong band was located at 1743 cm^{-1}. Moreover, the samples that reported a high FA (corresponding to a high fly attack) showed an absorbance at 1728 cm^{-1} which overlaps with the stretching vibration at 1746 cm^{-1}. According to Vlachos et al. (2006), the absorbance at 1728 cm^{-1} may be due to the production of saturated aldehyde functional groups or other secondary oxidation products. The sign of O–H deformation of water is evident near band 1640 cm^{-1} in the spectra, as illustrated in Fig. 3B. As mentioned by Hayati, Man, Tan, and Aini (2005), evaporation of water occurred in the oxidized samples, leading to a significant decrease in the absorbance of the band.

b) 1300–800 cm^{-1}; the wavelengths numbers of FTIR spectra at 827, 1039, 1115, 1143, 1286 cm^{-1} were assigned to C–H alkenes, –C–O alcohols, C–OH alcohols, –OH aromatic, C–O alcohols (Gorinstein et al., 2009). According to Bureau, Ścibisz, Le Bourvellec, and Renard (2012) the region 1225–950 cm^{-1} was assigned to aromatic C–H in plane bend.

Briefly, this region of fingerprinting (1330–800 cm^{-1}) was considered for the phenolic compounds estimation. Basically, the olive oils from highly fly attacked olives reported lower absorbance (Fig. 3C).

3.3. FTIR–PLS models for the fly attack

In order to correlate the level of fly attack in olive oils with their FTIR spectrum, a multivariate calibration model was built by the PLS algorithm (Fig. 2, parts A–D), using the full spectrum (4000–700 cm^{-1}). Mean centre and multiplicative scatter correction were used as data pretreatment. From the initial set of samples a calibration set and a validation set by random selection were performed (Table 1). Besides, a cross-validation process was carried out within the calibration set, using the "leave one out" procedure (Picard & Dennis Cook, 1984) in order to establish the PLS parameters. The appropriate number of PLS-factors were determined applying the criterion of Haaland and Thomas (1988) based on the minimal stable PRESS (Fig. 2, part C).

Table 4 reports the results regarding statistical summary and figures of merit of the model. Low values were obtained for RMSD (root mean square deviation, measures the average error in the analysis) and REC% (percentage relative error in calibration, evaluates the goodness of fit of the calibration data to the models), which measure the average error in the analysis and evaluate the goodness of fit of the calibration data to the models developed during calibration. Acceptables LODs (limit of detection) and R^2

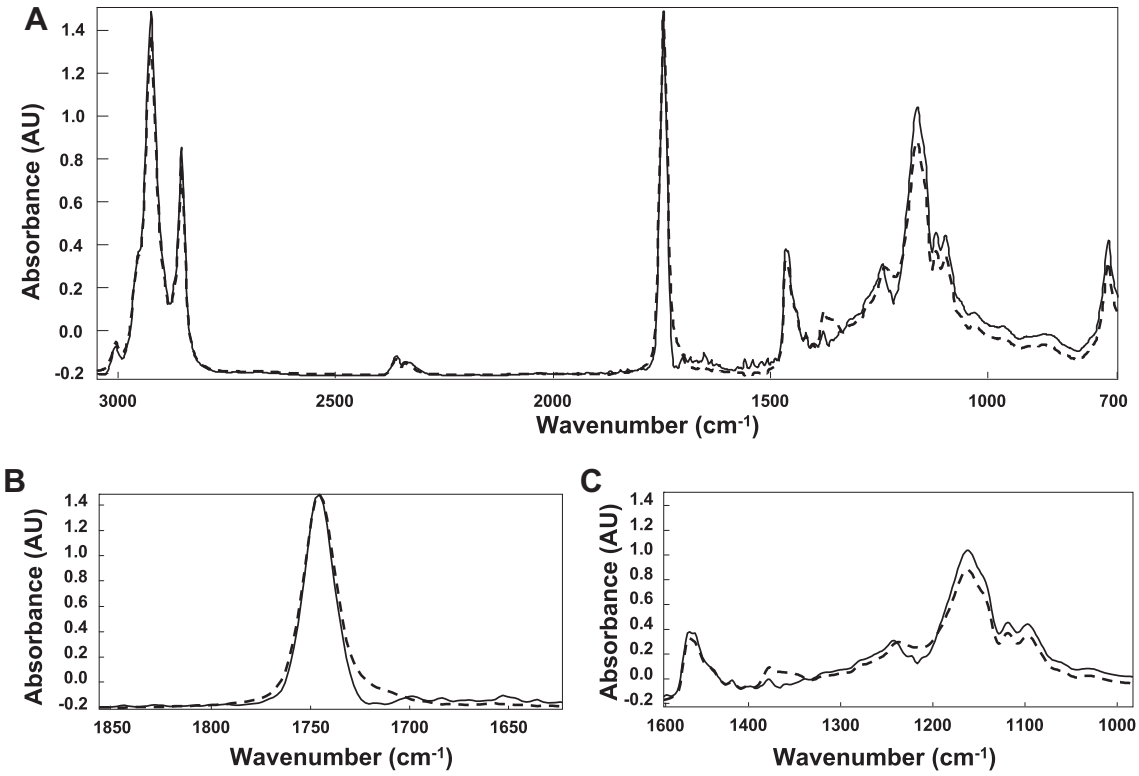

Fig. 3. Untreated FTIR spectra of two oils from olives with low (continue line spectra) and high (dotted line spectra) fly attack.

were also obtained. These later values were limited by the small number of actual samples that could be obtained.

3.4. Evaluation of validation samples

As stated above (Section 3.3. *FTIR–PLS models for the fly attack*), the validation set was constructed by random selection from initial set of samples (Table 1). In the Fig. 2, part A, the fitting curve of actual vs. predicted values (attack, %), shows a good correlation ($R^2 = 0.9084$). Additionally, the curve had a slope close to 1 and intercept close to 0 demonstrated by the joint confidence ellipse [containing the point (1, 0)]. This fact implies no bias or systematic errors may occur. Besides, prediction errors uniformly distributed (Fig. 2, part D) also validate the linearity of the model. Finally, during the evaluation of the validation set it was observed that the spectral residues were uniformly distributed, showing that the validation samples contain no unmodeled interference.

Thus, it can be concluded that the method can model the problem correctly and that FTIR represents an appropriate technique to quickly determine the extent of fly attack and to establish the healthy status of the olives. This fact is very useful because olive oils highly attacked by *B. oleae* are described to have worse quality characteristics and, therefore, in most cases extra virgin olive oils can not be obtained from these kinds of olives (Mraicha et al., 2010).

Table 4
Statistical summary and figures of merit of fly attack FTIR–PLS model.

Statistical summary		Figures of merit	
Factors	7	Sensitivity:	0.00058
RMSD[a]	6.06	Analytical sensitivity:	0.75
REC[a]	41.60	Selectivity	0.2
R^{2a}	0.9090	Mean spectral residue:	0.00069
		LOD = 3.3*SD	4.36

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

4. Conclusions

An ATR-FTIR–PLS based strategy for the determination of quality of olive fruits and consequently of VOO has been developed. This methodology is non-destructive and, at the same time, allows the level of *B. oleae* attack to be established by analysing directly the olive oil obtained. Therefore, this method could be very useful for the qualitative control of the raw material (olives) in the step prior to the production of olive oil. Besides, the FTIR instrument could be used together with a small laboratory mill (i.e. Abencor) and, in this way, the simultaneous determination of the quality of olive oil and the quality of the olives could be estimated.

It is also important to highlight that the principal advantages of the use of these instruments are that it would be possible to avoid consuming organic solvents and, besides, analysis could be performed even by non lab-qualified workers.

References

Angerosa, F., Di Giacinto, L., & Solinas, M. (1992). Influence of *Dacus oleae* infestation on flavor of oils, extracted from attacked olive fruits, by HPLC and HRGC analyses of volatile compounds. *Grasas y Aceites, 43*, 134–142.

Aparicio, R., Roda, L., Albi, M. A., & Gutiérrez, F. (1999). Effect of various compounds on virgin olive oil stability measured by Rancimat. *Journal of Agricultural and Food Chemistry, 47*, 4150–4155.

Beltrán Sanahuja, A., Prats Moya, M. S., Maestre Pérez, S. E., Grané Teruel, N., & Martín Carratala, M. L. (2009). Classification of four almond cultivars using oil degradation parameters based on FTIR and GC data. *Journal of American Oil Chemists' Society, 86*, 51–58.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A., et al. (2007). 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., Cichelli, A., & Lercker, G. (2008). Effect of *Bactrocera oleae* infestation on the aromatic profile of virgin olive oils. *Rivista Italiana delle Sostanze Grasse, 85*, 167–177.
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.
Bendini, A., Gómez-Caravaca, A. M., Cerretani, L., Del Carlo, M., Segura-Carretero, A., Compagnone, D., et al. (2007). Evaluation of contribution of micro and macro components to oxidative stability on virgin oils obtained from olives characterized by different health quality. *Progress in Nutrition, 9*, 210–215.
Bonoli, M., Bendini, A., Cerretani, L., Lercker, G., & Gallina-Toschi, T. (2004). Qualitative and semiquantitative analysis of phenolic compounds in extra virgin olive oils as a function of the ripening degree of olive fruits by different analytical techniques. *Journal of Agricultural and Food Chemistry, 52*, 7026–7032.
Bureau, S., Ścibisz, I., Le Bourvellec, C., & Renard, C. M. G. C. (2012). Effect of sample preparation on the measurement of sugars, organic acids, and polyphenols in apple fruit by mid-infrared spectroscopy. *Journal of Agricultural and Food Chemistry, 60*, 3551–3563.
Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2006). A simple and rapid electrophoretic method to characterize simple phenols, lignans, complex phenols, phenolic acids, and flavonoids in extravirgin olive oil. *Journal of Separation Science, 29*, 2221–2233.
Christie, W. W. (1998). The preparation of derivatives of fatty acids. In W. W. Christie (Ed.), *Gas chromatography and lipids* (pp. 64–84). Ayr, Scotland: The Oily Press.
Commission Regulation 1513/01. (July 23, 2001). *Official Journal of the European Union, L201*, 4–7.
Commission Regulation 2568/91. (July 11, 1991). *Official Journal of the European Union, L248*, 1–82.
Commission Regulation 61/2011. (January 24, 2011). *Official Journal of the European Union, L23/1*, 1–14.
De la Mata, P., Dominguez-Vidal, A., Bosque-Sendra, J. M., Ruiz-Medina, A., Cuadros-Rodríguez, L., & Ayora-Cañada, M. J. (2012). Olive oil assessment in edible oil blends by means of ATR-FTIR and chemometrics. *Food Control, 23*, 449–455.
El Riachy, M., Priego-Capote, F., León, L., Rallo, L., & Luque de Castro, M. D. (2011). Hydrophilic antioxidants of virgin olive oil. Part 2: biosynthesis and biotransformation of phenolic compounds in virgin olive oil as affected by agronomic and processing factors. *European Journal of Lipid Science and Technology, 113*, 692–707.
Gallina-Toschi, T., Cerretani, L., Bendini, A., Bonoli-Carbognin, M., & Lercker, G. (2005). Oxidative stability and phenolic content of virgin olive oil: an analytical approach by traditional and high resolution techniques. *Journal of Separation Science, 28*, 859–870.
Gómez-Caravaca, A. M., Cerretani, L., Bendini, A., Segura-Carretero, A., Fernández-Gutiérrez, A., Del Carlo, M., et al. (2008). Effects of fly attack (*Bactrocera oleae*) on the phenolic profile and selected chemical parameters of olive oil. *Journal of Agricultural and Food Chemistry, 56*, 4577–4583.
Gorinstein, S., Park, Y. S., Heo, B. G., Namiesnik, J., Leontowicz, H., Leontowicz, M., et al. (2009). A comparative study of phenolic compounds and antioxidant and antiproliferative activities in frequently consumed raw vegetables. *European Food Research and Technology, 228*, 903–911.
Guillén, M. D., & Cabo, N. (2002). Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chemistry, 77*, 503–510.
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*, 1193–1202.
Hayati, I. N., Man, Y. B. C., Tan, C. P., & Aini, I. N. (2005). Monitoring peroxide value in oxidized emulsions by Fourier transform infrared spectroscopy. *European Journal of Lipid Science and Technology, 107*, 886–895.
Kiritsakis, A. K. (1998). *Olive oil from the tree to the table*. Trumbull, Conneticut, USA: Food & Nutrition Press, Inc.
Koprivnjak, O., Dminić, I., Kosic, U., Majetić, V., Godena, S., & Valenčič, V. (2010). Dynamics of oil quality parameters changes related to olive fruit fly attack. *European Journal of Lipid Science and Technology, 112*, 1033–1040.
Lerma-García, M. J., Simó-Alfonso, E. F., Bendini, A., & Cerretani, L. (2011). Rapid evaluation of oxidized fatty acid concentration in virgin olive oil using Fourier transform infrared spectroscopy and multiple linear regression. *Food Chemistry, 124*, 679–684.
Maggio, R. M., Cerretani, L., Chiavaro, E., Kaufman, T. S., & Bendini, A. (2010). A novel chemometric strategy for the estimation of extra virgin olive oil adulteration with edible oils. *Food Control, 21*, 890–895.
Maggio, R. M., Valli, E., Bendini, A., Gómez-Caravaca, A. M., Gallina-Toschi, T., & Cerretani, L. (2011). A spectroscopic and chemometric study of virgin olive oils subjected to thermal stress. *Food Chemistry, 127*, 216–221.
Martens, H., & Naes, T. (1989). *Multivariate calibration*. Chichester, UK: Wiley.
Mraicha, F., Ksantini, M., Zouch, O., Ayadi, M., Sayadi, S., & Bouaziz, M. (2010). Effect of olive fruit fly infestation on the quality of olive oil from Chemlali cultivar during ripening. *Food and Chemical Toxicology, 48*, 3235–3241.
Muik, B., Lendl, B., Molina-Diaz, A., Valcarcel, M., & Ayora-Canada, M. J. (2007). Two dimensional correlation spectroscopy and multivariate curve resolution for the study of lipid oxidation in edible oils monitored by FTIR and FT-Raman. *Analytica Chimica Acta, 593*, 54–67.
Nunes, A., Martins, J., Barros, A. S., Galvis-Sánchez, A. C., & Delgadillo, I. (2009). Estimation of olive oil acidity using FT-IR and partial least squares regression. *Sensing and Instrumentation for Food Quality and Safety, 3*, 187–191.
Olivieri, A. C., Goicoechea, H. C., & Iñón, F. A. (2004). MVC1: an integrated Matlab toolbox for first-order multivariate calibration. *Chemometrics and Intelligent Laboratory Systems, 73*, 189–197.
Ozen, B. F., & Mauer, L. J. (2002). Detection of hazelnut oil using FT-IR spectroscopy. *Journal of Agricultural and Food Chemistry, 50*, 3898–3901.
Pereira, J. A., Alves, M. R., Casal, S., & Oliveira, M. B. P. P. (2004). Effect of olive fruit fly infestation on the quality of olive oil from cultivars Cobrançosa, Madural and Verdeal Transmontana. *Italian Journal of Food Science, 16*, 355–365.
Picard, R., & Dennis Cook, R. (1984). Cross-validation of regression models. *Journal of the American Statistical Association, 79*, 575–583.
Pirisi, F. M., Cabras, P., Falqui Cao, C., Migliorini, M., & Mugelli, M. (2000). Phenolic compounds in virgin olive oil. 2. Reappraisal of the extraction, HPLC separation, and quantification procedures. *Journal of Agricultural and Food Chemistry, 48*, 1191–1196.
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.
Tamendjari, A., Angerosa, F., Mettouchi, S., & Bellal, M. M. (2009). The effect of fly attack (*Bactrocera oleae*) on the quality and phenolic content of Chemlal olive oil. *Grasas y Aceites, 60*, 507–513.
Thomas, E. V. (1994). A primer on multivariate calibration. *Analytical Chemistry, 66*, 795A–804A.
Vlachos, N., Skopelitis, Y., Psaroudaki, M., Konstantinidou, V., Chatzilazarou, A., & Tegou, E. (2006). Applications of Fourier transform-infrared spectroscopy to edible oils. *Analytica Chimica Acta, 573–574*, 459–465.