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RESEARCH ARTICLE OPEN ACCESS

A multivariate data approach for FTIR-ATR monitoring of virgin olive oils: Lipid structure during autoxidation

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Abstract – In this study, fresh and 12 month stored virgin olive oils (VOOs) were monitored with Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) as a quick, non-destructive and no chemical used method. Principal component analysis (PCA) chemometric procedure was used to discriminate those in terms of diversity parameters (cultivar and growing area of olives) and detect possible chemical differences on lipid structure at the end of storage time. In this way, analyses were carried on at the beginning of the storage time and were repeated at the end of the storage time. 1488–924 cm^{-1} band gave the best (PC1: 98%) discrimination at the beginning of storage. It was also observed that symmetric and asymmetric stretching vibration of aldehydes, ketones, alcohols and hydroperoxides (3008, 2924, 1745 cm^{-1}) were found to be more intense at the end of the storage time. Moreover, 3080–2790 cm^{-1} band gave the best (PC1: 96%) discrimination after 12 month expiry. In conclusion, FTIR spectroscopy was demonstrated to be a rapid tool with chemometric evaluation for accurate discrimination of olive oils based on cultivar and geographic origin.

Keywords: FTIR / PCA / virgin olive oils / storage / multivariate data analysis

Résumé – Une approche de données multivariées pour la surveillance FTIR-ATR des huiles d'olive vierges : structure des lipides au cours de l'autoxydation. Dans cette étude, des huiles d'olive vierges (VOO) fraîches et stockées durant 12 mois ont été contrôlées par spectroscopie infrarouge à transformée de Fourier à réflexion totale atténuée (ATR-FTIR) en tant que méthode rapide, non destructive et sans produit chimique. Une procédure chimiométrique d'analyse en composantes principales (ACP) a été utilisée pour discriminer les paramètres de diversité (cultivar et zone de culture des olives) et détecter d'éventuelles différences chimiques sur la structure lipidique à la fin de la période de stockage. De cette manière, les analyses ont été effectuées au début de la durée de stockage et ont été répétées à la fin de la durée de stockage. La bande d'absorption 1488–924 cm^{-1} a donné la meilleure discrimination (PC1 : 98 %) au début du stockage. Il a également été observé que les vibrations d'élongation symétriques et asymétriques des aldéhydes, des cétones, des alcools et des hydroperoxydes (3008, 2924, 1745 cm^{-1}) étaient plus intenses à la fin du temps de stockage. De plus, la bande 3080–2790 cm^{-1} donnait la meilleure discrimination (PC1 : 96 %) après 12 mois d'expiration. En conclusion, la spectroscopie FTIR s'est révélée être un outil rapide d'évaluation chimiométrique permettant une discrimination précise des huiles d'olive en fonction du cultivar et de l'origine géographique.

Mots clés : FTIR / PCA / huiles d'olive vierges / stockage / analyse de données multivariées / autoxydation

1 Introduction

Cultivation of olive trees, production and consumption of table olives and olive oil, as well as assessment of wastes and by-products have significant technical and nutritional importance for Mediterranean countries. Thanks to olive oil's chemical composition, including high levels of monounsaturated fatty acid (oleic acid), minor components (biophenols, tocopherols), desired flavour compounds and colour, it is well established as having a large number of positive health effects. Olive oil not only has nutritional and economic impacts but

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Table 1. Sample definitions and label information on package.

Brand	Cultivar	Geographic origin	Other information
1.A	Ayvalık	Aegean Region (mixture)	Organic; cold press, dark glass bottled EVOO
2.B	Ayvalık	North part of Aegean Region (Ayvalık town, Balıkesir)	Dark glass bottled EVOO
3.C	Ayvalık	North part of Aegean Region (Ayvalık town, Balıkesir)	Stone mill; dark glass bottled; PDO signed EVOO
4.C	Ayvalık	North part of Aegean Region (Ayvalık town, Balıkesir)	Unfiltered; cold press, dark glass bottled; PDO signed EVOO
5.B	Ayvalık	North part of Aegean Region (Edremit town, Balıkesir)	Green-unripe olive extract; transparent glass bottled EVOO
6.B	Ayvalık	North part of Aegean Region (Edremit town, Balıkesir)	Well-ripe olive extract; transparent glass bottled EVOO
7.B	Ayvalık	North part of Aegean Region (Ayvalık town, Balıkesir)	Dark glass bottled EVOO
8.B	Ayvalık	North part of Aegean Region (Cunda Island, Balıkesir)	Dark glass bottled EVOO
9.B	Ayvalık	North part of Aegean Region (Edremit town, Balıkesir)	Dark glass bottled EVOO
10.B	Ayvalık	North part of Aegean Region (all town mixture)	Dark glass bottled EVOO
11.B	Ayvalık	North part of Aegean Region (all town mixture)	Transparent glass bottled EVOO
12.B	Memecik	South part of Aegean Region (all town mixture)	Transparent glass bottled EVOO
13.C	No information		Plastic packed EVOO
14.D			Plastic packed EVOO
15.E	Memecik	South part of Aegean Region (all town mixture)	Cold press, transparent glass bottled EVOO
16.E	Ayvalık	North part of Aegean Region (all town mixture)	Cold press, transparent glass bottled EVOO

also significant social importance such as recitation of olive legends to new generations. Therefore, it can be appropriately referred to as "oleoculture". Consumer perception regarding olive oil can be influenced by many factors such as geographic authenticity, compliance with regulations and special certifications related to olive oil quality. At the same time, these are the most important factors determining the commercial value of olive oils.

Classification, characterisation, detection of adulteration and deterioration of major and minor components of olive oils can be examined using a large range of analytical procedures such as chromatography, spectroscopy or a combination of them (Bajoub *et al.*, 2018). Even though many chromatographic methods such as gas chromatography have been the official techniques utilised, they are really time consuming and involve toxic chemicals. In this assay, a vibrational spectroscopic method termed FTIR was used to classify fresh VOOs and to detect probable storage defects at the end of oils' 12-month expiry.

FTIR spectroscopy has many advantages over other spectroscopic analysis methods. First, it gives fast results and its usage is simple. Secondly, sample preparation is easy. All functional chemical bonds give different molecular vibrations and intensities when using FTIR spectroscopy according to their motion type (*i.e.* stretching, bending, wagging, rocking, scissoring). Evaluation of spectral data requires some of software application with prediction or validation models. FTIR spectroscopy was used by several olive oil researchers for classification (Jimenez-Carvelo *et al.*, 2017), monitoring of adulteration (Rohman *et al.*, 2017), characterisation of cultivars (Abdallah *et al.*, 2016) and geographic origin (Hennessy *et al.*, 2009). Different data processing techniques were applied in these previous studies (Sinelli *et al.*, 2007; Maggio *et al.*, 2009; Lerma-García *et al.*, 2011). Particularly, researchers have focused on the thermal oxidation of VOOs (Selaimia *et al.*, 2017) due to the nutritional and economic losses, and also generation of toxic compounds. However in this study, it was aimed that classifying fresh VOOs based on cultivar and geographic origin, and to improve knowledge including observation of the effects of "slow-pace autoxidation" at room temperature in dark conditions on lipid chemical structure by FTIR spectroscopy using PCA chemometric data mining. To the best of our knowledge, there was no research about autoxidation monitoring of stored VOOs by FTIR without any thermal stress.

2 Materials and methods

2.1 Materials

16 virgin olive oil brands including "*Ayvalık*" and "*Memecik*" cultivars (*cv.*), which are leading cultivars for oil production in Turkey, were collected from different retail shops (signed as A, B, C, D and E) after the 2017–2018 harvest season on March 2018. Three bottles for each brand package were used and each oil sample bottle was analysed in triplicate (3 × 3 × 16), so that the number of analyse sample was 144.

Brand and label information of VOO samples were also provided in Table 1. It can be seen that there was 1 organic-processed VOO. The others were obtained from different geographical origins namely "North Aegean Region" and "South Aegean Region".

2.2 Methods

The FTIR spectra of every sample was scanned and recorded. Then, all commercial VOOs were stored between 22–26 °C in dark conditions during 12 months. FTIR analysis was again performed at the end of 12 months storage time.

2.2.1 FTIR spectroscopic analysis

After subtraction against air spectra, all VOOs were scanned with an ATR-FTIR spectrometer using OPUS NT 6.0 spectroscopic software (Bruker Tensor II, Bruker Optics Inc., Billerica, Milano, Italy) in the range from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} at 20 scans and at controlled ambient temperature (20 °C). A low volume of sample (5 μL)

Table 2. FTIR-ATR spectral zones for PCA data evaluation.

Detected frequency	Functional group	PCA scores % (fresh VOOs)	PCA scores % (stored VOOs)	Mode of vibration*
793–650	$–(CH_2)_n–$ –HC=CH– (*cis*)	PC1: 97 PC*: 3	PC1: 86 PC*: 10	Rocking Bending (out of plane)
1488–924	–HC=CH– (*cis*) –C–O– $–CH_2–$ –C–H– (terminal-CH_3) –C–H– (CH_2)	PC1: 98 PC*: 2	PC1: 89 PC*: 4	Bending (out of plane) Stretching Bending Asymmetric bending Scissoring
1814–1637	–C=O (free fatty acids, ester bonds from triglyceride) –C–H– (CH_2)	PC1: 77 PC*: 13	PC1: 66 PC2: 26	Stretching Asymmetric stretching
2936–2357	–C=O (anomeric structure) –C–H– (CH_2) –C–H– (CH_2)	PC1: 80 PC*: 16	PC1: 96 PC*: 2	Stretching Symmetric stretching Asymmetric stretching
3080–2790	=C–H (*cis* fatty acid) =C–H (*trans* fatty acid) –OH (hydroperoxides, alcohols)	PC1: 77 PC*: 12	PC1: 96 PC*: 3	Stretching

PC*: determines the sum of other PC scores;
*: Silverstein *et al.*, 1981; Guillèn and Cabo, 2000; Maggio *et al.*, 2009; Jiang *et al.*, 2016; Li and Wang, 2018; Tena *et al.*, 2018.

was dropped between the well-polished disks for getting a thin film. A deuterated triglycine sulphate detector was used.

The acquisition of FTIR spectra was performed in triplicate and displayed as mean spectra in both absorbance and transmittance mode. The data of each spectrum were obtained by OPUS NT 6.0 spectroscopic software in excel format for further multivariate analysis (PCA).

2.2.2 Multivariate analysis

PCA is a statistical test involving factor analysis, which has been accepted as an exploratory method in multivariate statistical analysis and is a mathematical tool which shows variations in the dataset using a small number of factors (Granato *et al.*, 2018). PCA assessment was carried out by Unscrambler software (version 9.7, Camo Company, Oslo, Norway) for statistical analysis. Every obtained data was examined and then, the spectral region was divided at 5 groups. These were 793–650, 1488–924, 1814–1637, 2936–2357 and 3000–2790 cm^{-1} for fresh VOOs and 793–650, 1488–924, 1814–1637, 2936–2357 and 3080–2790 cm^{-1} for stored VOOs (Tab. 2). The successful wave number bands for classification in terms of cultivar and geographic origin were given in Table 2 and were discussed above.

3 Results and discussions

3.1 Determination of FTIR spectra

Characteristic FTIR spectra of VOOs both at the beginning and at the end of storage were shown for the 3600–600 cm^{-1} region (Fig. 1). The peaks were basically dominated at ~3006, ~2952, ~2922, ~2873, ~2853, ~1744, ~1463, ~1417, ~1402, ~1377, ~1322, ~1305, ~1279, ~1238, ~1160, ~1118, ~1096, ~1032, ~968 and ~725 cm^{-1} wave numbers from “fresh VOOs”. The most prominent peaks of “stored VOOs” located at ~3006, ~2955, ~2922, ~2853, ~1744, ~1462, ~1236, ~1160, ~1118, ~1096, ~1062 and ~1032 cm^{-1} wave numbers. The detected frequency, their functional bond roots and mode of vibrations were also given at Table 2 and described here.

The peak at 725 cm^{-1} was explained by the CH_2 rocking and –HC=CH– (*cis*) bending mode of molecular vibrations. 1238–1032 cm^{-1} peak were associated with –C–O– stretching and $–CH_2–$ bending movements, while the 1462 cm^{-1} band arose from CH_2 scissoring vibrations. The major peaks at 1744 and 2922 cm^{-1} could be explained as C=O stretching and CH_2 asymmetric stretching motions, respectively (Silverstein *et al.*, 1981; Guillèn and Cabo, 2000; Maggio *et al.*, 2009). It could be newly observed in this work that ~2873, ~1417, ~1402, ~1377, ~1322 and ~1305 cm^{-1} peaks disappeared due to fragmentation or derivatization and ~1062 cm^{-1} peak generated after 12 month storage. It was inferred that some of symmetric stretching, asymmetric and symmetric bending vibrational motion broke down (–C–H– from $–CH_2$ and terminal carbon atom). On the other hand, the ~1062 cm^{-1} peak was newly occurred by –C–O– stretching movement. Moreover, the 3029–2989 cm^{-1} band was linked =CH (both -*cis* and -*trans* isomers) and –OH stretching of hydroperoxides and alcohols (Li and Wang, 2018; Tena *et al.*, 2018). Therefore, it could be considered that the band over 2952 cm^{-1} and ~1062 cm^{-1} peaks were a fingerprint of the slow-paced autoxidation process.

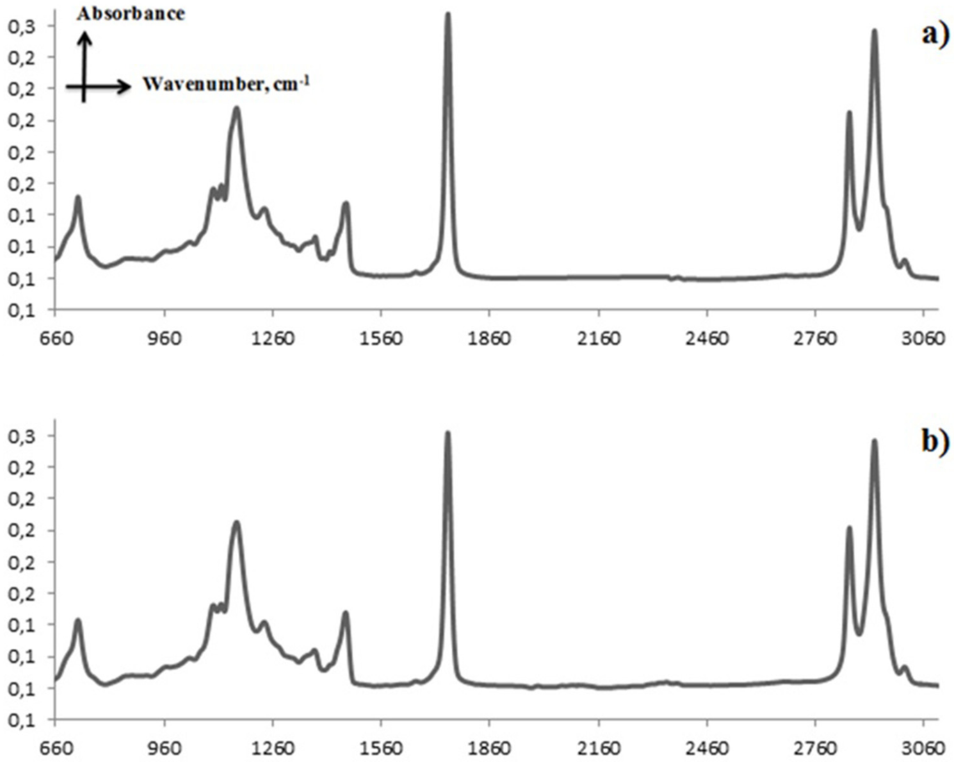

Fig. 1. The characteristic FTIR spectra of VOOs. a: defined as fresh VOOs; b: defined as 12 month stored at room temperature in dark conditions VOOs.

A previous study demonstrated that 1744 cm^{-1} (C=O stretching vibration of aldehydes and ketones), 2922 cm^{-1} (CH_2 asymmetric stretching) and 3006 cm^{-1} (OH stretching of hydroperoxides) bands could be used for differentiating VOOs in terms of chemical bond vibrational mode. 1463 cm^{-1} (scissoring mode of –CH in CH_2), 1160 cm^{-1} (stretching of C–O and bending of $–CH_2$), 1118 cm^{-1} (stretching of C–O), 1096 cm^{-1} (stretching of C–O) and 723 cm^{-1} (rocking mode of $–(CH_2)_n$ and out-of-plane bending mode of *cis* –HC=CH) bands showed similar loadings at PCA due to the intensity of the functional group (Li and Wang, 2018). The peaks measured at 1363 cm^{-1} and 1218 cm^{-1} belong to ether and epoxide structures, respectively, and were not observed to be intense. On the other hand, many "*FTIR spectroscopy and storage* studies concerning virgin olive oil" have been especially examined in terms of "thermal oxidation". On this direction, it was described before that after heat treatment, some peculiar infrared bands of an olive oil sample were changed (Guillèn and Cabo, 2000). The initial phase of the oxidation involves both the generation and increment of the hydroperoxides. Moreover, the next steps are characterised by the breaking of the *ester* bonds, which decreases the unsaturation degree and increases -*trans* isomer concentration (Navarra *et al.*, 2011). For this reason, frequency and absorbance of bands near 3006 cm^{-1} change when the thermal oxidation process advances (Guillèn and Cabo, 2002).

In this study, PCA was used to differentiate VOOs from two geographical origins (North and South Aegean Region), two olive cultivars (Memecik *cv.* and Ayvalık *cv.*) based on the extracted principal components (PCs). 1488–924 cm^{-1} band gave the best (PC1: 98%) discrimination of VOOs by PCA at the beginning of storage. 3080–2790 cm^{-1} band gave the best (PC1: 96%) discrimination of VOOs by PCA after 12 month expiry. Therefore, chemometric results were explained in terms of those spectral regions.

3.2 Principal component analysis

With the three principal components, 99.69% of total variance was explained (PC1: 98.2%, PC2: 1.1%, PC3: 0.4%) according to 1488–924 cm^{-1} band from fresh VOOs' FTIR data (Fig. 2); and with the three principal components, 99.65% of total variance was explained (PC1: 96%, PC2: 3%, PC3: 0.6%) according to 3080–2790 cm^{-1} band from stored VOOs' FTIR data (Fig. 3). Figure 2 showed that three main class, namely 1.A (the organic sample); 12.B and 15.E (Memecik *cv.* from South Aegean Region), and the others (Ayvalık *cv.* from North Aegean Region). After autoxidation at dark conditions, Figure 3 was obtained. It could be observed that 12.B and 15.E (Memecik *cv.* from South Aegean Region), and 3.C and 4.C (PDO signed samples) were clearly categorised.

This study is meaningful for monitoring chemical changes during autoxidation by FTIR analysis with chemometric data evaluation. The promising results could encourage new researches such as nutritional, sensorial and kinetical approach in order to support changes on FTIR spectrum.

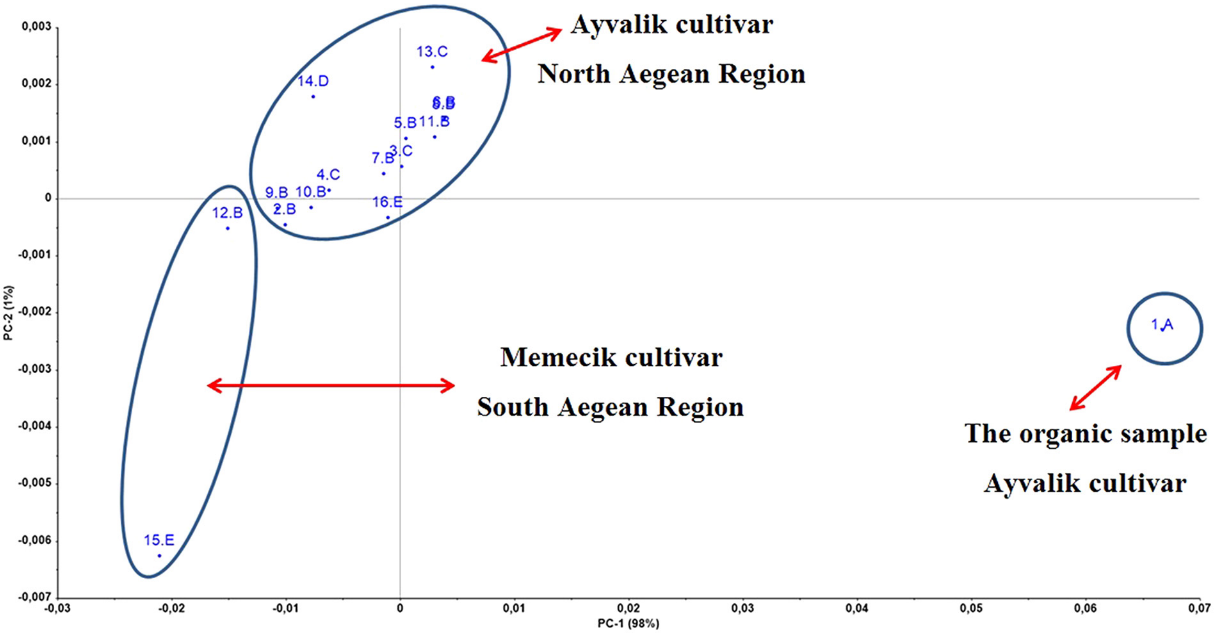

Fig. 2. PCA score plot for initial storage time (fresh VOOs).

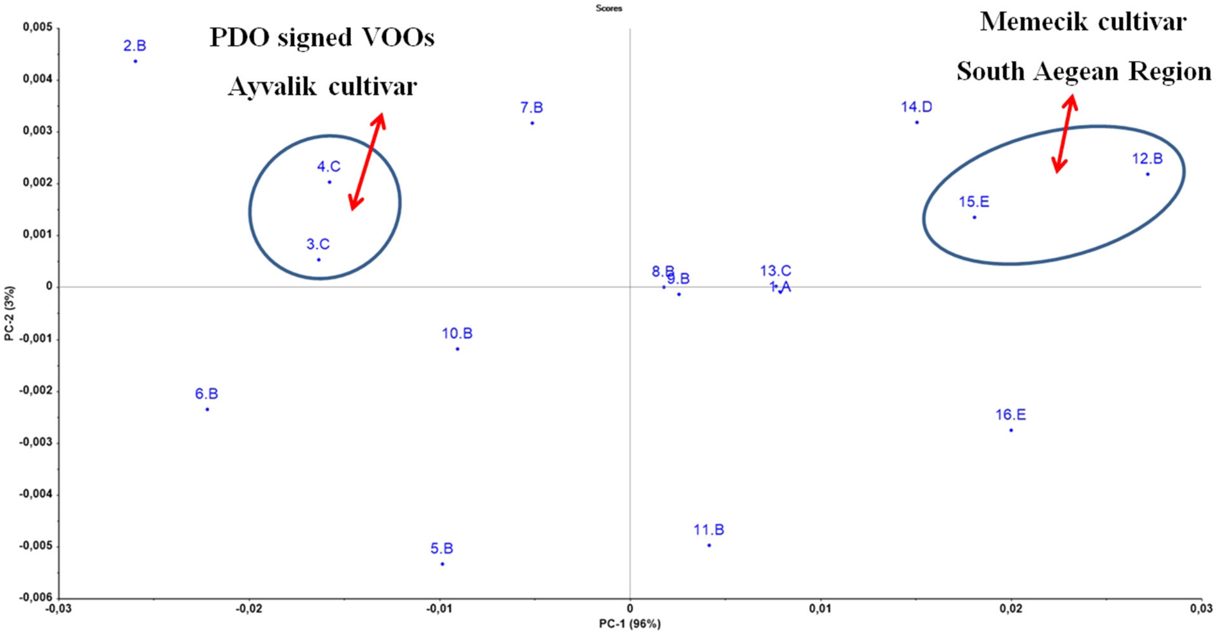

Fig. 3. PCA score plot for the end of storage time (stored VOOs).

4 Conclusion

This research concerned with high quality commercial virgin olive oils' vibrational motion monitoring by FTIR with a multivariate perspective. The FTIR fingerprints of virgin olive oils show strong molecular movements and good variability between samples. The discussed results demonstrate that FTIR spectroscopy with PCA tool is able to success for classifying virgin olive oils based on both cultivar and geographic origin. The potential of FTIR spectroscopy coupled with PCA chemometric model was also determined for stored samples (after 12 months at room temperature in dark conditions in their own package), with the proposed method also being found to be highly appropriate for classifying virgin olive oils. This study also pointed out the advantages of FTIR compared to the long-term official chemical composition analyses. Some of the superiority of FTIR related to its rapidity, non-destructive and low-cost characteristics.

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Conflicts of interest. The authors of the manuscript declare that there is no conflict of interest.

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