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GC-MS, FTIR and Raman spectroscopic analysis of fatty acids of *Pistacia vera* (Greek variety "Aegina") oils from two consecutive harvest periods and chemometric differentiation of oils quality

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ABSTRACT

Pistacia vera oil is a rich source of unsaturated fatty acids, whose presence is associated with high quality and nutritional value. According to the literature, fatty acid oil composition is not constant every harvest year, but varies mainly depending on climate conditions. Therefore, the knowledge of oil composition in fatty acids is necessary to assess both its quality and its nutritional value.

Twenty-two samples (11 samples from the harvest year 2017 and 11 samples from 2018) of the Greek variety "Aegina" were collected from four different Greek regions, from producers following the same cultivation and post-harvest cares. Extraction oil yields were found to be similar (61.7% w/w, 2017; 60.8% w/w, 2018). A reduction of the saturated fatty acids content was determined in 2018 (mean values 12.2% w/w against 13.8% w/w in 2017) by Gas Chromatography-Mass Spectrometry, accompanied by an increase of the unsaturated ones (mean values 87.9% w/w against 86.2% w/w in 2017). These results indicate that the harvest year 2018 may be considered superior to 2017 in terms of quality and nutritional value and may be correlated with an increased mean rain rate in 2018 and a slight decrease of the mean temperature. Fourier transform infrared (FTIR) and Raman spectroscopic studies of the oils were also performed. Three chemometric models were developed for the two consecutive harvest years of pistachio oil and the discrimination was based on GC–MS analysis, FTIR and Raman spectroscopic data combined with cross-validation techniques and comparison among them. The most successful chemometric model was that based on FTIR spectroscopy, which has the advantage of speed, simplicity and economy. Such a chemometric model may help in estimating the quality of *Pistacia vera* oils.

1. Introduction

Greece is among the leading countries that produces pistachio nuts (*Pistacia vera*), an agricultural product of great importance, which is exported as well as being largely consumed. Pistachio nuts are known as carriers of nutritious elements with a high oil content characterized by the abundance of unsaturated fatty acids (UFAs) and the presence of an array of bioactive phytochemicals with putative health-promoting actions, including phenols, flavonoids, proanthocyanidins, stilbenes, phytosterols and carotenoids (Ballistreri, Arena, & Fallico, 2011). Pistachio nuts are a rich source of fatty acids, such as oleic, linoleic, linolenic, palmitic, palmitoleic and stearic, which are essential for the human diet. A diet very rich in UFAs may reduce the concentration of cholesterol in blood and prevent cardiovascular diseases. Pistachio nuts also contain considerable amounts of protein, minerals (Ca, Mg, K, P, Cu etc.) and vitamins (A, B1, B2, B6, etc.) (Küçüköner & Yurt, 2003). As most recently shown, chronic intake of pistachio prevents obesity-associated chronic inflammation and improves gut microbiota composition in high-fat diet mice (Terzo et al., 2020).

The oil content of pistachio nut is generally more than 55.0% (w/w) and it is used in food, cosmetic and pharmaceutical industries. Different varieties and different agroclimatic factors result in statistically significant differences in the fatty acid contents (Küçüköner & Yurt, 2003; Mahmoodabadi, Panahi, Agharahimi, & Salajegheh, 2012; Satil, Azcan, & Baser, 2003). The unsaturated fatty acid content greatly affects the nutritional value of pistachio oil, making it susceptible to auto-oxidation, influencing negatively or positively its flavour and colour (Yildiz, Gürcan, & Ozdemir, 1998). Several studies have been conducted in order to characterize pistachio nuts of different geographic origins and the composition of pistachio oil cultivated in Greece is known to a

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Table 1
The oil yield of two consecutive harvest seasons (2017, 2018) pistachio (*Pistacia vera*, variety "Aegina") samples (g oil/100 g dried sample)[a].

Samples No	Geographical Origin	Harvest 2017	Harvest 2018
1-A[b]/1-B[c]	AEGINA	62.6 ± 1.3	63.2 ± 2.5
2-A/2-B	AEGINA	62.5 ± 3.3	57.5 ± 6.8
3-A/3-B	AEGINA	61.2 ± 2.2	60.0 ± 5.9
4-A/4-B	AEGINA	61.5 ± 1.7	57.7 ± 5.3
5-A/5-B	MEGARA	60.1 ± 3.0	61.5 ± 4.8
6-A/6-B	MEGARA	60.2 ± 0.3	62.4 ± 1.1
7-A/7-B	MEGARA	64.1 ± 2.2	60.7 ± 6.4
8-A/8-B	PHTHIOTIS	64.8 ± 2.4	61.2 ± 9.2
9-A/9-B	PHTHIOTIS	62.5 ± 10.7	60.3 ± 5.5
10-A/10-B	PHTHIOTIS	60.0 ± 1.1	59.0 ± 2.9
11-A/10-B	TRIZINA	59.7 ± 4.4	64.8 ± 18.4
Mean		**61.7**	**60.8**

[a] mean ± Standard Deviation (SD) (n = 3); [b]harvest 2017; [c]harvest 2018

certain extent. However, research on pistachio oil from the Greek variety "Aegina'' and its composition of fatty acids in relation to the year of harvest is limited (Arena, Campisi, Fallico, & Maccarone, 2007).

Fatty acids (FAs) are carboxylic acids with either saturated or unsaturated aliphatic chains, which may contain a different number of double bonds at different positions. Although fatty acids may be present in their free fatty acid (FFA) forms, they most often exist in esterified forms, such as triacylglycerols and phospholipids. The total FAs include FFAs and bound FAs.

Various analytical tools, including Gas Chromatography coupled with Mass Spectrometry (GC–MS) or Flame Ionization Detector (GC-FID), and Liquid Chromatography coupled with Mass Spectrometry (LC-MS), have been used to perform fatty acid analyses. Compared to GC-FID, GC–MS can provide more structural information. Moreover, GC–MS employs well-established databases for fatty acids' identification with higher efficiency and better selectivity compared to GC-FID. Therefore, GC–MS is the most frequently used method for fatty acid analysis (Chiu & Kuo, 2020).

Fourier transform infrared (FTIR) and Raman spectroscopy contribute to the identification of functional groups as well as the "skeletal structure'' of the compounds present in composite matrices. The use of the above techniques has significantly contributed to the study of vegetable oils as well as the detection of adulteration of olive oil with other lower-value oils (Uncu & Ozen, 2019). Both spectroscopic techniques are rapid, accurate, simple, non-destructive for the samples, environmentally friendly, and economical.

The economic importance of vegetable oils and the demands of consumers imply the need for appropriate rapid analytical techniques to evaluate their quality (Uncu & Ozen, 2019; Jentzsch & Ciobotă, 2014). Chemical models for the differentiation of oils, depending on their chemical composition of fatty acids, should be robust, simple, accurate, fast and economical. Infrared and Raman spectroscopy have already been applied (e.g. for wine, milk, etc.) having all the advantages mentioned above. However, GC–MS technique, which is mostly used to give an insight of fatty acids oils' composition, is neither fast nor economical.

The purpose of this work was: 1) the study of the fatty acids in oils, obtained from two consecutive years of pistachio (Pistacia vera) harvest (Greek variety "Aegina"), using three different analysis techniques (GC–MS, FTIR, Raman) and 2) the development of the oil's differentiation chemometric models, depending on the year of harvest and the quality, as well as a comparison of chemometric models with each other.

2. Materials and methods

2.1. Samples

Twenty-two *Pistacia vera* samples of the Greek variety "Aegina'' from production and marketing of pistachios Greek regions (Aegina, Megara, Phthiotis, Trizina) were harvested during two consecutive years (2017, 11 samples and 2018, 11 samples), by the same producers (Table 1). Each producer followed the same cultivation and post-harvest care in both seasons. All samples were dried by producers using mechanical or sun drying. The dried pistachios contained 5–7% w/w moisture, measured for each used sample according to the AOAC Official Method 925.40 (Georgiadou et al., 2015), and presented the typical standards of the edible variety. Each sample was peeled, finely ground in a laboratory mill at 20,000 rpm (IKA M 20, Königswinter, Germany), sieved at a particle size between 500 and 800 μm and the pistachio kernel flour was kept in the freezer (-20 °C).

2.2. Reagents

Petroleum ether, hexane (purity 99%), potassium hydroxide (KOH), methanol (MeOH), and magnesium sulfate ($MgSO_4$) were purchased from Sigma-Aldrich (Steinhein, Germany) and methyl decanoate (purity 99.5%) was purchase from Merck (Darmstadt, Germany). All chemicals and reagents used in this study were of analytical grade.

2.3. Oil extraction

According to the AOAC Official Method 948.22, approximately 4 g of pistachio kernel flour were mixed with 250 mL petroleum ether in a Soxhlet apparatus for 6 h at 50 °C and the extraction of the pistachio oil was completed after evaporation of the solvent at 35 °C under reduced pressure (Laborota 400 efficient, Link Lab, Heidolph). The previous procedure was repeated in triplicate, using a different portion of the sample each time, and the mean and standard deviation of oil yield were calculated. Pistachio oils were stored in freezing conditions (-20 °C).

2.4. Fatty acids methyl esters (FAMEs) analysis using GC–MS

2.4.1. Alkali-Catalyzed transesterification

Fatty acids were converted to FAMEs according to the AFNOR method (1984) modified as follows: Into a 10 mL volumetric flask, 1.12 g KOH were added and diluted to volume with MeOH and stirred (Grant, 35 kHz) until complete homogenization of the KOH/MeOH solution (2.00 M). 100 μL of pistachio oil, 200 μL of the KOH/MeOH solution and 1 mL of hexane were added in a sealed cap vial, vortexed and kept until separation of the polar and non-polar phases. The polar phase (lower layer) contained glycerol derived from the triglycerides of pistachio oil, while the non-polar phase (upper layer) included the FAMEs of pistachio oil together with hexane. In a sealed cap vial, 200 μL from the upper layer were carefully collected with a pipette and 775 μL hexane were added. A quantity of $MgSO_4$ was added to the vial to absorb any trace of moisture and the mixture was filtered through a non-polar 0.20 μm filter (Xtra PTFE 20/13 Chromafil). Methyl decanoate was used as an internal standard for quantification purpose.

2.4.2. GC–MS analysis

A Thermo Scientific system (Trace GC ULTRA) equipped with a mass detector (DSQ II) and a Trace GOLD TG-5MS low polarity capillary column (30 m, 0.25 mm ID, 0.25 μm film thickness) with a stationary phase made of 5% diphenyl/95% dimethyl polysiloxane were used with helium as the carrier gas with a flow rate of 1 mL/min under constant pressure. Analysis was conducted in split mode (1:50) with an injection volume of 1 μL. The GC oven temperature was programmed as follows: 110 °C to 205 °C at a rate of 4.0 °C/min, 205 °C to 210 °C; 1.0 °C/min, 210 °C to 212 °C; 0.5 °C/min, 212 °C to 250 °C ; 4.0 °C/min, then kept constant at 250 °C for 15 min. The injector and detector (MS transfer line) temperatures were at 240 °C.

Qualitative analysis was achieved through comparison (retention time and ion fragments) of the mass spectrum data of each fatty acid in the sample with the mass spectra obtained from GC–MS Libraries: XCalibur library search software and NIST Mass Spectral Library. The

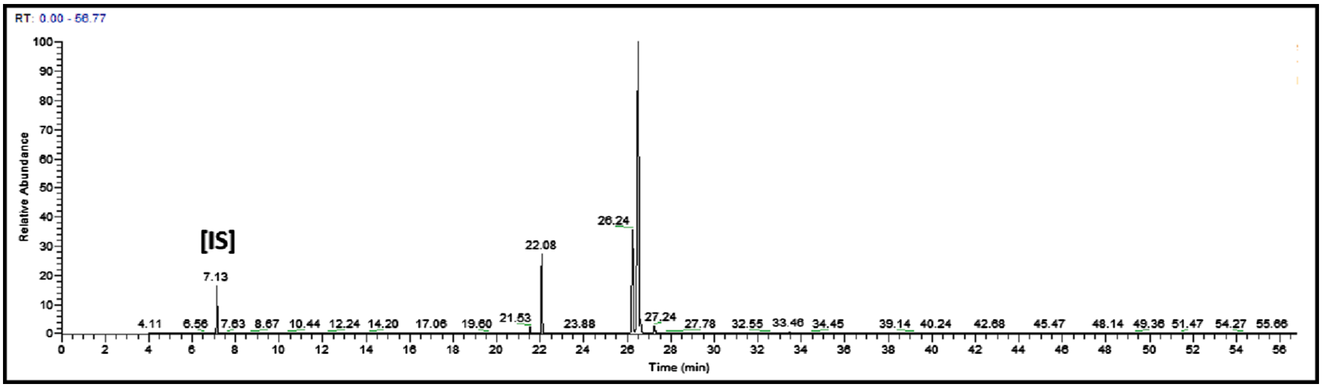

Fig. 1. Representative chromatogram of pistachio (*Pistacia vera*, variety “Aegina”) oil FAMEs. Peaks of fatty acids as eluted: 1, Myristic (RT ~ 20.08 min); 2, Palmitoleic (RT ~ 24.67 min); 3, Palmitic (RT ~ 25.20 min); 4, Heptadecenoic (RT ~ 27.53 min); 5, Margaric (RT ~ 28.24 min); 6, Linoleic (RT ~ 30.69 min); 7, Oleic (RT ~ 30.95 min); 8, Stearic (RT ~ 31.89 min); 9, Gondoic (RT ~ 38.31 min); 10, Arachidic (RT ~ 39.08 min); 11, Behenic (RT ~ 43.38 min).

quantification of fatty acids was performed using methyl decanoate as the internal standard in triplicate and the mean and standard deviation were measured.

2.5. FTIR spectra recording

A Thermo Nicolet 6700 FTIR spectrophotometer (Thermo Electron Corporation, Madison, WI, USA) equipped with a deuterated triglycine sulfate (DTGS) detector was used to obtain a total of three replicate spectra (three different sub-samples) from each sample. A Spectra-Tech Inc. horizontal ATR accessory (Stamford, CT, USA) with a trapezoid ZnSe-ATR crystal (45° angle of incidence) was employed for spectral recording from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} and 100 scans. The speed of the interferometer moving mirror was 0.6329 mm/s. The ATR crystal provided a background spectrum, which was subtracted from each sample spectrum. Each spectrum was manipulated using the corresponding functions of the software (OMNIC ver. 8.2.0.387; Thermo Fisher Scientific Inc., Waltham, MA, USA) that came with the spectrophotometer, as follows: Each spectrum was “automatically smoothed”, using the Savitzky–Golay algorithm (2nd order, 5-point window), baseline corrected using the “automatic baseline correction” (2nd order polynomial fit), averaged spectrum of each example triplet spectra was calculated, and the averaged spectra were normalized (absorbance from 0 to 1).

2.6. Raman spectra recording

All the Raman data were collected by a Raman spectrometer from a LabWrench (Midland, Ontario, Canada) Advantage 785 NIR model. The excitation wavelength of 785 nm was generated by a diode laser (80 mW power at sample). The Raman signal was recorded with the NuSpec program over the wavenumber range 200–2000 cm^{-1}, the region containing the C–C, C = C, C–O, and C = O stretches and bends as well as the C–H bends at a resolution of 3–5 cm^{-1}. Raman spectra were obtained directly from the pistachio oil samples at ambient temperature. The sample was oriented with the surface perpendicular to the incident radiation. Every measurement consisted of 10 averaged signal accumulations each with an actual exposure time of 10 s to improve the signal-to-noise ratio, and each sample was analysed in triplicate (three Raman spectra for separate sub-samples). The Raman spectra were processed as the FTIR spectra, using the same software.

2.7. Statistical analysis

Discriminant analysis was performed using IBM SPSS Statistics 22 (ver. 8.0.0.245) and MetaboAnalyst 5.0 software for integrative data analysis.

3. Results and discussion

The storage of samples in the freezer maintains the initial chemical characteristics of the pistachio oils and does not allow degradation of the more important content. The temperature of −20 °C prevents chemical alterations to happen (Rowshan, Bahmanzadegan, & Saharkhiz, 2013).

3.1. Oil extraction

The oil content determines the quality of pistachio nut, as the oil contains unsaturated (monounsaturated and polyunsaturated) FAs, which in specific amounts are beneficial to human health. The oil yield using the Soxhlet extraction method is presented in Table 1. The oil yield of the 2017 growing season fluctuated between 59.7 and 64.8 g oil/100 g dried sample (% w/w) and the mean was found to be 61.7% w/w. For the 2018 growing season, the oil yield ranged from 57.5 to 64.8% w/w with a mean value of 60.8% w/w. Considering the averages and the corresponding standard deviations, the differences are not significant. The 22 samples, 11 of each of the two consecutive growing seasons, came from the same growers, underwent the same growing care and were dried in the same way by each grower. Therefore, the small differences of the oil yield are more due to the variety, the cultivation care followed by each producer, the harvesting time, and climatic and geographical factors**.**

These results were in line with the study of Arena et al. (2007), where the oil yield of Greek pistachios was found to be 55.4% w/w. In addition, the average oil yield of Turkey pistachio samples was found to be 59.7% w/w (Yildiz et al., 1998). Another study by Satil et al. (2003) on pistachio nuts from Turkey showed an oil yield ranging from 57.0 to 62.0% w/w. Martínez et al. (2016) concluded to an average oil yield of around 50.0% w/w.

Pistachios have lower percentage of total oil content than walnuts, whose oil content is 65.2% w/w according to Bulló, Juanola-Falgarona, Hernández-Alonso, and Salas-Salvadó (2015). The percentages of total oil of raw pecan peanuts and macadamia are higher than 70.0% w/w, while those of hazelnuts and almonds are 60.8% w/w and 49.9% w/w approximately, respectively. However, compared to a study by Vukajlović et al. (2019) with oils derived from different nuts, pistachios seemed to have higher oil content than walnuts (60.5% w/w), hazelnuts (56.2% w/w) and almonds (45.4% w/w).

3.2. Fames analysis using GC–MS

A representative chromatograph of pistachio oil FAMEs is presented in Fig. 1. The fatty acid composition of pistachio oils obtained from the GC–MS analysis of the FAMEs (expressed as g of fatty acid/100 g total fatty acids (%)) is presented in Tables 2 and 3.

Table 2
Fatty acid composition (g of fatty acid/100 g total fatty acids) of pistachio (*Pistacia vera*, variety "Aegina") oils.

Samples No	Myristic (14:0)	Palmitoleic (16:1)	Palmitic (16:0)	Heptadecenoic (17:1)	Margaric (17:0)	Linoleic (18:2)	Oleic (18:1)	Stearic (18:0)	Gondoic (20:1)	Arachidic (20:0)	Behenic (22:0)	Tricosylic (23:0)	**Lignoceric (24:0)**
1-A	0.1 ± 0.0	0.8 ± 0.2	12.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	14.2 ± 1.4	71.1 ± 2.0	1.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2-A	0.1 ± 0.0	0.7 ± 0.1	10.5 ± 1.7	0.1 ± 0.0	0.0 ± 0.0	13.6 ± 0.3	73.7 ± 1.9	1.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3-A	0.1 ± 0.0	1.0 ± 0.1	11.8 ± 1.2	0.1 ± 0.0	0.0 ± 0.0	15.1 ± 0.1	70.2 ± 0.9	1.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
4-A	0.1 ± 0.0	1.0 ± 0.3	10.3 ± 1.9	0.1 ± 0.0	0.1 ± 0.0	11.6 ± 8.2	74.5 ± 1.7	1.7 ± 0.1	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
5-A	0.1 ± 0.0	0.9 ± 0.1	12.8 ± 0.7	0.1 ± 0.0	0.1 ± 0.0	12.8 ± 0.1	71.2 ± 0.6	1.8 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
6-A	0.1 ± 0.0	0.8 ± 0.3	14.2 ± 4.0	0.1 ± 0.0	0.1 ± 0.0	10.9 ± 0.2	72.1 ± 4.4	1.5 ± 0.4	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
7-A	0.1 ± 0.0	0.8 ± 0.1	11.2 ± 0.5	0.1 ± 0.0	0.0 ± 0.0	14.4 ± 0.4	71.7 ± 0.1	1.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8-A	0.1 ± 0.0	0.9 ± 0.1	12.6 ± 0.8	0.1 ± 0.0	0.0 ± 0.0	14.1 ± 0.5	70.6 ± 0.3	1.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
9-A	0.1 ± 0.0	0.9 ± 0.0	12.2 ± 0.6	0.1 ± 0.0	0.1 ± 0.0	14.3 ± 0.4	70.5 ± 1.2	1.5 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
10-A	0.1 ± 0.0	1.1 ± 0.1	14.0 ± 0.5	0.1 ± 0.0	0.1 ± 0.0	12.0 ± 0.1	71.3 ± 0.5	1.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
11-A	0.1 ± 0.0	1.0 ± 0.0	11.9 ± 1.0	0.1 ± 0.0	0.0 ± 0.0	13.7 ± 0.7	71.3 ± 0.3	1.8 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Mean ± SD[a]	**0.1 ± 0.0**	**0.9 ± 0.1**	**12.2 ± 1.2**	**0.1 ± 0.0**	**0.0 ± 0.0**	**13.3 ± 1.3**	**71.7 ± 1.3**	**1.4 ± 0.2**	**0.2 ± 0.1**	**0.1 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
Mean ± SE[b]	**0.1 ± 0.0**	**0.9 ± 0.0**	**12.2 ± 0.4**	**0.1 ± 0.0**	**0.0 ± 0.0**	**13.3 ± 0.4**	**71.7 ± 0.4**	**1.4 ± 0.1**	**0.2 ± 0.0**	**0.1 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
1-B	0.1 ± 0.0	0.8 ± 0.0	10.1 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	20.6 ± 0.0	66.9 ± 0.1	1.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2-B	0.1 ± 0.0	0.6 ± 0.0	9.8 ± 0.2	0.1 ± 0.0	0.0 ± 0.0	16.0 ± 0.4	72.1 ± 0.5	1.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3-B	0.1 ± 0.0	0.8 ± 0.1	10.5 ± 1.5	0.1 ± 0.1	0.1 ± 0.0	19.0 ± 0.5	68.1 ± 1.6	0.9 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
4-B	0.1 ± 0.0	0.9 ± 0.1	10.8 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	17.7 ± 0.3	69.3 ± 0.1	1.1 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5-B	0.1 ± 0.1	1.0 ± 0.1	11.3 ± 0.3	0.1 ± 0.1	0.0 ± 0.0	16.2 ± 0.1	69.8 ± 0.7	1.1 ± 0.4	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
6-B	0.1 ± 0.0	0.6 ± 0.0	10.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	16.6 ± 0.0	70.6 ± 0.2	1.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
7-B	0.1 ± 0.0	0.9 ± 0.1	11.0 ± 0.4	0.1 ± 0.0	0.1 ± 0.0	15.6 ± 0.1	71.1 ± 0.5	1.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8-B	0.0 ± 0.0	0.7 ± 0.2	10.7 ± 1.6	0.0 ± 0.0	0.0 ± 0.0	12.5 ± 0.1	74.4 ± 1.8	1.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
9-B	0.1 ± 0.0	1.0 ± 0.1	11.1 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	13.8 ± 0.1	72.5 ± 0.6	1.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
10-B	0.1 ± 0.0	0.7 ± 0.2	11.3 ± 2.2	0.1 ± 0.0	0.0 ± 0.0	10.6 ± 0.9	75.6 ± 3.4	1.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
11-B	0.1 ± 0.0	0.8 ± 0.0	11.0 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	13.5 ± 0.7	72.7 ± 0.3	1.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Mean ± SD	**0.1 ± 0.0**	**0.8 ± 0.1**	**10.7 ± 0.5**	**0.1 ± 0.0**	**0.0 ± 0.0**	**15.7 ± 2.9**	**71.2 ± 2.6**	**1.2 ± 0.2**	**0.2 ± 0.1**	**0.1 ± 0.0**	**0.1 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
Mean ± SE	**0.1 ± 0.0**	**0.8 ± 0.0**	**10.7 ± 0.2**	**0.1 ± 0.0**	**0.0 ± 0.0**	**15.7 ± 0.9**	**71.2 ± 0.8**	**1.2 ± 0.1**	**0.2 ± 0.0**	**0.1 ± 0.0**	**0.1 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**

[a] SD = Standard Deviation (n = 11); [b]SE = Standard Error (n = 11)

Table 3
Content of pistachio (*Pistacia vera*, variety “Aegina”) oils in saturated, unsaturated, and monounsaturated fatty acids (g of fatty acid/100 g total fatty acids).

Samples No	Saturated (SFA)[c]	Unsaturated (UFA)[d]	Monounsaturated (MUFA)[e]
1-A	13.6	86.4	72.2
2-A	11.7	88.3	74.7
3-A	13.4	86.6	71.6
4-A	12.3	87.7	76.0
5-A	14.8	85.2	72.4
6-A	16.0	84.0	73.2
7-A	12.8	87.2	72.8
8-A	14.2	85.8	71.7
9-A	13.9	86.1	71.8
10-A	15.5	84.5	72.6
11-A	13.9	86.1	72.4
Mean[a]	**13.8**	**86.2**	**72.8**
1-B	11.4	88.6	68.0
2-B	11.0	89.0	73.0
3-B	11.8	88.2	69.2
4-B	12.0	88.0	70.3
5-B	12.8	87.2	71.0
6-B	11.8	88.2	71.6
7-B	12.3	87.7	72.1
8-B	12.3	87.8	75.3
9-B	12.6	87.4	73.6
10-B	12.9	87.1	76.5
11-B	12.7	87.3	73.8
Mean[b]	**12.2**	**87.9**	**72.2**

[a] mean (n = 11) of the 2017 eleven samples; [b]mean (n = 11) of the 2018 eleven samples; [c]SFA consist of myristic, palmitic, margaric, stearic, arachidic, behenic, tricosylic and lignoceric acids; [d]UFA consist of palmitoleic, heptadecenoic, linoleic, oleic and gondoic acids; [e]MUFA consist of palmitoleic, heptadecenoic, oleic and gondoic acids.

The major fatty acids found in the pistachio oil samples were oleic (71.7 ± 1.3% for 2017 and 71.2 ± 2.6% for 2018), linoleic (13.3 ± 1.3% for 2017 and 15.7 ± 2.9% for 2018) and palmitic (12.2 ± 1.2% for 2017 and 10.7 ± 0.5% for 2018). We observed that the oleic acid content of the oils was the same in both growing seasons, the linoleic acid content increased slightly, while the palmitic content presented a small decrease. The stearic acid content was found to be 1.4 ± 0.2% for 2017 and 1.2 ± 0.2% for 2018, also showing a small decrease. The content of other fatty acids was<1.0%. Tricosylic and lignoceric acids, in one sample of 2018, were identified in trace amounts. Furthermore, in six samples from harvest 2017 behenic acid was not detected. These results are in accordance with literature (Satil et al., 2003; Ozturk, Sagdic, Yalcin, Capar, & Asyali, 2016; Agar, Kaşka, & Kafkas, 1995).

The oleic acid was the dominant fatty acid in all samples and together with linoleic acid constituted 85.9 ± 1.4% of the total fatty acids. These results present great similarities with those of Yildiz et al. (1998), Okay (2002) and Luh, Wong, and El-Shimi (1981). Oleic acid is the main fatty acid found in olive, safflower, and rapeseed oils. Olive oil has the highest oleic acid content (81.5%). Linoleic acid is the predominant fatty acid found in corn and soybean oils. In addition, corn oil has the highest linoleic acid content (58.4%) (Liu et al., 2018).

The total UFAs content was found to be 86.2% and 87.9% for the years 2017 and 2018, respectively. These findings are similar to those of Kamangar, Farrohi, and Mehran (1975), who studied the Iranian pistachio nuts (88.0%). Oleic and linoleic acid, belonging to UFAs, are important from a nutritional point of view. Their high levels make pistachio nut oil more stable to oxidative alterations. In the present study, the SFAs, UFAs, monounsaturated fatty acids (MUFAs) were found at 13.8%, 86.2%, 72.8% and 12.2%, 87.9%, 72.2% of the total fatty acids for 2017 and 2018 harvest, respectively (Table 3). These findings agree with those of Maskan and Karatas (1999). Given the fact of the slightly higher percentage of UFAs in 2018 harvest, it is assumed that the harvest of 2018 was superior in nutritional value compared to 2017.

Collection of meteorological data from the two years for the months of June, July and August, the environmental conditions of which are crucial for the fruiting and the growth of the pistachio tree, showed that the average temperature (T) and the mean rain rate (RR) were T = 28.70 °C, RR = 15.33 mm and T = 28.17 °C, RR = 36.50 mm for 2017 and 2018, respectively (Table S1) (Michelaraki, 2017, 2018). Reduced T and increased RR (2018 harvest) caused an increase in lipid unsaturation, which is in accordance with many reported studies in plants (Neidleman, 1987; Zhang et al., 2020).

The distribution of pistachio oil and olive oil fatty acids is similar (Arena et al., 2007). Except from coconut oil, the olive, safflower, rapeseed, corn, and soybean oils contain large amounts of UFAs (83.5–95.3%). Rapeseed oil has the highest content of UFAs (95.3%) and the lowest content of SFAs (4.7%). On the contrary, coconut oil has the

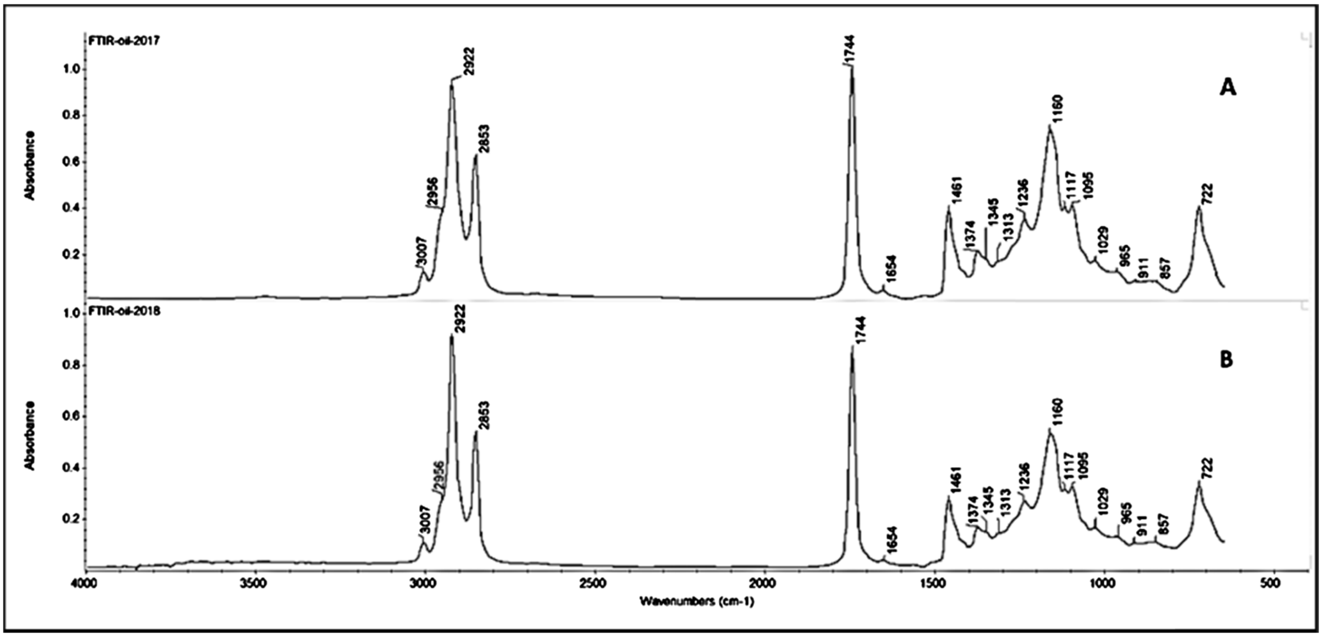

Fig. 2. Representative FTIR spectra of pistachio (*Pistacia vera*, variety “Aegina”) oil samples from the same producer, but from different years of harvest, 2017 (A) and 2018 (B).

Table 4
Peak correspondence of pistachio (*Pistacia vera*, variety "Aegina") oil FTIR spectra.

Wavenumber (cm^{-1})	Function Group	Abbreviation	Reference
3007	C-H symmetric in-plane stretching vibration of = C-H (olefinic double bonds of unsaturated fatty acids)	v_s (=C-H)	[Schulz & Baranska, 2007; Rohman & Che Man, 2010; Uncu et al., 2019]
2956	C-H asymmetric in-plane stretching vibration of $–CH_3$	$v_{as}(CH_3)$	[Rohman & Che Man, 2010; Uncu et al., 2019]
2922	C-H asymmetric in-plane stretching vibration of $–CH_2$-	$v_{as}(CH_2)$	[Rohman & Che Man, 2010; Uncu et al., 2019; Gurdeniz, Ozen, & Tokatli, 2010]
2853	C-H symmetric in-plane stretching vibration of $–CH_2$-	$v_s(CH_2)$	[Rohman & Che Man, 2010; Uncu et al., 2019; Dymińska, Calik, Albegar, Zajac, Kostyń, Lorenc, & Hanuza, 2017]
1744	C = O in-plane stretching vibration	v(C = O)	[Gurdeniz et al., 2010]
1654	>C = C < *cis*-olefinic in-plane stretching vibration	v(C = C)	[Schulz & Baranska, 2007]
1461	C-H in-plane bending vibration of $–CH_2$- (scissoring)	$\delta_s(CH_2)$	[Schulz & Baranska, 2007; Uncu et al., 2019; Gutiérrez, Quiñones-Segura, Sanchez-Reinoso, Díaz, & Abril, 2017]
1374	C-H symmetric in-plane bending vibration of $–CH_3$(mono-, di-, tri-glycerides)	$\delta(CH_3)$	[Dymińska et al, 2017]
1345, 1313	$–CH_2$- out-of-plane bending vibration (wagging)	$\omega(CH_2)$	[Rohman & Che Man, 2010]
1236, 1160, 1117	C-O stretching vibration (esters)	ν (C-O)	[Uncu & Ozen, 2019; Rohman & Che Man, 2010; Uncu et al., 2019; Dymińska et al, 2017]
1095, 1029	in-plane-C-C stretching vibration	ν(C-C)	[Dymińska et al, 2017; Gutiérrez et al., 2017]
965	C-H in-plane bending vibration (scissoring) (*trans*-olefins)	$\delta_s(C = C = C)$	[Rohman & Che Man, 2010]
911, 857	$–CH_2$- out-of-plane vibration	$\gamma(CH_2)$	[Rohman & Che Man, 2010]
722	out-of-plane vibration	γ(C = C)	[Dymińska et al, 2017]
	in-plane bending vibration of $–CH_2$- (rocking)	$\rho(CH_2)$	[Farber et al., 2019]

lowest polyunsaturated fatty acids (PUFAs, 1.3%) and UFAs (7.1%) contents, and the highest SFAs (92.9%) content (Liu et al., 2018).

3.3. Spectroscopic study

The infrared (IR) and Raman spectroscopies are complementary techniques and can provide useful qualitative and quantitative information on the chemical composition of edible oils. These two techniques of analysis allow the quick determination of the total unsaturation of oils, the content of FFAs (El-Abassy, Donfack, & Materny, 2009; Muik, Lendl, Molina-Díaz, & Ayora-Cañada, 2003), the distinction and the classification of oils (Yang, Irudayaraj, & Paradkar, 2005), the detection of oil adulteration (Baeten et al., 2005), and the stages of ripening of the kernels of the oils (López-Sanchez, Ayora-Cañada, & Molina-Díaz, 2010).

3.3.1. Ftir spectroscopic study

Two representative FTIR spectra of two pistachio oil samples (same producer, different harvesting years) are depicted in Fig. 2 and the interpretation of each vibration is summarized in Table 4.

Pistachio oil mostly contains triglycerides, which consist of one molecule of glycerol esterified with three FAs. The peaks at 2922 and 2853 cm^{-1} are due to the asymmetric and symmetric stretching vibration of the C-H bonds of the non-aromatic methylene group. We noticed that the peaks were high and relatively wide. On the one hand, this is mainly due to the high number of methylene groups on the fatty acid (at least 12) and on the other hand due to the high content of fatty acids in pistachio oils. The stretching C-H vibrations of the terminal methyl groups $–CH_3$ are noticeably weaker than those of the more abundant $–CH_2$- groups. The weak right-hand shoulder at the strong 2922 cm^{-1} band probably represents the weaker symmetric stretching C-H vibrations in $–CH_3$, $\nu_s(–CH_3)$, which is expected to appear at around ~ 2875 cm^{-1} (Kamnev et al., 2021). Two other important peaks appear at 1744 and 1160 cm−1, which are correlated to the stretching vibration of the carbonyl (C = O) of the ester bonds, and to the asymmetric stretching vibration of the C-O ester bonds, respectively (Uncu, Ozen, & Tokatli, 2019). The spectral intensity of the peaks at 1236, 1160 and 1117 cm−1 are strongly dependent on the date of harvest. According to an olive oil study by López-Sanchez et al. (2010), these peaks are observed more intense at the beginning and decrease with the ripening of the olive oil.

3.3.2. Raman spectroscopic study

Two representative Raman spectra of pistachio oil samples from the same origin but of two consecutive years of harvest and the correspondence of their most important spectrum peaks are presented in Fig. 3 and Table 5, respectively.

As depicted in Table 5, the characteristic peak of *cis*-double bonds (C = C) is an acute peak at 1657 cm^{-1}. Also, significant, sharp, and wide peaks are present at 1442 and 1305 cm^{-1}, which represent the scissor bending vibration (in-plane) and the twisting bending vibration (out-of-plane), of the bonds C-H of $–CH_2$-, respectively.

The comparison of the FTIR and Raman spectra of the pistachio oil samples shows that the wavenumbers of some characteristic peaks were found to be similar, while the intensities differed significantly. For the same vibration modes, these differences can be explained by different physical rules, which FTIR and Raman spectroscopies are based upon. For example, the peaks at 1654–1657 and 1442 cm^{-1}, which are attributed to the *cis*-double bonds of UFAs and the bending vibrations of the C-H bonds of the methylene group, respectively, appeared weak in the IR, but were stronger in the Raman spectra. In contrast, the Raman scatter signal at 732 cm^{-1} was quite weak, but the corresponding infrared absorption peak (722 cm^{-1}) was strong. In crystalline samples this peak is split in two (e.g. 720 and 730 cm^{-1}), while in non-crystalline samples, such as pistachio oil samples, a single intermediate peak is observed (Farber et al., 2019).

3.4. Multivariate statistical analysis

The differentiation of pistachio oils according to the year of harvest of pistachios and therefore according to the quality (2017, 2018) was carried out using multivariate statistical analysis. The independent variables (fatty acid content for GC–MS and peak intensities for FTIR and Raman) and the dependent variable (harvest year) were initially determined.

The independent variables were datasets containing large number of variables (chemometrics data). For this kind of datasets data filter is strongly recommended. These variables were filtered by the software, allowing a reduction in the number of data sets and a corresponding

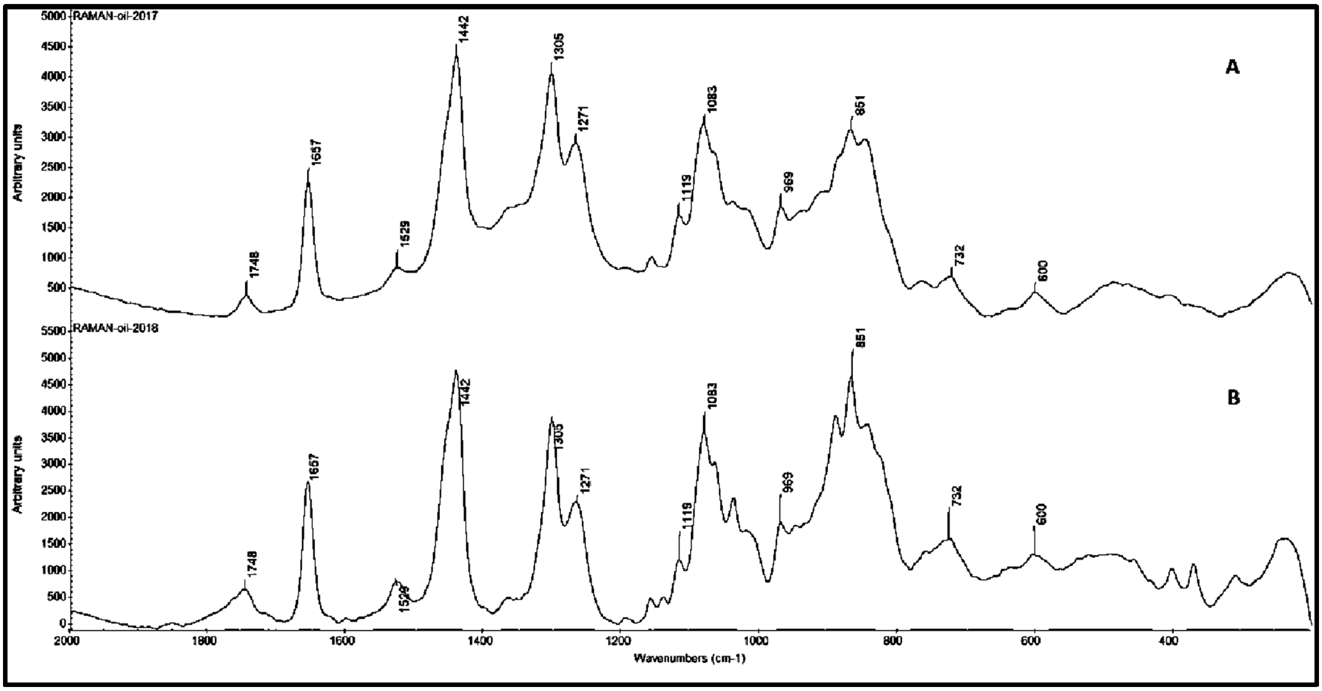

Fig. 3. Representative Raman spectra of pistachio (*Pistacia vera*, variety "Aegina") oil samples from the same producer but from different years of harvest, 2017 (A) and 2018 (B).

increase in the power of the Partial Least Squares (PLS) regression models that were developed. The interquartile range (IQR) filter of MetaboAnalyst was used to detect independent variables' values, which were non-informative, prevented a symmetric data distribution and were identified as outliers. Independent variables$<$250 were subjected to 5% filtering, while these between 250 and 500 and 500–1000 to 10% and 25% filtering, respectively (Hackstadt & Hess, 2009).

After the removal of the non-informative variables, the remaining 95% of GC–MS fatty acids content, 90% of FTIR and 75% of Raman intensities were normalized by MetaboAnalyst software. The samples normalization allowed the better discrimination of differences among the samples; data logarithmic transformation and auto scaling (mean-centering and dividing by the SD of each variable) were two different approaches that were combined to make the individual features follow a normal distribution and be more comparable.

Then, three PLS regression models were developed based on: 1) the qualitative and quantitative composition of the oils, as determined by GC–MS, 2) the FTIR spectra, and 3) the Raman spectra. PLS method used multivariate regression techniques to extract via linear combination of original independent variables the information that could predict the dependent variable. Each PLS regression model generated different number of latent factors. Each latent factor determined the relationship between the dependent variable and the independent variables. The latent factor, which had more variance explained, was chosen for the model generation.

For each model, the test of equality of group means using SPSS measured each independent variable's potential to discriminate data between two years of harvest, before the model was created. For independent variables, *t*-test was performed using the year of harvest as the grouping variable. If the significance value (S.V.) was greater than 0.05, the variable was rejected, because it did not contribute to the model. Wilks' lambda was another measure of a variable's potential to discriminate using the year of harvest as the grouping variable. Smaller values of Wilks' lambda indicated that the variable was better at discriminating between groups and resulted in an accurate and correct function, which represented a robust discriminant model with all the significant variables in it.

As for the canonical discriminant function, the eigenvalues table of SPSS provided information about its relative efficacy. The canonical correlation value measured the extent of association between the discriminant scores and the groups. Wilks' lambda was also a measure of how well the function separated cases into groups. Higher values of canonical correlation and smaller values of Wilks' lambda indicated greater discriminatory ability of the function. The associated chi-square statistic tested the hypothesis that the function was equal across the groups. Low correlation between the two years of harvest resulted in high values for chi-square. The small significance value indicated that the discriminant function could successfully separate the two groups.

For validation of the three predictive PLS models, cross-validation using SPSS and permutation tests using MetaboAnalyst were performed. Both types of test statistics assessed the significance of class discrimination. Cross-validation tested the model's ability to predict a new independent dataset in order to flag problems like overfitting or selection bias. The permutation tests of MetaboAnalyst software (Figs. 4, 6, 8) presented the distribution derived from the permuted samples. The black highlighted pointer represented the original sample. The further to the right of the distribution was the black highlighted pointer, the more significant from a statistical point of view was the separation between the two groups (Barberini et al., 2016).

3.4.1. *Discrimination based on GC–MS analysis*

Applying the Orthogonal Partial Least Squares-Discrimination Analysis (orthoPLS-DA), the model used one latent factor to explain the variances of the independent (cumulative X variance) and the dependent (cumulative Y variance) variables. The test of equality of group means together with the Variable Importance in the Projection (VIP) table, which exhibits the importance of each variable in the projection, proved that five from the total fatty acids were statistically significant for the discrimination between 2017 and 2018 harvest. T-tests showed that palmitic acid followed by stearic, linoleic, behenic and palmitoleic played the most important role, S.V. < 0.05 and small value of Wilks' lambda (Table 6). The canonical discriminant function (S.V. $<$ 0.05) indicated that the discriminant function separated the two groups of harvesting year. From the original grouped cases, 86.4% were

Table 5
Peak correspondence of pistachio (*Pistacia vera*, variety “Aegina”) oil Raman spectra.

Wavenumber (cm^{-1})	Function Group	Abbreviation	Reference
1748	C = O in-plane stretching vibration (esters of triglycerides)	v(C = O)	[Schulz & Baranska, 2007; Vaskova & Buckova, 2017; Liu, Chen, Shi, Yang, & Han, 2020; Portarena et al., 2019]
1657, 1529	>C = C < *cis*-olefinic in-plane stretching vibration (carotenoids in extra virgin oils)	v(C = C)	[Vaskova & Buckova, 2017; Liu et al., 2020; Carmona, Lafont, Jiménez-Sanchidrián, & Ruiz, 2014]
1442	C-H in-plane bending vibration of $–CH_2$- (scissoring)	$\delta_s(CH_2)$	[Vaskova & Buckova, 2017; Liu et al., 2020; Carmona et al., 2014]
1305	C-H out-of-plane bending vibration of $–CH_2$- (twisting)	$\tau(CH_2)$	[Carmona et al., 2014]
1271	C-H out-of-plane bending vibration of $–CH_2$- (wagging)	$\omega(CH_2)$	[Schulz & Baranska, 2007]
1119, 1083	C-O stretching vibration (esters) C-C in-plane stretching vibration (esters)	ν(C-O) ν(C-C)	[Jentzsch & Ciobotă, 2014; Liu et al., 2020] [Portarena et al., 2019; Czamara, Majzner, Pacia, Kochan, Kaczor, & Baranska, 2014]
969	C-H in-plane bending vibration (scissoring) (*trans*-olefins)	δ_s(H-C = C-H)	[Liu et al., 2020; Ali, Nawaz, Saleem, Nurjis, & Ahmed, 2016]
851	C-H in-plane bending vibration of $–CH_2$- (scissoring) C-C in-plane stretching vibration	$\delta_s(CH_2)$ v(C-C)	[Czamara et al, 2014] [Jentzsch & Ciobotă, 2014; Liu et al., 2020]
732	=C-H out-of-plane bending vibration (*cis*-olefins)	γ(C = C)	[Dymińska et al, 2017; Liu et al., 2020]
600	C-H in-plane bending vibration of $–CH_2$- (rocking) C-C out-of-plane bending vibration	$\rho(CH_2)$ γ(C-C-C)	[Ali et al., 2016]

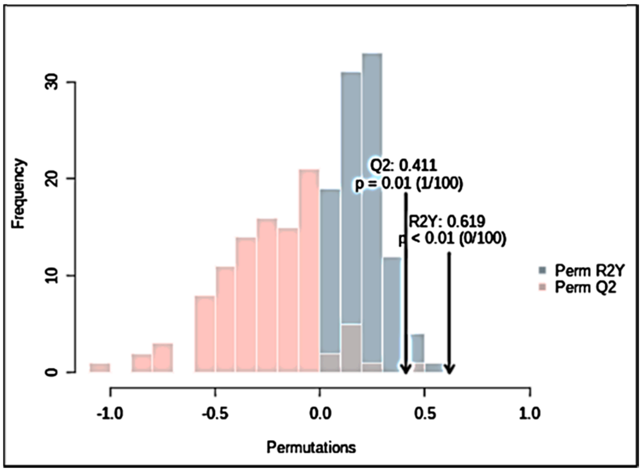

Fig. 4. Orthogonal Partial Least Squares - Discriminant Analysis (orthoPLS-DA) permutation test for the discrimination model of GC–MS fatty acids using the year of harvest as grouping variable (MetaboAnalyst); (permutation numbers:100; $p < 0.01$).

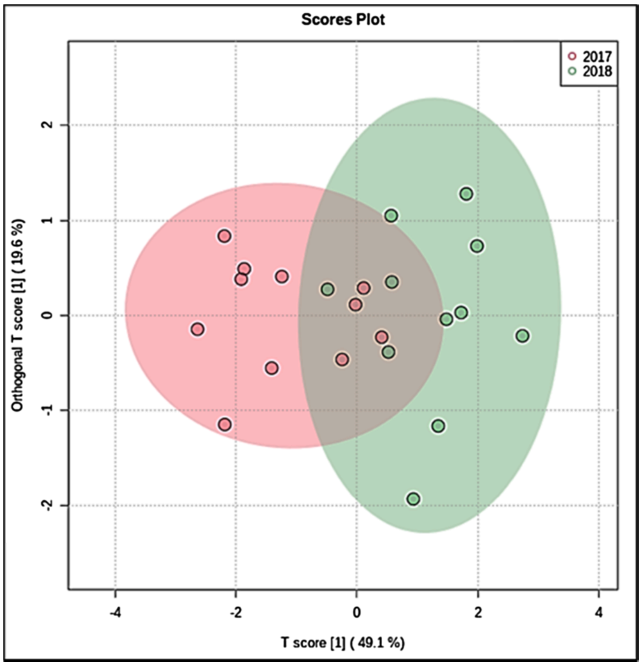

Fig. 5. Orthogonal Partial Least Squares-Discrimination (orthoPLS-DA) for the discrimination model of GC–MS fatty acids using the year of harvest as grouping variable (MetaboAnalyst).

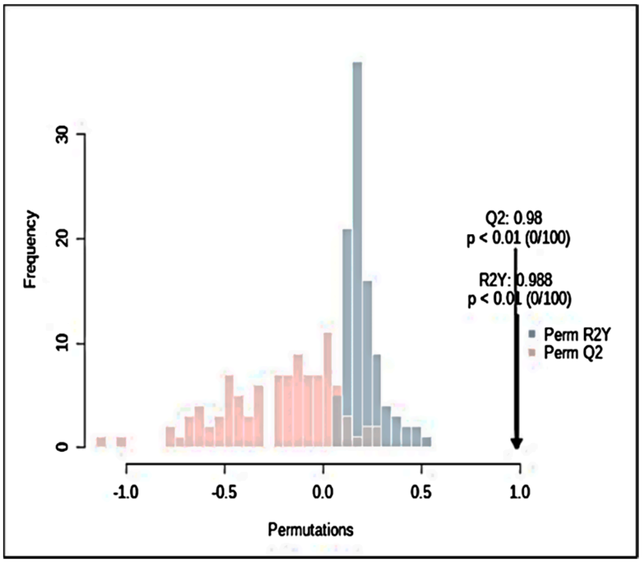

Fig. 6. Orthogonal Partial Least Squares - Discriminant Analysis (orthoPLS-DA) permutation for the discrimination model of FTIR variables using the year of harvest as grouping variable (MetaboAnalyst) (permutation numbers:100; $p < 0.01$).

classified correctly, while from the cross-validated ones, 72.7% with $R^2 = 0.619$ and $Q^2 = 0.411$ (Figs. 4 and 5).

3.4.2. *Discrimination based on FTIR spectroscopic study*

FTIR and Raman spectral data showed a similar distribution of characteristic peaks from year to year. It was observed that the spectra of the samples showed visually significant similarities with peaks in all major spectral regions. Thus, only multivariate statistical analysis can prove with certainty whether the samples show statistically significant differences between the two years of harvest.

The function created by the total spectral region 400–4000 cm^{-1}

Table 6
Performance of *t*-test for Equality of Means.

Fatty Acids		Levene's Test for Equality of Variances		*t*-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
tetradecanoic acid	Equal variances assumed	16.244	0.001	0.947	20	0.355	0.00909	0.00960	-0.01092	0.02911
	Equal variances not assumed			0.947	12.337	0.362	0.00909	0.00960	-0.01175	0.02993
palmitoleic acid[a]	Equal variances assumed	0.400	0.534	2.184	20	0.041	0.10545	0.04828	0.00475	0.20616
	Equal variances not assumed			2.184	19.396	**.041**[a]	0.10545	0.04828	0.00455	0.20636
palmitic acid[a]	Equal variances assumed	4.096	0.057	3.587	20	0.002	1.46273	0.40782	0.61203	2.31343
	Equal variances not assumed			3.587	13.297	**.003**[a]	1.46273	0.40782	0.58368	2.34177
heptadecenoic acid	Equal variances assumed	7.120	0.015	-0.149	20	0.883	-0.00091	0.00608	-0.01360	0.01178
	Equal variances not assumed			-0.149	14.961	0.883	-0.00091	0.00608	-0.01388	0.01206
margaric acid	Equal variances assumed	0.131	0.721	-0.714	20	0.484	-0.00364	0.00509	-0.01426	0.00699
	Equal variances not assumed			-0.714	19.505	0.484	-0.00364	0.00509	-0.01428	0.00701
linoleic acid[a]	Equal variances assumed	3.923	0.062	−2.404	20	0.026	−2.32000	0.96526	−4.33349	-0.30651
	Equal variances not assumed			−2.404	14.047	**.031**[a]	−2.32000	0.96526	−4.38962	-0.25038
oleic acid	Equal variances assumed	4.617	0.044	0.544	20	0.592	0.48273	0.88710	−1.36773	2.33319
	Equal variances not assumed			0.544	14.823	0.594	0.48273	0.88710	−1.41005	2.37551
stearic acid[a]	Equal variances assumed	0.166	0.688	3.062	20	0.006	0.27545	0.08996	0.08781	0.46310
	Equal variances not assumed			3.062	19.160	**.006**[a]	0.27545	0.08996	0.08728	0.46363
gondoic acid	Equal variances assumed	1.508	0.234	1.325	20	0.200	0.04909	0.03704	-0.02818	0.12636
	Equal variances not assumed			1.325	14.583	0.205	0.04909	0.03704	-0.03006	0.12824
araxidic acid	Equal variances assumed	0.029	0.868	−1.866	20	0.077	-0.02455	0.01316	-0.05199	0.00290
	Equal variances not assumed			−1.866	19.892	0.077	-0.02455	0.01316	-0.05200	0.00291
behenic acid[a]	Equal variances assumed	1.761	0.199	−2.196	20	0.040	-0.03182	0.01449	-0.06204	-0.00160
	Equal variances not assumed			−2.196	17.335	**.042**[a]	-0.03182	0.01449	-0.06234	-0.00129
tricosanoic acid	Equal variances assumed	4.938	0.038	−1.000	20	0.329	-0.00182	0.00182	-0.00561	0.00197
	Equal variances not assumed			−1.000	10.000	0.341	-0.00182	0.00182	-0.00587	0.00223
tetracosanoic acid	Equal variances assumed	4.938	0.038	−1.000	20	0.329	-0.00091	0.00091	-0.00281	0.00099
	Equal variances not assumed			−1.000	10.000	0.341	-0.00091	0.00091	-0.00293	0.00112

[a] Fatty acids which played the most important role for the discrimination between the two years of harvest, S.V. < 0.05.

showed great discriminatory ability (Wilks' lambda = 0.002) between 2017 and 2018 harvests' samples. The Classification Function Coefficients table indicated how strongly (higher magnitudes) the discriminating variables effected the score. The wavenumbers at 2922, 2853, 1744, 1654 and 722 cm^{-1} had the greatest impact on the discriminant score. From cross-validated grouped cases, 100.0% were classified correctly according to their FTIR variables and year of harvest (Figs. 6 and 7). The values of $R^2 = 0.988$ and $Q^2 = 0.980$, demonstrated in Fig. 6, indicated a high predictive accuracy.

3.4.3. *Discrimination based on Raman spectroscopic study*

The whole spectra range was selected for orthoPLS-DA. The 22 pistachio oil samples were not sufficiently classified into two groups (2017 and 2018 harvest year) (Fig. 8). The S.V. greater than 0.05 showed that the discrimination was not successful. Furthermore, the bad predictive accuracy of the discrimination model was demonstrated through the $R^2 = 0.644$ and $Q^2 = 0.270$ values (Fig. 9). The results indicate that only the 59.1% of original and cross-validated group cases were correctly classified.

From the above statistical analysis, it appears that the proposed chemometric models of differentiation of pistachio oils, which come from two consecutive harvest periods (2017, 2018), based on cross-validation and: a) GC–MS analysis and b) in FTIR spectroscopy are equivalent, but FTIR spectroscopy is faster, cheaper, more environmentally friendly and does not require specialized personnel. The corresponding chemometric model based on Raman spectroscopy is considered unsuccessful.

4. Conclusions

The fatty acid composition of *Pistacia vera* (Greek variety "Aegina") oils for two consecutive harvest years (2017, 2018) was analyzed by GC–MS, FTIR and Raman techniques. Twenty-two samples (2017, 11 samples and 2018, 11 samples) originated from four different pistachios production and marketing Greek regions, namely Aegina, Megara, Phthiotis and Trizina, were collected. The producers followed the same cultivation and post-harvest cares in both seasons. The extracted oil mean yield was found to be similar for the two growing seasons (61.7 and 60.8 g/100 g dried sample for 2017 and 2018, respectively). The oil analysis performed by GC–MS showed that the SFAs content, in all samples, reduced in 2018, with the mean value decreasing from 13.8% (2017) to 12.2% w/w (2018). On the contrary, the content of UFAs was found to increase in all samples in 2018, with the mean value varying from 86.2% (2017) to 87.2% w/w (2018). Regarding the MUFAs

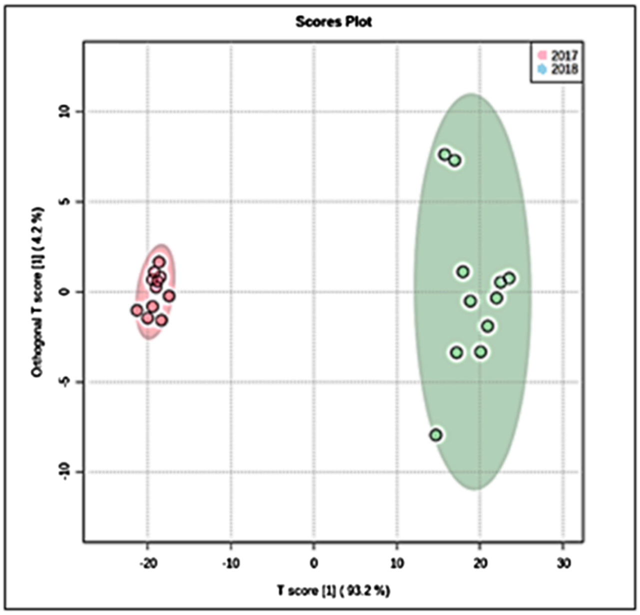

Fig. 7. Orthogonal Partial Least Squares-Discrimination (orthoPLS-DA) for the discrimination model of FTIR variables using the year of harvest as grouping variable (MetaboAnalyst).

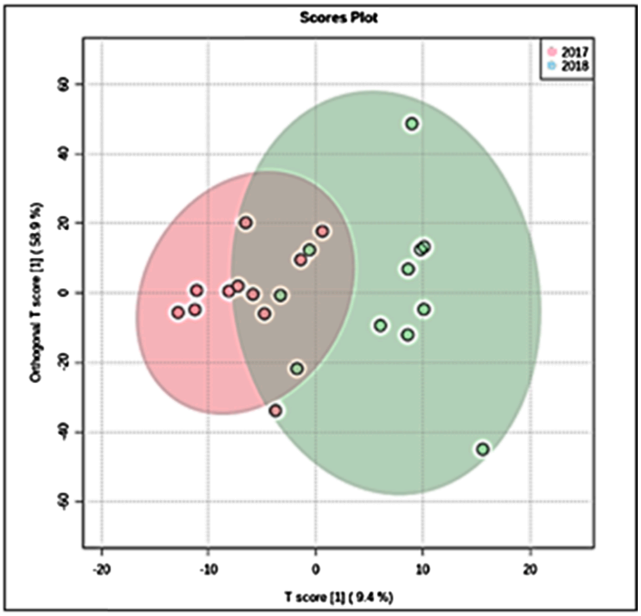

Fig. 8. Orthogonal Partial Least Squares-Discrimination (orthoPLS-DA) for the discrimination model of Raman variables using the year of harvest as grouping variable (MetaboAnalyst).

content, the slight decrease is not systematic in each sample and therefore no safe conclusions can be drawn. The quality superiority of 2018 harvest year was proven due to the higher content of unsaturated fatty acids compared to 2017.

Three chemometric models were also developed, in order to discriminate the oils of the two growing seasons, and therefore according to their quality, based on GC–MS, FTIR and Raman data combined with cross-validation techniques. The Raman chemometric model was unsuccessful (S.V. greater than 0.05). The differentiation chemometric model based on FTIR spectroscopy and cross-validation technique was found to be more successful (100.0% successful discrimination; $R^2 = 0.988$; $Q^2 = 0.980$) than the corresponding of GC–MS (72.3% successful discrimination; $R^2 = 0.619$; $Q^2 = 0.411$). Furthermore, FTIR spectroscopic technique is fast, more economical, environmentally friendly, and does not require special staff.

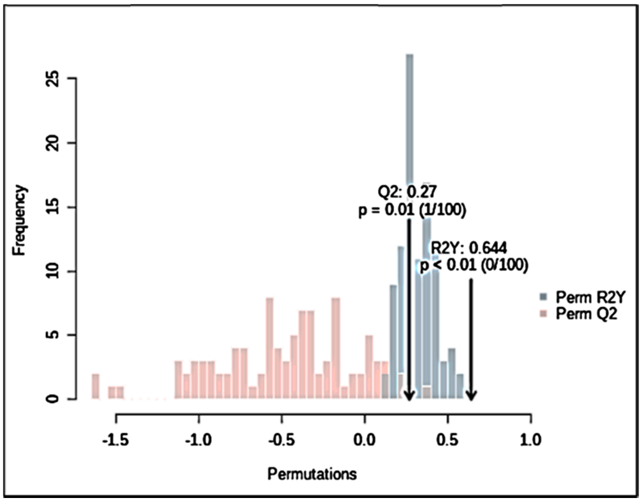

Fig. 9. Orthogonal Partial Least Squares - Discriminant Analysis (orthoPLS-DA) permutation for the discrimination model of Raman variables using the year of harvest as grouping variable (MetaboAnalyst) (permutation numbers:100; $p < 0.01$).

CRediT authorship contribution statement

Lydia Valasi: Conceptualization, Software, Formal analysis, Investigation, Resources, Writing – original draft. **Maroula G. Kokotou:** Validation, Formal analysis, Investigation, Writing – review & editing. **Christos S. Pappas:** Conceptualization, Methodology, Investigation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2021.110590.

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