Food Research International 103 (2018) 426–437

Contents lists available at ScienceDirect

Food Research International

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

Chemometric characterization of virgin olive oils of the two major Cypriot cultivars based on their fatty acid composition

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ARTICLE INFO

Keywords:
Cypriot virgin olive oil
Variety
FAME
GC–MS
GC-FID
Chemometrics
Altitude
Microclimate

ABSTRACT

The effects of the geographical region and the botanical origin of olive oils on the profile of fatty acids isolated from monovarietal virgin olive oils from Cyprus were investigated, in order to establish, for the first time, promising models for authentication and classification of monovarietal virgin olive oils produced on the island. The two dominant Cypriot olive cultivars, Cypriot (ladoelia), and Koroneiki (lianolia), were analyzed for fatty acid composition by GC/FID after preparation of Fatty Acid Methyl Esters from olive oil, using a cold esterification method. The data obtained were analyzed statistically using multivariate and univariate ANOVA, principal components analysis (PCA) and hierarchical cluster analysis. Results showed that the olive oils can be separated into two distinct groups using the FAME data bank obtained from 225 samples. Significant differences in the proportion of some variables such as polyunsaturated linoleic acid, and the ratio C18:1/C18:2 from oils of different varieties and geographical regions were detected. The ratios C18:1/C18:2 and MUFA/PUFA, as well SFA, ω-9 and PUFA showed the higher discriminant power based on the cultivar.

1. Introduction

Cyprus is a small country located at the Mediterranean basin and considered a small size producer of virgin olive oils compared to the production of European countries such as Spain (40%), Italy (21%) and Greece (13%), which constitute 74% of the total worldwide olive oil production, with 1.275, 395 and 285 thousand tons per year respectively for the harvesting six-year period 2009/10-2014/15 (IOOC, 2015a); Vossen, 2013).

Despite of its small size Cyprus is a virgin olive oil producer of high potency, with an average annual production of 5.5 thousand tons for the six-year crops 2009/10-2014/15 (FAOStat, 2011), which corresponds to 0.84 tons of olive oil per km^2 of land. This is quite challenging when compared with the major olive oil producing countries, Spain, Italy and Greece which produce 2.5, 1.3 and 2.2 tons olive oil/km^2 of land respectively, showing the high potency of Cyprus to contribute to the international olive oil market.

The olive oil production has been a very important labor for Cyprus since the ancient years. The cultivation of olive tree dates back to 4800 B.C. at Fyllia village in Northern part of Cyprus, while archeological evidence showed that olive domestication started at previous time for many areas of the Mediterranean basin (Zohary, Hopf, & Weiss, 2012).

Olive oil is a product of great importance because of its unique sensory characteristics, nutritional and therapeutic properties. It is derived from the fruit *Olea Europea* L. using only physical or mechanical methods exhibiting high redox stability during long-term storage. The high nutritional value of the olive oil consumption arises from the high content of monounsaturated fatty acids, such as oleic acid (56–84%), and the presence of minor components such as phytosterols, squalene, vitamins and antioxidants as polar phenols and tocopherols (Boskou, Blekas & Tsimidou, 2006).

Health beneficial effects to humans have also been correlated with the consumption of olive oil. Oleic acid protects human body against several types of cancer, including chest, colon and lung, has neurotrophic properties, and lowers LDL oxidation slowing the progression of atheromatic lesion on the artery walls while minor biomolecules of olive oil have anti-inflammatory activity, anticarcinogenetic potency, chemopreventive properties and exert protection against neurogenerative conditions such as Alzheimer and Parkinson diseases (Dewapriya, Himaya, Li, & Kim, 2013; Mateos et al., 2013; Medina & Tabernero, 2010).

It is noteworthy, that the rate for incidents such as heart attacks, different types of cancer and hypertension is smaller for the Mediterranean countries compare to other countries. This has been attributed to the high consumption ratio of unsaturated fatty acids to saturated fatty acids from the locals at the countries located in the

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http://dx.doi.org/10.1016/j.foodres.2017.10.064
Received 9 August 2017; Received in revised form 18 October 2017; Accepted 28 October 2017
Available online 31 October 2017

Mediterranean Basin (Keys et al., 1986; Trichopoulou & Trichopoulou, 2009). Recently, two health claims for food labeling, one on oleic acid and one on olive oil polyphenols been approved by EC Regulation 432/2012 (EEC, 2012), and US FDA permission for olive oil product labeling (US, 2004) constitute the high significance of diets rich in olive oil for human health.

The dependence of olive oil quality on cultivar, geographic origin and pedoclimatic conditions results in the production of monovarietal olive oils exerting unique chemical composition and organoleptic properties (D'Imperio, Dugo, Alfa, Mannina, & Segre, 2007; Luna, Morales, & Aparicio, 2006). However, due to high commercial value such monovarietal olive oils may be subjected to fraud.

European Community has established a framework for the development of geographical indications and designations of origin to enhance the credibility of the products bearing such indications in the consumers' eyes, and to ensure fair competition between the producers (ECC, 2006).

In this line, the incorporation of Cyprus in the European Union in 2004 put into force the Regulation of the European Union EC 2081/92 on the protection of geographical indications (PGI) and designations of origin (PDO) of agricultural products and food. Today the upgraded Regulation EC 510/2006 of the European Union is applied (ECC, 2006). Therefore, this research could provide support for future applications of the Cypriot olive oils as PDO or PGI products.

Two olive genetic varieties, Cypriot (ladoelia) and Koroneiki (lianolia or psiloelia) are the predominant cultivars on the island. Cypriot cultivar is autochthonous, adopted to drought and hot conditions of the island, and produces fruits of medium size, appropriate for table olives and olive oil of delicate taste (Gregoriou, 2006). Cypriot cultivar is considered as one of the most aromatic varieties of olives in the world because of its characteristic flavor. The maturation of the fruit at the lowlands occurs in October- early November, while at hilly areas in late November - early December. There are 31 local accessions of the Cypriot variety grown at different areas, most of them count very small population, getting their names from the names of villages at which the specific accessions were identified. This intra-varietal diversity has also been observed for other olive varieties spread in Mediterranean basin, a fact attributed to the crosses of wild local oleasters with a specific domestic olive cultivar (Gregoriou, 1996).

Koroneiki variety is a Greek variety, the predominant one in Peloponnisos and Crete, it was introduced to Cyprus in 1977. The tree produces small-sized fruits which have high yield in oil of exceptional flavor and aroma, up to 27% oil, depending on growing conditions, in a stable and sustainable way. It is considered the best variety for oil production, since it is a productive tree that has a stable bearing and overproduces every second year. The maturation of the fruit at the lowlands occurs in November–December, while at hilly areas until the end of February.

Authenticity studies have been reported for the classification of olive oils according to their botanical or geographical origin based on determinations of variables count for their major or minor constituents, such as: fatty acids profile of olive oils (D'Imperio et al., 2007; García-González, Luna, Morales, & Aparicio, 2009; Mannina et al., 2003; Stefanoudaki, Kotsifaki, & Koutsaftakis, 1999), phytosterols (Matos et al., 2007), phenols (Alonso-Salces et al., 2010; Petrakis, Agiomyrgianaki, Christophoridou, Spyros, & Dais, 2008), squalene (D'Imperio et al., 2007), volatiles (Luna et al., 2006) or a combination of two or more components (Aparicio, Morales, Aparicio-Ruiz, Tena, & García-González, 2013; Karabagias et al., 2013; Longobardi et al., 2012; Matos et al., 2007; Merchak et al., 2017; Ollivier, Artaud, Pinatel, Durbec, & Guérere, 2006; Ollivier, Artaud, Pinatel, Durbec, & Guérère, 2003). Several multi-informative techniques quantifying more than one components simultaneously, such as ^{1}H, ^{13}C, ^{31}P NMR (Alonso-Salces et al., 2010; Merchak et al., 2017; Petrakis et al., 2008), Fourier Transform Infra-Red (FT-IR) spectroscopy (Tapp, Defernez, & Kemsley, 2003), and $\delta\ ^{13}C$ $\delta\ ^{2}H$ and/or their isotopic ratio (Spangenberg, Macko, & Hunziker, 1998) have been used.

Up to now, geographical origination of olive oils has been limited to selected isolated areas within a province, regarding specific locations. No information related to the oils composition for the bulk olive oil production of a province/country is provided although olive oils produced at different locations may be affected by several other factors, such as different altitude and microclimate of olive orchards or different irrigation regimes of trees, ripeness of collected olives, and other agronomic practices followed by the local farmers. To our knowledge there are no chemometric studies on fatty acid composition of cultivars for discrimination of olive oils from a broad area of a country combining adjacent locations of continuous altitude levels, and adjacent provinces, encompassing various irrigation systems and degrees of olive fruit maturation. The effect of Altitude on the composition of fatty acids of VOOs and its possible potency as discrimination factor of VOOs, for the first time, is explored for extended elevation zones covering several adjacent provinces all over the country. In this work the fatty acid composition of virgin olive oils (VOOs) of Cypriot and Koroneiki cultivars are mapped all over the free provinces of Cyprus, including different districts and altitude zones. Furthermore, there is no previous data concerning the chemical composition in fatty acids of the virgin olive oils of the island. The aim of this work is categorized into (a) the recording of the fatty acid content of VOOs from the predominant cultivars of Cypriot and Koroneiki (b) the investigation for authenticity of the botanical origin of Cypriot VOOs to differentiate between cultivars, (c) the investigation for authenticity of Cypriot VOOs regarding geographical origin exploring the impact of altitude or district on the composition of VOOs in fatty acids.

In both cases, determination of indicators (components of distinct power) is complicated, since chemical composition of virgin olive oil is influenced by various factors such as: botanical origin, climatic conditions, soil, degree of ripening of olives and the way of extraction (Montealegre, Marina Alegre, & García-Ruiz, 2010; Tapp et al., 2003). The concept of 'indicators' in this research, refers to those variables (fatty acids, and the six groups of fatty acids) which have the ability to discriminate VOOs among cultivars or different geographical areas.

2. Materials and methods

2.1. Olive oil samples

A total of two hundred and twenty-five (n = 225) authentic samples of monovarietal VOOs of Cypriot and Koroneiki cultivars were obtained over three harvesting periods (2013/2014, 2014/2015, 2015/2016), and collected from 124 different villages/locations all over the free areas of the island. Three altitude classes (a) < 100 m, (b) 100–450 m, (c) > 450 m, were chosen based on the terrain of the island and the areas of olive growing, and taking into account literature reports regarding olive oil dependence on the elevation. Nicosia is considered a hilly to mountainous area, with the lowest altitude of 163 m at Solea village.

This research focuses on the central and south part of Cyprus (71.4%) because the rest part of Cyprus (28.6%) including Kyrenia province and parts of Famagusta and Nicosia regions are under Turkish occupation, therefore unavailable for study. Although Cyprus has 6 provinces, for the purposes of this study samples have been assigned to four Cypriot districts, namely, Limassol, Larnaca/Famagusta (combined), Nicosia, and Paphos, matching more or less the area of provinces of the free part of the island, Fig. 1.

2.2. Quality indices

Free acidity expressed as % oleic acid, peroxide value expressed as meq O_2/kg, and specific extinction (K_{232} and K_{268}) and its variation, ΔK, calculated from the absorption at 232–272 nm, were determined based on the experimental procedures according to the Commission

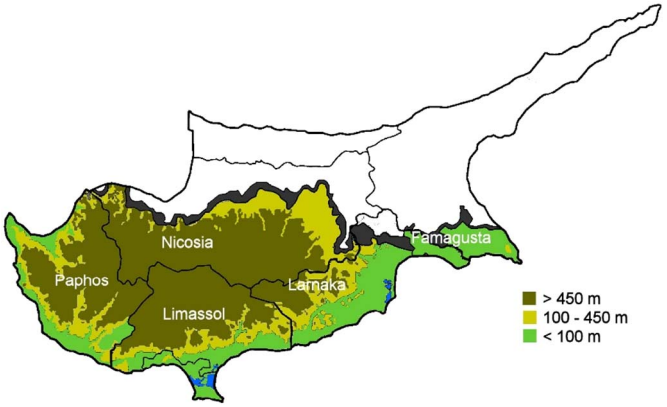

Fig. 1. Cyprus sampling map for this work, with districts and altitude classes indicated.

Regulation (EEC) No. 2568/91 and later amendments (ECC, 1991).

2.3. Determination of Fatty Acid Methyl Esters (FAME), by GC/FID and GC/MS

2.3.1. Preparation of FAME

Fatty acids were measured as FAME by gas chromatography. Experimental procedure was performed according to COI/T.20/Doc. No.33 (IOOC, 2015b). More specific, olive oil (0.100 g) dissolved in n-heptane (4.50 ml) was cold transmethylated with a solution of KOH 2.0 N (0.100 ml). The mixture was centrifuged at 4000 rpm for 3 min. The upper layer was isolated and measured by GC/FID. Each sample was analyzed in triplicate.

2.3.2. FAME analysis

Identification of FAME was performed by GC/MS and quantification was done by GC/FID gas chromatograph. Qualitative analysis was performed by (i) comparing the retention time with those of the 37 components reference standard mixture (37-Component FAME Mix, Supelco) analyzed under exactly the same operational conditions, and (ii) mass spectra obtained from GC–MS Libraries: NIST21/NIST107/NIST08. For quantitative analysis, each fatty acid was expressed as a percentage by mass of methyl esters, by determining the percentage represented by the area of the corresponding peak relative to the sum of the areas of all the peaks, and the results were expressed as a percentage of total fatty acids.

2.3.3. GC/MS instrumentation

Identification of FAME was conducted by Shimadzu GC-2010 gas chromatograph equipped with a split/splitless injector, and GCMS QP 2010 Plus mass spectrometer detector, and an AOC-20i autosampler. The temperatures of the injector and detector were 250 °C and 240 °C respectively. FAME separation was achieved using a Supelco-WAX 10 fused silica capillary column of length 60 m, I.D. 0.32 μm, film thickness 0.25 mm and poly(ethylene)glycol phase. Helium was used as a carrier gas at an inlet pressure of 65.1 kPa. The split ratio was 1: 20 and the flow rate was 1.00 ml/min. Temperature program: The initial oven temperature was 150 °C and increased to 180 °C at a rate of 0.60 °C/min, then increased to 200 °C at a rate of 1 °C/min and held for 10 min. Finally, increased to 220 °C at a rate of 5 °C/min and held for 25 min.

2.3.4. GC/FID instrumentation

Quantification of FAME was conducted by Agilent Technologies 7890A GC system equipped with a split/splitless injector (250 °C) and a flame ionization detector (FID) (250 °C). The column used for the quantification of the peaks was SP-2560 of length 100 m, I.D. 0.20 μm, film thickness 0.25 mm and poly(bicyanopropyl siloxane) phase. Hydrogen was used as a carrier gas at an inlet pressure of 254.7 kPa. The split ratio was 1: 100 and the flow rate was 1.00 ml/min. Temperature program: the initial oven temperature was 155 °C and increased to 220 °C at a rate of 1.5 °C/min, and held for 7 min.

2.4. Chemometric methods

Data for fatty acids composition (%) and 6 groups of fatty acids were analyzed with the ANOVA method, within the methodological frame of General Linear Models (Rencher, 2000). The corresponding models involved the effects (main and interactions) of three factors; factor "Cultivar" with two levels (Cypriot and Koroneiki), factor "altitude class" with three levels (< 100 m, 100–450 m, and > 450 m), and factor "District" with four levels (Limassol, Larnaca/Famagusta, Nicosia, and Paphos). Initially, data were analyzed, according to the previously mentioned model, with the MANOVA method (Hair, Anderson, Tatham, & William, 1995) in order: (a) to highlight significant effects, taking into account the correlation between the dependent variables and (b) to enhance the protection against the inflation of Error Type I rate of the followed (univariate) ANOVA's and multiple comparisons' procedures. Means' separation was accomplished with the Least Significant Difference–LSD criterion (protected LSD) in order to achieve high statistical power levels of the multiple comparisons tests and, consequently, not to miss interesting differences (Saville, 1990). All the previously analyses were performed with $\log_{10}(X + 1)$ transformed data values (X), in order to achieve normality and homoscedasticity of linear models' residuals. Reported results presented in tables (e.g. mean values) have been back-transformed. The correlation between 13 fatty acids (set 1) and the chemical parameters SFA, MUFA, PUFA, C18:1/C18:2, MUFA/PUFA, and ω-9 (6 groups of fatty acids, set 2) were examined by evaluating the significance and the magnitude of the corresponding Spearman's *rho* rank correlation coefficients. Principal Component Analysis (PCA), with Varimax rotation, was performed on the fatty acids data sets (set 1 and set 2) in order to detect latent structures of fatty acids (Hair et al., 1995). Finally, Hierarchical Cluster Analysis (HCA) was performed for examining the possibility of classification of samples to clusters, based on the similarities observed for the chemical parameters SFA, MUFA, PUFA, C18:1/C18:2, MUFA/PUFA, and ω-9, between sets of observations. Ward's minimum variance criterion was applied as a general agglomerative hierarchical clustering procedure on the squared Euclidean distances among observations (Sharma, 1996). Resulted clusters' profiles relative to the above mentioned six chemical parameters were compared by means of a series of *t*-tests. Distributions of cultivars, altitude classes, and districts within clusters were compared with the χ^2 test. In all statistical hypothesis testing procedures the significance level was preset at $\alpha = 0.05$ ($p \leq 0.05$). Results are displayed as figures and tables. All the statistical analyses were done with the SPSS v.15.0 statistical software (SPSS Inc., Ill: Chicago).

2.5. Nomenclature

2.5.1. Fatty acids

C16:0, palmitic acid (hexadecanoic acid); C16:1(7) or C16:1ω-9, hypogeic acid (cis-7-hexadecenoic acid); C16:1(9) or C16:1ω-7, palmitoleic acid (cis-9-hexadecenoic acid); C17:0, margaric acid (heptadecanoic acid); C17:1(9) or C17:1ω-8, margaroleic acid (cis-9-heptadecenoic acid); C18:0, stearic acid (octadecanoic acid); C18:1(9) or C18:1ω-9, oleic acid (cis-9-octadecenoic acid); C18:1(11) or C18:1ω-7, cis-vaccenic acid (cis-11-octadecenoic acid); C18:2(9,12) or C18:2ω-6, linoleic acid (cis,cis-9,12-octadecadienoic acid); C18:3(9,12,15) or C18:3ω-3, α-linolenic acid (all cis-9,12,15-octadecatrienoic acid); C20:0, arachidic acid (eicosanoic acid); C20:1(11) or C20:1ω-9, gondoic acid (11-eicosenoic acid); C22:0, behenic acid (docosanoic acid); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω-9, omega-9, ω-9 fatty acids.

3. Results and discussion

3.1. Quality indices

The conventional analyses for quality indices carried out, allowed for all samples to be classified as extra virgin and virgin olive oils according to EC regulation (ECC, 1991), Codex Alimentarius (Alimentarius, 2015), and the IOOC (IOOC, 2015c). Free acidity (% m/m expressed in oleic acid) of the samples are in the range from 0.2 to 1.9%, which are in the limits ≤ 0.8 and ≤ 2.0 for extra virgin and virgin olive oil respectively. Peroxide value (meq O_2/kg oil) of the analyzed samples ranging from 6 to 20 meq O_2/kg oil are in conformity to those described for both extra virgin and virgin olive oils (≤ 20). Limits for the Specific extinction parameters K_{232}, K_{268}, and ΔK, according to EC regulation, are ≤ 2.50, ≤ 0.22, and ≤ 0.01 for the extra virgin olive oil, and ≤ 2.60, ≤ 0.25, and ≤ 0.01 for virgin olive oil. Determination of spectroscopic values of the olive oils samples resulted in K_{232} from 0.5 to 2.60, K_{268} from 0.05 to 0.25, and ΔK from 0.00 to 0.01 for all samples analyzed, classifying oils as VOOs.

3.2. Chemometrics

Tables 1, 2, 3, and 4 report the results obtained for each fatty acid and the six groups of fatty acids combined, identified for the two cultivars under evaluation and for three years' harvest 2013/2014, 2014/2015, and 2015/2016 at the different altitude classes, and districts. The observed fatty acids composition's great variability is well known and attributed to several factors such as botanical origin (Ollivier et al., 2006; Stefanoudaki et al., 1999), pedoclimatic conditions, soil characteristics, olive maturity(Gutiérrez, Jimenez, Ruiz, & Albi, 1999), altitude, etc. (Karabagias et al., 2013; Longobardi et al., 2012; Merchak et al., 2017; Ollivier et al., 2003).

3.2.1. Multivariate analysis

MANOVA has been used to identify significant effects such as the main effects, and to detect significant differences between the dependent variables of the two or more groups defined by the treatment, when all variables are considered jointly. MANOVA results for the set of 13 fatty acids showed significant effects for up to two-way interactions. In particular, for "Cultivar × District" (Wilk's λ = 0.605, $p < 0.001$, $\eta^2 = 0.154$), for "Cultivar × Altitude class" (Wilk's λ = 0.778, p = 0.016, $\eta^2 = 0.118$), and for "District × Altitude class" (Wilk's λ = 0.473, $p < 0.001$, $\eta^2 = 0.117$). Multivariate tests were also performed for the set of six groups of fatty acids and resulted to significant effects for up to two-way interactions. Specifically, for "Cultivar × District" (Wilk's λ = 0.844, p = 0.013, $\eta^2 = 0.055$) and for "District × Altitude class" (Wilk's λ = 0.729, p = 0.003, $\eta^2 = 0.051$). Index "eta squared" (η^2) is a measure of the corresponding effect size (magnitude of the effect).

Table 1
Fatty acid composition (%) of 225 Cypriot VOO samples resulting from the two main Cypriot cultivars.

Cultivar	Cypriot (ladoelia)			Koroneiki (lianolia)		
Samples no.	104			121		
Fatty acid[a]	Mean (%)	Range		Mean (%)	Range	
		Min	Max		Min	Max
C16:0	11.18a	7.15	17.27	11.33a	6.14	16.05
C16:1(7)	0.01a	0.00	0.10	0.02a	0.00	0.28
C16:1(9)	0.39a	0.00	1.12	0.53a	0.00	1.27
C17:0	0.04a	0.00	0.21	0.01b	0.00	0.11
C17:1(9)	0.02a	0.00	0.21	0.01b	0.00	0.15
C18:0	3.48a	1.72	7.41	2.86b	0.49	5.47
C18:1(9)	70.97b	55.35	82.63	73.65a	61.04	82.90
C18:1(11)	1.95a	0.57	3.73	2.32a	0.00	3.88
C18:2(9,12)	10.94a	4.88	15.66	8.22b	3.24	13.15
C18:3(9,12,15)	0.46a	0.00	1.22	0.48a	0.00	1.14
C20:0	0.38a	0.00	1.30	0.37a	0.00	1.01
C20:1(11)	0.12a	0.00	0.41	0.13a	0.00	0.42
C22:0	0.06a	0.00	0.29	0.09a	0.00	0.32
Σ^{SFAs}	15.14a	9.33	24.61	14.65b	6.63	22.11
Σ^{MUFAs}	73.46b	59.61	84.29	76.66a	65.96	84.34
Σ^{PUFAs}	11.41a	5.09	16.78	8.70b	3.24	13.53
$C_{18:1}/C_{18:2}$	7.09b	3.79	17.23	10.15a	5.42	25.58
MUFA/PUFA	6.86b	3.64	16.56	9.71a	5.29	25.58
ω-9	71.10b	55.63	82.86	73.80a	61.41	82.90

Values were determined as the % of the total fatty acids. Significance letters (a,b) in the same row indicate significant differences (p ≤ 0.05) according to the LSD criterion.
[a] All unsaturated fatty acids are cis isomers, Σ - sum.

3.2.2. Univariate analysis

ANOVA, as a univariate procedure, has been used to assess group differences on a single metric variable such as a fatty acid or a group of fatty acids. Univariate-ANOVAs, followed MANOVA, resulted to significant effects ($p < 0.05$) up to two-way interactions between the independent variables ("Cultivar", "Altitude class", and "District"). The main effect of "Cultivar" was significant for: margaric, margaroleic, stearic, oleic, linoleic acids, SFA, MUFA, PUFA, C18:1/C18:2, MUFA/PUFA, and ω-9 fatty acids, shown in Table 1. Independent variable "District" as main effect was significantly important for: palmitic, palmitoleic, cis-vaccenic, linoleic, arachidic, gondoic acids, and SFA. The main effect of "Altitude class" was significant in the case of: palmitoleic, cis-vaccenic, linolenic, and arachidic acids.

Three combinations of two-way interactions were examined for the three independent variables; Interaction of "Cultivar × Altitude", "Cultivar × District", and "District × Altitude".

3.2.2.1. "Cultivar × Altitude". The two-way interactions of "Cultivar × Altitude" showed significant effects for margaric acid, as shown in Table 2. More specific, for the elevation zones 100–450 m and > 450 m, the concentration of margaric acid is higher for VOOs of Cypriot than the Koroneiki cultivar. No significant effect observed for either the rest of the fatty acids or the groups of fatty acids. Several studies in the literature have reported the effect of the fatty acids in the differentiation of olive oils of only one cultivar by the altitude (Aparicio, Ferreiro, & Alonso, 1994; Mousa, Gerasopoulos, Metzidakis, & Kiritsakis, 1996). According to those studies oleic acid, linoleic acid, and the ratio unsaturated: saturated fatty acids or linoleic acid alone in oil were found to increase by increasing the altitude. It was reported that selected altitude levels, regarding isolated areas, were either 100 m and 800 m, or ≤ 400 m and 700 m ≤, characterized by high inter-level distance, because the influence of the altitude is only strong enough to find a discrete relationship with the chemical composition and not a continuous one(R. Aparicio et al., 1994). In other studies three different altitude levels have been used for discriminating oils extracted from olives of isolated trees located at those altitudes (Merchak et al., 2017). In the current work, all altitude levels were taken into consideration to reflect the total olive oil production of the described areas of Cyprus for investigating possible variables with highest discriminant power for geographical differentiation within the island. However, the fatty acid composition of olive oil for a specific cultivar is also dependent on several other factors such as the pedoclimatic effect, reflecting the microclimate of the surrounding environment, the soil, the humidity, the rain-fed/irrigation system, and degree of olives maturation, which may have a more primary effect than just the altitude in itself, making the discrimination of olive oils more complicated, as previously reported (Aparicio et al., 1994; Dag et al., 2015; D'Imperio et al., 2007; Gutiérrez et al., 1999).

Another silent feature of Table 2 is that each altitude level is extended to several provinces/districts, with the high elevation, > 450 m, and the mediate elevation, 100–450 m to show the highest

Table 2
Fatty acid composition (%) of the two Cultivars at different altitude classes.

Cultivar				Cypriot (ladoelia)									Koroneiki (lianolia)					
Altitude class	< 100 m			100–450 m			> 450 m			< 100 m			100–450 m			> 450 m		
Samples no.	22			54			28			41			62			18		
Fatty acid[a]	Mean (%)	Range		Mean (%)	Range		Mean (%)	Range		Mean (%)	Range		Mean (%)	Range		Mean (%)	Range	
		Min	Max		Min	Max		Min	Max		Min	Max		Min	Max		Min	Max
C16:0	12.08 a;w	8.62	17.27	10.89 a;w	7.15	16.49	11.05 a;w	7.61	14.28	11.74 a;w	6.88	15.33	11.31 a;w	6.14	15.38	10.46 a;w	6.48	16.05
C16:1(7)	0.02 a;w	0.00	0.06	0.01 a;w	0.00	0.08	0.01 a;w	0.00	0.10	0.02 a;w	0.00	0.06	0.03 a;w	0.00	0.28	0.01 a;w	0.00	0.07
C16:1(9)	0.58 a;w	0.19	1.12	0.34 a;w	0.00	0.98	0.35 a;w	0.00	1.05	0.61 a;w	0.00	1.15	0.53 a;w	0.00	1.27	0.39 a;w	0.00	0.82
C17:0	0.02 b;w	0.00	0.07	0.04 a;w	0.00	0.21	0.04 a;w	0.00	0.16	0.01 a;x	0.00	0.05	0.01 a;x	0.00	0.11	0.01 a;x	0.00	0.05
C17:1(9)	0.02 a;w	0.00	0.09	0.02 a;w	0.00	0.17	0.03 a;w	0.00	0.21	0.01 a;w	0.00	0.07	0.01 a;w	0.00	0.15	0.00 a;w	0.00	0.05
C18:0	3.22 a;w	2.30	4.15	3.46 a;w	1.77	7.41	3.73 a;w	1.72	5.83	2.82 a;w	0.72	3.95	2.94 a;w	0.49	5.47	2.64 a;w	0.84	5.05
C18:1(9)	69.58 a;w	62.74	77.97	71.33 a;w	55.35	82.08	71.36 a;w	58.82	82.63	73.04 a;w	65.75	81.28	73.24 a;w	61.04	82.90	76.42 a;w	65.68	82.50
C18:1(11)	2.38 a;w	1.43	3.73	1.81 a;w	0.57	3.24	1.88 a;w	0.76	3.17	2.55 a;w	0.39	3.85	2.24 a;w	0.00	3.88	2.07 a;w	0.00	3.42
C18:2(9,12)	10.88 a;w	7.10	14.54	11.18 a;w	7.17	15.66	10.53 a;w	4.88	14.67	8.06 a;w	3.63	12.07	8.66 a;w	3.24	13.15	7.12 a;w	3.62	10.86
C18:3(9,12,15)	0.58 a;w	0.33	0.95	0.41 a;w	0.00	1.13	0.48 a;w	0.00	1.22	0.51 a;w	0.00	0.82	0.48 a;w	0.00	1.14	0.39 a;w	0.00	0.73
C20:0	0.43 a;w	0.21	0.69	0.36 a;w	0.00	1.30	0.39 a;w	0.00	0.88	0.40 a;w	0.00	0.64	0.37 a;w	0.00	1.01	0.29 a;w	0.00	0.79
C20:1(11)	0.11 a;w	0.00	0.37	0.11 a;w	0.00	0.38	0.13 a;w	0.00	0.41	0.14 a;w	0.00	0.33	0.13 a;w	0.00	0.42	0.13 a;w	0.00	0.40
C22:0	0.09 a;w	0.00	0.26	0.05 a;w	0.00	0.29	0.05 a;w	0.00	0.28	0.10 a;w	0.00	0.32	0.09 a;w	0.00	0.32	0.08 a;w	0.00	0.20
Σ^{SFAs}	15.84 a;w	11.15	20.48	14.79 a;w	9.40	24.61	15.27 a;w	9.33	20.34	15.06 a;w	7.59	19.56	14.73 a;w	6.63	21.59	13.47 a;w	7.32	22.11
Σ^{MUFAs}	72.70 a;w	67.51	80.22	73.63 a;w	59.61	82.64	73.75 a;w	63.76	84.29	76.37 a;w	70.33	82.89	76.17 a;w	65.96	84.01	79.03 a;w	68.97	84.34
Σ^{PUFAs}	11.46 a;w	7.72	14.97	11.59 a;w	7.80	16.78	11.00 a;w	5.09	15.90	8.56 a;w	3.63	12.87	9.14 a;w	3.24	13.53	7.51 a;w	3.62	11.58
$C_{18:1}/C_{18:2}$	7.00 a;w	4.73	11.00	6.86 a;w	3.79	10.69	7.58 a;w	4.22	17.23	10.24 a;w	5.72	16.39	9.52 a;w	5.42	2558	12.16 a;w	6.55	22.40
MUFA/PUFA	6.67 a;w	4.61	10.15	6.69 a;w	3.64	10.44	7.33 a;w	4.01	16.56	9.75 a;w	5.46	15.69	9.12 a;w	5.29	25.58	11.69 a;w	6.24	22.40
ω-9	69.71 a;w	63.16	78.02	71.45 a;w	55.63	82.08	71.50 a;w	59.33	83.86	73.20 a;w	66.13	81.46	73.39 a;w	61.41	82.90	76.56 a;w	65.91	82.79

Values were determined as the % of the total fatty acids. Significance letters (a–c) in the same row concerning the same cultivar indicate significant differences ($p \leq 0.05$) according to the LSD criterion. Significance letters (w, x) in the same row concerning the same altitude class indicate significant differences ($p \leq 0.05$) according to the LSD criterion.

[a] All unsaturated fatty acids are cis isomers, Σ - sum.

Table 3
Fatty acid composition (%) of the two Cultivars at different district.

Cultivar	Cypriot (ladoelia)				Koroneiki (lianolia)			
District	Larnaca/Famagusta	Limassol	Nicosia	Paphos	Larnaca/Famagusta	Limassol	Nicosia	Paphos
Samples no.	33	12	39	20	52	17	36	16
Fatty acid[a]	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)
C16:0	12.15 a;w	10.91 ab;w	10.17 b;x	11.71 ab;w	11.74 a;w	9.67 c;x	11.42 b;w	11.41 b;w
C16:1(7)	0.02 a;w	0.01 a;w	0.01 a;w	0.01 a;w	0.02 a;w	0.01 a;w	0.04 a;w	0.01 a;w
C16:1(9)	0.59 a;w	0.28 a;w	0.28 a;w	0.35 a;w	0.62 a;w	0.29 a;w	0.59 a;w	0.38 a;w
C17:0	0.05 a;w	0.04 ab;w	0.03 b;w	0.02 b;w	0.01 a;x	0.00 a;x	0.02 a;w	0.00 a;w
C17:1(9)	0.03 a;w	0.02 a;w	0.02 a;w	0.02 a;w	0.01 a;w	0.00 a;w	0.02a;w	0.00 a;w
C18:0	3.72 a;w	3.59 a;w	3.35 a;w	3.27 a;w	2.86 ab;x	2.18 c;x	3.16 a;w	2.85 ab;w
C18:1(9)	67.64 b;x	72.23 ab;x	73.11 a;w	71.54 ab;w	73.13 b;w	78.09 a;w	72.22 ab;w	74.13 ab;w
C18:1(11)	2.45 a;w	1.76 ab;w	1.61 b;x	1.90 b;w	2.55 a;w	1.74 b;w	2.37 a;w	2.01 ab;w
C18:2(9,12)	12.08 a;w	10.22 a;w	10.55 a;w	10.27 a;w	7.89 a;x	7.34 a;x	8.97 a;x	8.55 a;x
C18:3(9,12,15)	0.62 a;w	0.36 a;w	0.39 a;w	0.43 a;w	0.53 a;w	0.36 a;w	0.53 a;w	0.31 a;w
C20:0	0.45 a;w	0.37 a;w	0.34 a;w	0.35 a;w	0.42 a;w	0.21 b;x	0.42 a;w	0.24 b;w
C20:1(11)	0.17 a;w	0.10 ab;w	0.10 ab;w	0.07 b;w	0.15 a;w	0.07 c;x	0.17 a;w	0.05 b;w
C22:0	0.07 a;x	0.07 a;w	0.05 a;w	0.05 a;w	0.10 a;w	0.05 b;x	0.11 ab;w	0.04 ab;w
Σ^{SFAs}	16.45 a;w	14.99 ab;w	13.94 b;w	15.41 ab;w	15.13 a;w	12.10 d;x	15.13 a;w	14.54 a;w
Σ^{MUFAs}	70.89 b;x	74.40 ab;x	75.13 a;w	73.89 ab;w	76.47 a;w	80.20 a;w	75.40 a;w	76.59 a;w
Σ^{PUFAs}	12.70 a;w	10.58 a;w	10.93 a;w	10.69 a;w	8.41 a;x	7.71 a;x	9.49 a;x	8.87 a;x
C18:1/C18:2	6.23 b;x	7.84 ab;x	7.36 a;w	7.52 ab;x	10.60 ab;w	12.18 a;w	8.80 ab;w	9.68 b;w
MUFA/PUFA	6.01 b;x	7.60 ab;x	7.16 a;w	7.23 ab;x	10.08 a;w	11.75 a;w	8.40 a;w	9.42 a;w
ω-9	67.82 b;x	72.34 ab;x	73.22 a;w	71.62 ab;w	73.29 b;w	78.16 a;w	72.42 ab;w	74.19 b;w

Values were determined as the % of the total fatty acids. Significance letters (a–d) in the same row concerning the same cultivar indicate significant differences ($p \leq 0.05$) according to the LSD criterion. Significance letters (w, x) in the same row concerning the same district indicate significant differences ($p \leq 0.05$) according to the LSD criterion.

[a] All unsaturated fatty acids are cis isomers, Σ - sum.

heterogeneity because they are extended along to the slopes of the central mountain of Cyprus, Troodos, and also to plateau areas, enabling some locations to face the sea while others to be in the interior of the island. This peculiarity of the high and the mediate altitude zones may also be a reason for the composition of fatty acids of these samples to decline from the formation of a clear pattern with the respective values from the other altitude zone under study.

The "Altitude" variable, in the one-way ANOVA analysis, showed that only palmitoleic acid is a marker for the differentiation of olive oils, whereas its percentage composition decreases with increasing altitude, as following: at low heights < 100 m 0.60%, at mediate heights 100–450 m 0.44%, and at high elevation, > 450 m, 0.36%.

Apparently the district variable, which is strongly associated with the natural geographical separation of the provinces is expected to have

Table 4
Fatty acid composition (%) of 225 Cypriot VOO samples at different altitude classes for the four districts.

District	Larnaca/Famagusta			Limassol			Nicosia		Paphos		
Altitude class	< 100 m	100–450 m	> 450 m	< 100 m	100–450 m	> 450 m	100–450 m	> 450 m	< 100 m	100–450 m	> 450 m
Samples no.	44	22	19	6	10	13	61	14	14	16	6
Fatty acid[a]	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)
C16:0	12.05 a;w	12.69 a;w	10.63 b;x	10.16 a;x	10.53 a;x	9.96 a;x	10.55 b;x	12.06 a;w	11.84 a;wx	11.47 a;wx	11.14 a;wx
C16:1(7)	0.02 a;w	0.02 a;w	0.01 a;w	0.01 a;w	0.01 a;w	0.01 a;w	0.02 a;w	0.01 a;w	0.02 a;w	0.01 a;w	0.00 a;w
C16:1(9)	0.66 a;w	0.65 a;w	0.42 a;w	0.44 a;w	0.34 a;w	0.19 a;w	0.42 a;w	0.48 a;w	0.45 a;w	0.31 a;w	0.30 a;w
C17:0	0.01 b;w	0.04 a;wx	0.04 a;w	0.01 b;w	0.03 a;w	0.02 b;wx	0.02 a;xy	0.04 a;w	0.02 a;w	0.01 a;y	0.00 a;x
C17:1(9)	0.01 a;w	0.02 a;w	0.02 a;w	0.01 a;w	0.02 a;w	0.01 a;w	0.02 a;w	0.02 a;w	0.02 a;w	0.01 a;w	0.00 a;w
C18:0	2.91 b;wx	3.58 a;w	3.40 ab;wx	2.49 b;x	2.98 a;w	2.75 b;x	3.15 b;w	3.91 a;w	3.31 a;w	2.97 a;w	2.90 a;wx
C18:1(9)	71.69 a;w	68.36 a;w	72.51 a;w	75.14 a;w	74.28 a;w	76.74 a;w	73.02 a;w	70.71 a;w	71.08 a;w	73.62 a;w	73.50 a;w
C18:1(11)	2.62 a;w	2.73 a;w	2.01 b;wx	2.17 a;w	1.78 a;x	1.56 a;x	1.91 a;x	2.33 a;w	2.19 a;w	1.81 b;x	1.89 b;x
C18:2(9,12)	8.84 a;w	10.59 a;w	9.80 a;w	8.62 a;w	9.06 a;w	8.19 a;w	9.89 a;w	9.19 a;w	9.92 a;w	9.20 a;w	9.73 a;w
C18:3(9,12,15)	0.53 a;w	0.63 a;w	0.55 a;wx	0.49 a;w	0.41 ab;xy	0.28 b;y	0.44 a;x	0.50 a;x	0.54 a;w	0.28 b;y	0.29 b;xy
C20:0	0.41 a;w	0.50 a;w	0.40 a;w	0.31 ab;w	0.37 a;wx	0.20 b;x	0.36 a;wx	0.48 a;w	0.43 a;w	0.23 b;x	0.21 b;x
C20:1(11)	0.15 a;w	0.17 a;w	0.16 a;wy	0.04 a;w	0.12 a;wx	0.07 a;xy	0.12 b;wx	0.18 a;w	0.09 a;w	0.05 b;x	0.03 b;x
C22:0	0.11 a;w	0.09 a;w	0.05 a;wx	0.06 a;w	0.08 a;wx	0.04 a;x	0.07 b;wx	0.11 a;w	0.07 a;w	0.04 b;x	0.01 b;x
Σ^{SFAs}	15.49 ab;w	16.89 a;w	14.54 b;w	13.03 ab;x	13.99 a;wx	12.96 b;w	14.15 b;wx	16.59 a;w	15.68 a;wx	14.71 a;wx	14.27 a;w
Σ^{MUFAs}	75.14 a;w	71.96 a;w	75.14 a;w	77.80 a;w	76.55 a;w	78.58 a;w	75.53 a;w	73.73 a;w	73.85 a;w	75.80 a;w	75.73 a;w
Σ^{PUFAs}	9.37 a;w	11.22 a;w	10.35 a;w	9.11 a;w	9.46 a;w	8.46 a;w	10.33 a;w	9.70 a;w	10.46 a;w	9.49 a;w	10.01 a;w
$C_{18:1}/C_{18:2}$	9.37 a;w	7.78 a;w	9.18 a;w	9.97 a;w	10.05 a;w	10.66 a;w	8.01 a;w	8.33 a;w	7.86 a;w	8.83 a;w	8.79 a;w
MUFA/PUFA	8.95 a;w	7.37 a;w	8.82 a;w	9.40 a;w	9.81 a;w	10.32 a;w	7.72 a;w	7.98 a;w	7.46 a;w	8.61 a;w	8.66 a;w
ω-9	71.86 a;w	68.55 a;w	72.68 a;w	75.18 a;w	74.41 a;w	76.82 a;w	73.17 a;w	70.90 a;w	71.19 a;w	73.68 a;w	73.53 a;w

Values were determined as the % of the total fatty acids. Significance letters (a–c) in the same row concerning the same district indicate significant differences ($p \leq 0.05$) according to the LSD criterion. Significance letters (w, x, y) in the same row concerning the same altitude class indicate significant differences ($p \leq 0.05$) according to the LSD criterion.

[a] All unsaturated fatty acids are cis isomers, Σ – sum.

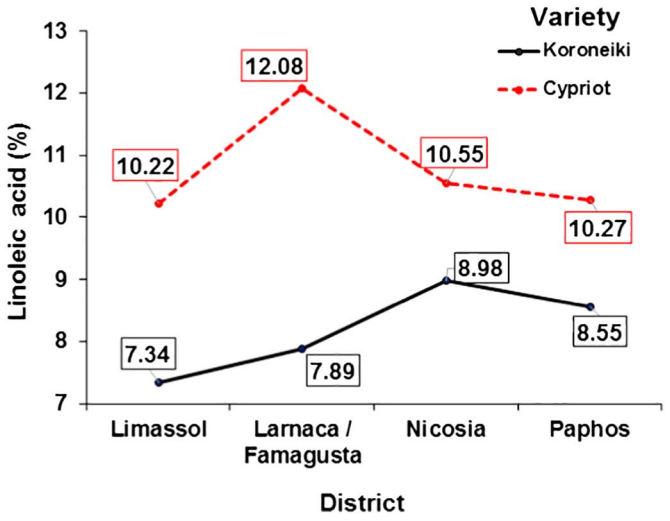

Fig. 2. Mean Linoleic acid (%) across districts.

a higher impact to the discrimination of oils for each cultivar (vide infra).

3.2.2.2. "Cultivar × District". Interaction of "*Cultivar × District*" indicated that oils of Cypriot cultivar are characterized by higher concentrations of SFA and PUFA, and lower concentrations of MUFA, and ω-9 fatty acids, when compared to the oils of Koroneiki cultivar in most districts, with data be presented in Table 3. In particular, saturated fatty acids such as margaric, stearic, and arachidic presented higher mean values for oils of Cypriot cultivar in most districts, compare to those of Koroneiki. Polyunsaturated linoleic acid presented higher mean values for Cypriot oils in all four districts (Fig. 2). In contrast, VOOs of Koroneiki cultivar had higher amounts of the monounsaturated fatty acids (MUFA), oleic, and cis-vaccenic acids than the VOOs of Cypriot cultivar. These results show a primary dependence of the fatty acids composition of VOOs on the cultivar.

It has been reported that either oleic (or MUFA) or linoleic acid (or PUFA) content or both or their ratio in olive oils can serve as strong discriminant factors among varieties.(D'Imperio et al., 2007; Mannina et al., 2003; Matos et al., 2007; Ollivier et al., 2003; Ollivier et al., 2006). Based on the results, discrimination of VOOs of each cultivar by oleic acid, MUFA or linoleic acid, PUFA or their ratio based on the district occurs at a moderate level, which shows that the cultivar is a primary factor for VOOs separation than the district, which has been associated with the microclimate effect on VOOs composition in fatty acids (D'Imperio et al., 2007; Ollivier et al., 2003).

Significant differences regarding the ratio C18:1/C18:2 among the two cultivars in the different districts revealed that the Koroneiki cultivar possessed higher value, from 8.80 to 12.18, over Cypriot cultivar ranging from 6.23 to 7.84 (Fig. 3). It is well known that the C18:1/C18:2 ratio is used as a stability parameter for olive oils, indicating higher values of C18:1/C18:2 for an increased oxidative stability (E Stefanoudaki et al., 1999). The higher the ratios of oleic to linoleic (C18:1/C18:2) of VOOs from olive cultivars are, the higher their oxidative stability has been. Based on the results for this ratio for all districts shown in Fig. 3, the VOOs from Koroneiki cultivar are considered more resistant to oxidative degradation compared to oils from Cypriot cultivar. Susceptibility of oils of Cypriot cultivar to oxidation is also verified by the higher content of PUFA (range: 10.58–12.70) when compared to Koroneiki cultivar (range: 7.71–9.49) in all districts (Fig. 4). However, the unsaturated linoleic and α-linolenic acids are the precursors of compounds contributing to the green notes aroma of fresh olive oils through the pathway involving lipoxygenase and hydroxide lyase activation during olives crushing and malaxation process (Luna et al., 2006; Olias, Perez, Rios, & Sanz, 1993). Therefore, the high

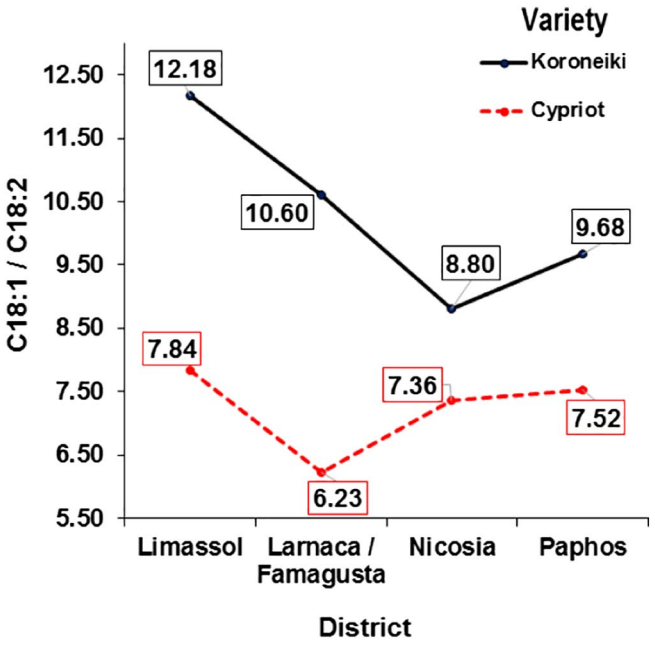

Fig. 3. Mean C18:1/C18:2 across districts.

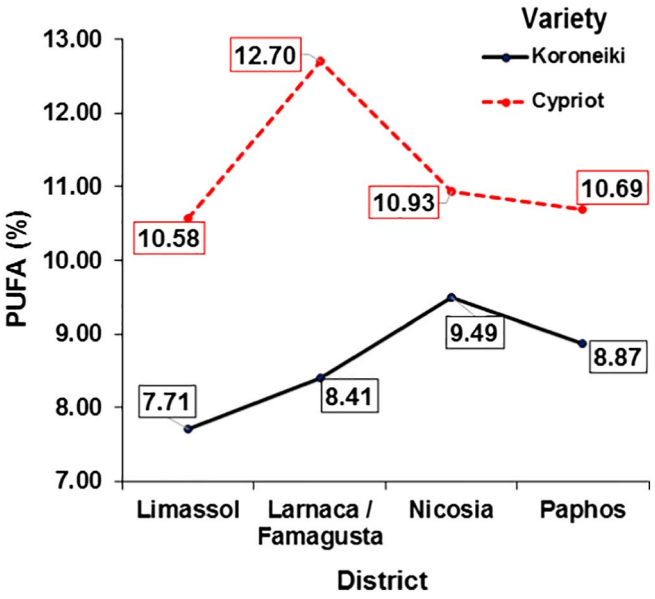

Fig. 4. Mean PUFA (%) across districts.

linoleic content may strongly support the fine fresh olive oil aroma of the VOOs from Cypriot cultivar attracting the local's preference.

The monounsaturated fatty acids margaroleic C17:1(9) and gondoic C20:1(11) are found in very low concentrations (≤ 0.1%) in olive oils. VOOs of the Cypriot cultivar possessed higher average value of the aforementioned fatty acids when compared to those of Koroneiki cultivar.

One-way interaction due to variety showed significant differences only for the fatty acid margaroleic, indicating higher concentration (0.02%) for Cypriot variety when compared to Koroneiki (0.01%), regardless altitude and district.

3.2.2.3. "Altitude × District". The two-way interaction for "Altitude × District", showed substantial differences in fatty acids concentration (Table 4). A trend was observed for all olive oils regarding the palmitic, margaric, stearic, arachidic, and behenic

acids, and SFA concentration to increase from low to intermediate elevation zone and then slightly decreased at the higher elevation zone. These results are in partial agreement with a trend been recorded in the literature, referred to the decrease of the saturated fatty acids content of VOOs by increasing the altitude (Mousa et al., 1996; E Stefanoudaki et al., 1999). Monounsaturated palmitoleic, cis-vaccenic and gondoic acids, as well polyunsaturated α-linolenic acid, presented lower concentrations with increased altitude. In a similar way, it has been reported in the literature that SFA is a medium strength factor of differentiation for oils extracted from olives of different ripeness grade, described as mixed samples of oils from black and green olives (Merchak et al., 2017). The differentiation of the oils regarding the district variable for each altitude level indicates that the microclimate of each district plays a significant role in fatty acids composition of the olive oils, which is in agreement with the literature (R. Aparicio et al., 1994; D'Imperio et al., 2007; Luna et al., 2006). Other factors such as the maturity index (Gutiérrez et al., 1999) of olives and the irrigation regimes(Dag et al., 2015; Stefanoudaki, Williams, Chartzoulakis, & Harwood, 2009) for the olive tree watering have been recorded to affect the composition of VOOs in fatty acids, and these can explain the results obtained for oleic acid, which found to retain its composition in olive oils among different districts for each altitude level, thus, reducing its discriminant power in the current work.

In addition, palmitoleic acid showed only one-way interaction due not only to the altitude class but also to the district variable. Significant differences across districts showed higher concentration of this fatty acid in Larnaca/Famagusta (0.60%), when compared to Limassol (0.28%) and Paphos (0.36%), and higher concentration at Larnaca/Famagusta (0.60%) when compared with Nicosia (0.43%). Similar effect on the differentiation of oils based on the climate effect has been previously observed (R. Aparicio et al., 1994; D'Imperio et al., 2007; Karabagias et al., 2013; Longobardi et al., 2012; E Stefanoudaki et al., 1999).

3.2.3. Spearman's rho correlation coefficients

Spearman's rho Correlation Coefficients have been used to detect correlations of the fatty acids concentration to each other through kinetics, assigned to bio-transformations between fatty acids by the enzymes stearate desaturase (stearic to oleic acid conversion), oleate desaturase (oleic to linoleic acid conversion) and linoleate desaturase (linoleic to α-linolenic acid conversions) (Hernández, Padilla, Mancha, & Martínez-Rivas, 2009). In each cultivar, the fatty acids biosynthesis goes through the expression of several oleate desaturase genes, located in chloroplasts (plasmid) and in endoplasmic reticulum (microsomal) (Banilas, Moressis, Nikoloudakis, & Hatzopoulos, 2005).

Strong correlations coefficients ($p \leq 0.01$) have been recorded between fatty acids composing VOOs, as shown in Table 5. Strong positive correlations were observed between palmitic-palmitoleic (*rho* = 0.748), palmitoleic-cis-vaccenic (*rho* = 0.957), stearic-arachidic (*rho* = 0.836), cis-vaccenic-linolenic (*rho* = 0.835), and linolenic-arachidic (*rho* = 0.818). In contrast, strong negative correlation coefficients were found between stearic-oleic (*rho* = − 0.753). These results demonstrate the kinetic differences of reactions during the biosynthetic path of palmitic, stearic, oleic and linoleic acids. It has been recorded that palmitic and α-linolenic acid concentrations are lowered during ripening, in contrast to linoleic acid percentage which is increased (Gutiérrez et al., 1999), attributed to the continuous process of biosynthesis of triglycerides and the formation of oleic acid, through the pathway of oleate or linoleate desaturases activation. Therefore, in the biosynthesis of fatty acids in olives the oleic acid is formed first, and is converted to linoleic acid during the maturation of the fruit, in an inverse relationship, which indicates that any increase of the one of them will result in the decrease of the other. Similarly, strong antagonistic relationships between palmitic and oleic, stearic and oleic, and oleic and α-linolenic are recorded in this work, also supported by reports in

Table 5
Spearman's *rho* Correlation Coefficients between the Different Fatty Acids.

	C16:0	C16:1(7)	C16:1(9)	C17:0	C17:1(9)	C18:0	C18:1(9)	C18:1(11)	C18:2(9,12)	C18:3(9,12,15)	C20:0	C20:1(11)	C22:0
C16:0	1.000												
C16:1(7)	0.357(**)	1.000											
C16:1(9)	0.748(**)	0.662(**)	1.000										
C17:0	0.426(**)	0.500(**)	0.486(**)	1.000									
C17:1(9)	0.325(**)	0.716(**)	0.502(**)	0.680(**)	1.000								
C18:0	0.623(**)	0.309(**)	0.453(**)	0.701(**)	0.411(**)	1.000							
C18:1(9)	− 0.670(**)	− 0.431(**)	− 0.583(**)	− 0.660(**)	− 0.481(**)	− 0.753(**)	1.000						
C18:1(11)	0.711(**)	0.629(**)	0.957(**)	0.491(**)	0.458(**)	0.495(**)	− 0.570(**)	1.000					
C18:2(9,12)	0.326(**)	0.234(**)	0.304(**)	0.530(**)	0.371(**)	0.580(**)	− 0.675(**)	0.245(**)	1.000				
C18:3(9,12,15)	0.572(**)	0.532(**)	0.840(**)	0.579(**)	0.485(**)	0.561(**)	− 0.577(**)	0.835(**)	0.390(**)	1.000			
C20:0	0.723(**)	0.505(**)	0.743(**)	0.697(**)	0.498(**)	0.836(**)	− 0.657(**)	0.798(**)	0.393(**)	0.818(**)	1.000		
C20:1(11)	0.565(**)	0.637(**)	0.747(**)	0.663(**)	0.555(**)	0.611(**)	− 0.604(**)	0.786(**)	0.386(**)	0.668(**)	0.784(**)	1.000	
C22:0	0.513(**)	0.647(**)	0.654(**)	0.467(**)	0.526(**)	0.457(**)	− 0.372(**)	0.704(**)	0.061	0.617(**)	0.735(**)	0.668(**)	1.000

** Correlation is significant at the 0.01 level.

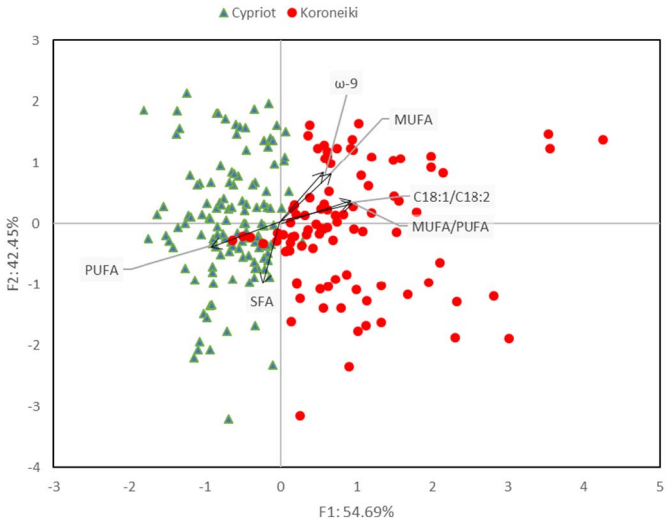

Fig. 5. Bi-plot presentation of 225 samples factor scores on the 2-factor solution, and the loadings of the six groups of fatty acids-Markers, by variety.

the literature (Hernández et al., 2009; Stefanoudaki et al., 1999). Beyond the kinetics taking place in the olives of a single cultivar, large differences in the amount of the linoleic acid produced from different cultivars and in the levels of relative genes expression for several cultivars have been recorded.(Banilas et al., 2005; Hernández et al., 2009) Based on those reports, in the mesocarp compartment of the drupe, the *OeFAD2* desaturase is constitutively expressed with maximum transcript accumulation, and the richer composition in linoleic acid for Picual compared with that of the Arbequina cultivar has been positively correlated with the higher level of the *FAD2-2* gene expression. This strongly correlates the composition of VOO in fatty acids with the cultivar of olives they originated.

3.2.4. PCA analysis

Principal Component Analysis has been used to examine the specificity of the samples by detecting the fatty acid or the group of fatty acids that are specific or characteristic of a VOO sample, resulting in its distinction within a polymorphic complex system. PCA results showed that two factors were enough to explain almost all the variability (97.13%). Factor loadings are shown in Fig. 5. Factor 1 (F1) presents high loadings for variables C18:1/C18:2 (0.925), MUFA/PUFA (0.916), and PUFA (− 0.896). Increased ratios of C18:1/C18:2 and MUFA/PUFA relate more to Factor 1 rather than Factor 2. Due to the negative loading, low values of PUFA relate more to F1 rather than F2. Factor 2 presents high loadings for variables SFA (− 0.968), ω-9 (0.805), and MUFA (0.771). Rich concentrations of ω-9 and MUFA relate more to F2 rather than F1. Due to the negative loading, low values of SFA relate more to F2 rather than F1. Furthermore, the 2-factor model can explain 98.8% of variance for SFA, 99.0% for MUFA, 92.6% for PUFA, 97.4% for C18:1/C18:2, 96.7% for MUFA/PUFA, and 98.3% for ω-9.

The bi - plot of 225 olive oil samples projected on the plane F1 × F2 of the two factorial axes extracted by PCA is presented for the six groups of fatty acids, in Fig. 5. The two big distinctive groups, concluding the discrimination of Cypriot olive samples regarding the botanical origin, are observed. In particular, the factor scores of the Koroneiki cultivar are mainly gathered to high values of F1. On the other hand, factor scores of the Cypriot cultivar seem to gather more at low values of F1.

All samples of the current study, provided by the olive oil mills and producers, represent the VOOs released in the olive oil market, thus, (i) they do not correspond to a single maturation index which strongly effects on the content of fatty acids of VOOs (Gutiérrez et al., 1999) (ii) have emerged from different rain-fed/irrigation regimes which interfere with the composition of fatty acids and were not taken into account. Consequently, some heterogeneity regarding the composition of the VOOs in each fatty acid was observed, leading to a separation, more or less declined from an ideal discrimination according to the cultivar.

Similar bi-plots were also performed using altitude classes and district as points' markers. However, the separation was not efficient and the bi - plots are not displayed. Possibly, the selection of continuous values of the elevation of the 3 altitude levels rather than large distant elevation altitude zones did not support a discrimination pattern to overcome differences in fatty acids content related to other factors such rain-fed/irrigation regimes and different degree of ripening of olives used for VOOs production. Moreover, three out of four districts of Cyprus, as defined in the experimental part, have villages/areas located at < 100 m and up to > 450 m, except Nicosia region which has no areas at < 100 m elevation. Not efficient separation in scatter plots for the district marker may also be attributed to the strong interference of various maturation indices of olives used for VOOs production by the microclimate of each district. This is in agreement with recent studies, which showed that altitude in itself is not a strong discriminant factor for differentiation of oils derived from olives of different fruit maturity index (Merchak et al., 2017).

3.2.5. Hierarchical cluster analysis

Cluster analysis resulted in the formation of two Clusters CA and CB between the VOOs samples. Significant differences ($p < 0.001$) between the two clusters were found for all fatty acids groups when *t*-test was applied. It was observed that both clusters presented large statistically significant differences regarding the content level of the six fatty acids groups (Table 6, Fig. 6). In particular, the concentrations of SFA (16.30 ± 2.37) and PUFA (11.71 ± 1.90) in CA are higher than those of CB, (12.93 ± 2.60) ($t = 10.28$, $p < 0.001$) and (7.54 ± 1.55) ($t = 16.38$, $p < 0.001$) respectively.

The ratios C18:1/C18:2 (6.59 ± 1.28) and MUFA/PUFA (6.34 ± 1.20) in CA are lower than those in CB, (11.67 ± 3.56) ($t = -12.60$, $p < 0.001$), and (11.21 ± 3.52) ($t = -12.19$, $p < 0.001$) respectively.

The concentrations of MUFA (72.01 ± 3.25) and ω-9 (68.98 ± 3.82) in CA are lower than those of CB, (79.53 ± 3.27) ($t = -15.43$, $p < 0.001$) and ω-9 (77.44 ± 4.02), ($t = -15.92$, $p < 0.001$).

Cluster analysis *t*-test results are in line with the findings of univariate-ANOVA for the six groups of fatty acids preceded, where concentration of SFA and PUFA were higher for Cypriot variety, similarly as in CA where Cypriot variety has the higher distribution (Table 6, Fig. 6). Variables of MUFA, C18:1/C18:2, MUFA/PUFA and ω-9 showed higher values for Koroneiki than Cypriot cultivar in Hierarchical Cluster analysis and are the same with those differentiated VOOs by the ANOVA analysis. This is in conformity with the findings for CB cluster where Koroneiki prevails over Cypriot.

Furthermore, the relationship between the clusters and the three independent variables (cultivar, altitude, and district) were investigated. Specifically, the distribution of these three variables within the two clusters was checked. Regarding the distribution of the altitude classes and the districts there were no statistically significant differentiation between the two clusters (Altitude class: $\chi^2 = 0.847$,

Table 6
Average concentration (% ± SD) of fatty acids groups in each cluster.

Fatty acids	CA	CB	*t*-test (223 df)	
	Mean (%)	Mean (%)	*t*	p
SFA	16.30 ± 2.37	12.93 ± 2.60	10.28	< 0.001
MUFA	72.01 ± 3.25	79.53 ± 3.27	− 15.43	< 0.001
PUFA	11.71 ± 1.90	7.54 ± 1.55	16.38	< 0.001
C18:1/C18:2	6.59 ± 1.28	11.67 ± 3.56	− 12.60	< 0.001
MUFA/PUFA	6.34 ± 1.20	11.21 ± 3.52	− 12.19	< 0.001
ω-9	68.98 ± 3.82	77.44 ± 4.02	− 15.92	< 0.001

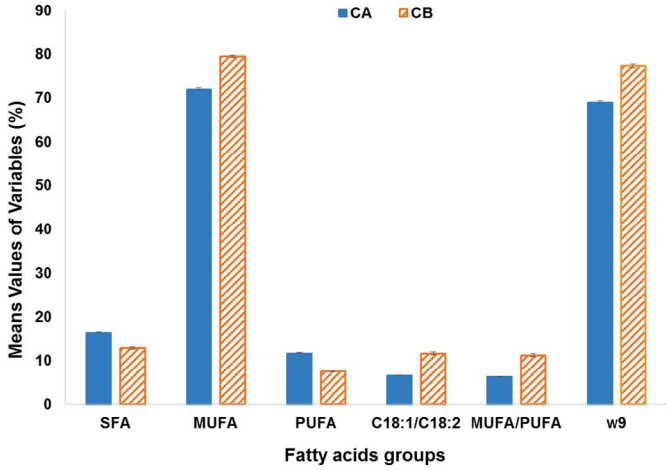

Fig. 6. Average concentration (%) of fatty acids groups in each cluster (CA, CB). Vertical bars correspond to Standard Error.

p = 0.520, District: χ^2 = 3.255, p = 0.332). On the other hand, chi-square test showed significant association between cultivar and the two clusters (χ^2 = 16.535, p < 0.001). For cluster CA, cultivar allocation is: Koroneiki, 38.5% and Cypriot cultivar, 61.5%, while in cluster CB 74.7% is Koroneiki and the 25.3% is Cypriot cultivar (Table 7).

Dendrogram of Hierarchical Cluster Analysis displays the amalgamation of clusters, in the form of binary tree, exhibiting the clustering of the virgin olive oil samples grouped on the basis of the set of parameters: SFA, MUFA, PUFA, C18:1/C18:2, MUFA/PUFA and ω-9, and is presented in Fig. S1. The dendrogram of the cluster analysis, without incorporation of any previous knowledge of the class membership, shows the number of the clusters and lists all 225 samples indicating the level of similarity of any two joined clusters. The population in the majority of clusters is a mixture of both varieties, Koroneiki and Cypriot. Heterogeneity was observed for the distribution of each cultivar inside each cluster for the majority of the clusters. At least 3 clusters consist a single cultivar, 2 of them Koroneiki, and 1 cluster consists Cypriot variety, at low dissimilarity level.

Similar heterogeneity in cultivar distribution inside the cluster as a result of the application of unsupervised statistical methods have also been observed in other studies (Matos et al., 2007). On the other hand, satisfactory classification of cultivars has been recorded in studies where, beyond fatty acids, other discriminant factors, such as phenolic composition and sterols content, have been used for optimizing differentiation of olive samples among the different provinces (Abu-Reidah, Yasin, Urbani, Servili, & Montedoro, 2013; Ollivier et al., 2003; Ollivier et al., 2006).

Microclimate, rain-fall, temperature variances during olives maturation and its maximum and minimum values, as well other meteorological characteristics effect the yield of olive oil production and the content of fatty acids on annual base. Although the three years crops study of fatty acids is more representative for the discrimination of VOOs between Cypriot and Koroneiki, it may also account for the observed fatty acid content variability of VOOs from the two cultivars cultivated on the island.

In general, chemometrics applied to this research included three types of statistical approaches providing different perspectives of the statistical analyses. Mainly, ANOVA analysis showed that VOOs of Cypriot cultivar have higher mean values for SFA, PUFA, and linoleic, stearic, margaroleic and lower values for MUFA, ω-9, C18:1/C18:2, and MUFA/PUFA, and the oleic acid than those of Koroneiki cultivar. Significant effects of cultivar, district and altitude on each fatty acid and each group of fatty acids was assessed for one- and two - way interactions. PCA analysis, applied on the 6 groups of fatty acids for the 225 samples, showed that the variables with the higher discriminant power, in decreasing order, are: C18:1/C18:2, MUFA/PUFA, PUFA, MUFA, and ω-9, for separation of the samples based on the cultivar. Hierarchical Cluster Analysis sufficient classified the 225 samples into two clusters allocated for the Cypriot and Koroneiki Cultivar. These results have high impact on classification of samples base on the cultivar because the method is based on the distance of metric values of the 6 groups of fatty acids, with the dissimilarity of the sets been specified by the Ward's linkage criterion, suggesting a natural grouping of the data themselves.

3.3. Koroneiki cultivar within Mediterranean basin

Koroneiki variety is wide spread around Mediterranean basin, and beyond Greece and Cyprus, it has also been planted in other countries, such as Israel, Tunisia, Italy, Australia and USA. Cypriot VOOs of Koroneiki cultivar have high average oleic acid content, similarly as VOOs from Greece, such as those from Crete (Stefanoudaki et al., 2009), Ionia islands (Longobardi et al., 2012), and Argolida province (Katsoyannos et al., 2015). The linoleic acid and SFA content of VOOs of Koroneiki cultivar produced in Cyprus, reach lower values compared with those of olive oils produced in Negev (Chapagain & Wiesman, 2009), and Golan Heights (Dag et al., 2015) areas in Israel, and Boughara (Zarrouk et al., 2009) and Benjaoua (Dabbou et al., 2009) in Tunisia, where the high temperatures in combination with the intensive tree water stress or poor rain-fed cultivation conditions increased the content of linoleic acid. Although Cyprus is also in the southern region of Mediterranean basin produces VOOs of Koroneiki variety rich in oleic acid, with intermediate/low content in linoleic acid, exerting a competitive profile of Cypriot VOOs to the olive oil market.

4. Conclusions

In general, VOOs are differentiated mainly due to the cultivar, Koroneiki or Cypriot based on the fatty acids composition. Across all four districts, VOOs from Cypriot cultivar are characterized by higher concentrations of SFA and PUFA compared to those from Koroneiki. VOOs from Koroneiki cultivar present higher concentrations of MUFA, and ω-9 fatty acids, when compared to the oils of Cypriot cultivar, thus exhibiting high oxidative stability.

Geographical discrimination, through altitude and district factors, was not satisfactory, mainly because of the different parameters such as rain-fed/irrigation systems and degree of olive ripeness throughout the four districts, the three altitude zones and the small land size of the country, that interfere with the fatty acid composition of VOOs.

The results show that for purposes of authentication of VOOs produced in a large area extended to several provinces of the country and

Table 7
Distribution of variety, altitude class, and district in each cluster.

Cluster	Variety		Altitude Class (m)			District			
	Koroneiki	Cypriot	< 100	100–450	> 450	Limassol	Larnaca /Famagusta	Nicosia	Paphos
CA (n = 130)	38.5%	61.5%	25.4%	53.8%	20.8%	10.0%	38.4%	36.2%	15.4%
CB (n = 95)	74.7%	25.3%	31.6%	48.4%	20.0%	15.8%	37.9%	29.5%	16.8%

altitude zones, for three consequent crop years, based on their fatty acid content, the factor of the cultivar exerts the most discriminant power.

Further studies for the evaluation of other VOOs components of the 2 olive tree cultivars grown in Cyprus are under way.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2017.10.064.

Acknowledgements

Authors are grateful to the owners of Cypriot olive oil mills and producers for kindly providing the VOOs samples over three harvesting periods 2013/2014, 2014/2015 and 2015/2016, and useful information, and for their collaboration.

Author contributions

The manuscript was written through contributions of all authors. /All authors have given approval to the final version of the manuscript.

References

Abu-Reidah, I. M., Yasin, M., Urbani, S., Servili, M., & Montedoro, G. (2013). Study and characterization of Palestinian monovarietal Nabali virgin olive oils from northern West Bank of Palestine. *Food Research International, 54*(2), 1959–1964. https://doi.org/10.1016/j.foodres.2013.09.004.

Alimentarius, C. (2015). *Codex standard for olive oils, and olive pomace oils.* ([standard]. CODEX STAN 33-1981).

Alonso-Salces, R. M., Héberger, K., Holland, M. V., Moreno-Rojas, J. M., Mariani, C., Bellan, G., ... Guillou, C. (2010). Multivariate analysis of NMR fingerprint of the unsaponifiable fraction of virgin olive oils for authentication purposes. *Food Chemistry, 118*(4), 956–965. https://doi.org/10.1016/j.foodchem.2008.09.061.

Aparicio, R., Ferreiro, L., & Alonso, V. (1994). Effect of climate on the chemical composition of virgin olive oil. *Analytica Chimica Acta, 292*(3), 235–241. https://doi.org/10.1016/0003-2670(94)00065-4.

Aparicio, R., Morales, M. T., Aparicio-Ruiz, R., Tena, N., & García-González, D. L. (2013). Authenticity of olive oil: Mapping and comparing official methods and promising alternatives. *Food Research International, 54*(2), 2025–2038. https://doi.org/10.1016/j.foodres.2013.07.039.

Banilas, G., Moressis, A., Nikoloudakis, N., & Hatzopoulos, P. (2005). Spatial and temporal expressions of two distinct oleate desaturases from olive (*Olea europaea* L.). *Plant Science, 168*(2), 547–555. https://doi.org/10.1016/j.plantsci.2004.09.026.

Boskou, D., Blekas, G., & Tsimidou, M. (2006). *Olive oil composition* (2nd ed.). AOCS PRESS.

Chapagain, B. P., & Wiesman, Z. (2009). MALDI-TOF/MS fingerprinting of triacylglycerols (TAGs) in olive oils produced in the Israeli Negev Desert. *Journal of Agricultural and Food Chemistry, 57*(4), 1135–1142. http://dx.doi.org/10.1021/jf8025277.

Dabbou, S., Issaoui, M., Servili, M., Taticchi, A., Sifi, S., Montedoro, G. F., & Hammami, M. (2009). Characterisation of virgin olive oils from European olive cultivars introduced in Tunisia. *European Journal of Lipid Science and Technology, 111*(4), 392–401. http://dx.doi.org/10.1002/ejlt.200800032.

Dag, A., Naor, A., Ben-Gal, A., Harlev, G., Zipori, I., Schneider, D., ... Kerem, Z. (2015). The effect of water stress on super-high-density 'Koroneiki' olive oil quality. *Journal of the Science of Food and Agriculture, 95*(10), 2016–2020. http://dx.doi.org/10.1002/jsfa.6913.

Dewapriya, P., Himaya, S. W. A., Li, Y.-X., & Kim, S.-K. (2013). Tyrosol exerts a protective effect against dopaminergic neuronal cell death in in vitro model of Parkinson's disease. *Food Chemistry, 141*(2), 1147–1157 https://doi.org/10.1016/j.foodchem.2013.04.004.

D'Imperio, M., Dugo, G., Alfa, M., Mannina, L., & Segre, A. L. (2007). Statistical analysis on Sicilian olive oils. *Food Chemistry, 102*(3), 956–965. https://doi.org/10.1016/j.foodchem.2006.03.003.

ECC (1991). Commission regulation no. 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal L, 248*, 1–83.

ECC (2006). Council regulation, no 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. *Official Journal L, 93*, 12–25.

EEC (2012). *Council regulation no. 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health*.

FAOStat. Food and Agriculture Organization of United Nations (2011). Retrieved August 2015, 2015, from http://faostat.fao.org/.

García-González, D. L., Luna, G., Morales, M. T., & Aparicio, R. (2009). Stepwise geographical traceability of virgin olive oils by chemical profiles using artificial neural network models. *European Journal of Lipid Science and Technology, 111*(10), 1003–1013. http://dx.doi.org/10.1002/ejlt.200900015.

Gregoriou, C. (1996). Assessment of variation of landraces of olive tree in Cyprus. *Euphytica, 87*(3), 173–176.

Gregoriou, C. (2006). *Application of good agricultural and environmental practices in olive.* (Nicosia).

Gutiérrez, F., Jimenez, B., Ruiz, A., & Albi, M. (1999). Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. *Journal of Agricultural and Food Chemistry, 47*(1), 121–127.

Hair, J. F., Jr., Anderson, R. E., Tatham, R. L., & William, C. (1995). *Black (1995), multivariate data analysis with readings.* New Jersey: Prentice Hall.

Hernández, M. L., Padilla, M. N., Mancha, M., & Martínez-Rivas, J. M. (2009). Expression analysis identifies FAD2-2 as the olive oleate desaturase gene mainly responsible for the linoleic acid content in virgin olive oil. *Journal of Agricultural and Food Chemistry, 57*(14), 6199–6206. http://dx.doi.org/10.1021/jf900678z.

IOOC (2015a). International olive oil council, world olive oil figures – Production. Retrieved 20/03/17, 2017, from http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oilfigures.

IOOC (2015b). *International olive oil council, COI/T.20/doc. No.33. Determination of fatty acid methyl ester by gas chromatography.* (Madrid, Spain).

IOOC (2015c). Trade standards applying to olive oil and olive pomace oil, Madrid, Spain. *International Olive Oil Council, COI/T.15/NC No 3/Rev.8*.

Karabagias, I., Michos, C., Badeka, A., Kontakos, S., Stratis, I., & Kontominas, M. (2013). Classification of Western Greek virgin olive oils according to geographical origin based on chromatographic, spectroscopic, conventional and chemometric analyses. *Food Research International, 54*(2), 1950–1958.

Katsoyannos, E., Batrinou, A., Chatzilazarou, A., Bratakos, S. M., Stamatopoulos, K., & Sinanoglou, V. J. (2015). *Quality parameters of olive oil from stoned and nonstoned Koroneiki and Megaritiki Greek olive varieties at different maturity levels. [Fatty acids; Koroneiki and Megaritiki varieties; Olive oil; Pitting; Ripening process]. 66*. (1) https://doi.org/10.3989/gya.0711142 (2015) .

Keys, A., Mienotti, A., Karvonen, M. J., Aravanis, C., Blackburn, H., Buzina, R., ... Toshima, H. (1986). The diet and 15-year death rate in the seven countries study. *American Journal of Epidemiology, 124*(6), 903–915.

Longobardi, F., Ventrella, A., Casiello, G., Sacco, D., Tasioula-Margari, M., Kiritsakis, A., & Kontominas, M. (2012). Characterisation of the geographical origin of Western Greek virgin olive oils based on instrumental and multivariate statistical analysis. *Food Chemistry, 133*(1), 169–175.

Luna, G., Morales, M. T., & Aparicio, R. (2006). Characterisation of 39 varietal virgin olive oils by their volatile compositions. *Food Chemistry, 98*(2), 243–252. https://doi.org/10.1016/j.foodchem.2005.05.069.

Mannina, L., Dugo, G., Salvo, F., Cicero, L., Ansanelli, G., Calcagni, C., & Segre, A. (2003). Study of the cultivar – composition relationship in Sicilian olive oils by GC, NMR, and statistical methods. *Journal of Agricultural and Food Chemistry, 51*(1), 120–127. http://dx.doi.org/10.1021/jf025656l.

Mateos, R., Pereira-Caro, G., Bacon, J. R., Bongaerts, R., Sarriá, B., Bravo, L., & Kroon, P. A. (2013). Anticancer activity of olive oil hydroxytyrosyl acetate in human adenocarcinoma Caco-2 cells. *Journal of Agricultural and Food Chemistry, 61*(13), 3264–3269. http://dx.doi.org/10.1021/jf305158q.

Matos, L. C., Cunha, S. C., Amaral, J. S., Pereira, J. A., Andrade, P. B., Seabra, R. M., & Oliveira, B. P. (2007). Chemometric characterization of three varietal olive oils (Cvs. Cobrançosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. *Food Chemistry, 102*(1), 406–414.

Medina, J. M., & Tabernero, A. (2010). Chapter 156 - the neurotrophic effect of oleic acid: Implications for olive oil in health and disease A2 - Preedy, victor R. In R. R. Watson (Ed.). *Olives and olive oil in health and disease prevention* (pp. 1405–1412). San Diego: Academic Press.

Merchak, N., El Bacha, E., Bou Khouzam, R., Rizk, T., Akoka, S., & Bejjani, J. (2017). Geoclimatic, morphological, and temporal effects on Lebanese olive oils composition and classification: A 1H NMR metabolomic study. *Food Chemistry, 217*, 379–388. https://doi.org/10.1016/j.foodchem.2016.08.110.

Montealegre, C., Marina Alegre, M. L., & García-Ruiz, C. (2010). Traceability markers to the botanical origin in olive oils. *Journal of Agricultural and Food Chemistry, 58*(1), 28–38. http://dx.doi.org/10.1021/jf902619z.

Mousa, Y. M., Gerasopoulos, D., Metzidakis, I., & Kiritsakis, A. (1996). Effect of altitude on fruit and oil quality characteristics of 'Mastoides' olives. *Journal of the Science of Food and Agriculture, 71*(3), 345–350. http://dx.doi.org/10.1002/(SICI)1097-0010(199607)71:3<345::AID-JSFA590>3.0.CO;2-T.

Olias, J. M., Perez, A. G., Rios, J. J., & Sanz, L. C. (1993). Aroma of virgin olive oil: Biogenesis of the "green" odor notes. *Journal of Agricultural and Food Chemistry, 41*(12), 2368–2373. http://dx.doi.org/10.1021/jf00036a029.

Ollivier, D., Artaud, J., Pinatel, C., Durbec, J. P., & Guérère, M. (2003). Triacylglycerol and fatty acid compositions of French virgin olive oils. Characterization by chemometrics. *Journal of Agricultural and Food Chemistry, 51*(19), 5723–5731.

Ollivier, D., Artaud, J., Pinatel, C., Durbec, J.-P., & Guérere, M. (2006). Differentiation of French virgin olive oil RDOs by sensory characteristics, fatty acid and triacylglycerol compositions and chemometrics. *Food Chemistry, 97*(3), 382–393.

Petrakis, P. V., Agiomyrgianaki, A., Christophoridou, S., Spyros, A., & Dais, P. (2008). Geographical characterization of Greek virgin olive oils (cv. Koroneiki) using 1H and 31P NMR fingerprinting with canonical discriminant analysis and classification binary trees. *Journal of Agricultural and Food Chemistry, 56*(9), 3200–3207. http://dx.doi.org/10.1021/jf072957s.

Rencher, A. (2000). *Linear models in statistics.* New York: John Willey & Sons, Inc.

Saville, D. J. (1990). Multiple comparison procedures: The practical solution. *The American Statistician, 44*(2), 174–180.

Sharma, S. (1996). *Applied multivariate techniques, New York.* John Willey & Sons. Inc. (OpenURL).

Spangenberg, J. E., Macko, S. A., & Hunziker, J. (1998). Characterization of olive oil by carbon isotope analysis of individual fatty acids: Implications for authentication. *Journal of Agricultural and Food Chemistry, 46*(10), 4179–4184. http://dx.doi.org/10.

1021/jf980183x.
Stefanoudaki, E., Kotsifaki, F., & Koutsaftakis, A. (1999). Classification of virgin olive oils of the two major Cretan cultivars based on their fatty acid composition. *Journal of the American Oil Chemists' Society, 76*(5), 623–626.
Stefanoudaki, E., Williams, M., Chartzoulakis, K., & Harwood, J. (2009). Effect of irrigation on quality attributes of olive oil. *Journal of Agricultural and Food Chemistry, 57*(15), 7048–7055. http://dx.doi.org/10.1021/jf900862w.
Tapp, H. S., Defernez, M., & Kemsley, E. K. (2003). FTIR spectroscopy and multivariate analysis can distinguish the geographic origin of extra virgin olive oils. *Journal of Agricultural and Food Chemistry, 51*(21), 6110–6115. http://dx.doi.org/10.1021/jf030232s.
US (2004). *Department of Health and Human Services, Letter responding to health claim petition dated August 28, 2003: Monounsaturated fatty acids from olive oil and coronary heart disease. Docket No 2003Q-0559, 2017(20/03/2017)*.
Vossen, P. (2013). *Growing olives for oil handbook of olive oil.* Springer19–56.
Zarrouk, W., Baccouri, B., Taamalli, W., Trigui, A., Daoud, D., & Zarrouk, M. (2009). *Oil fatty acid composition of eighteen Mediterranean olive varieties cultivated under the arid conditions of Boughrara (southern Tunisia). [Fatty acid composition; Mediterranean varieties; olive oil]. 60*, 9. (5) https://doi.org/10.3989/gya.021109 (2009) .
Zohary, D., Hopf, M., & Weiss, E. (2012). *Domestication of plants in the old world: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin.* OUP Oxford.
Trichopoulou, D. T., & Trichopoulou, A. (2009). Traditional Mediterranean diet and health. In D. Boskou (Ed.). *Olive oil. Minor constituents and health.* CRC Press.