Scientific Papers. Series B, Horticulture. Vol. LX, 2016
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOIL CHARACTERISTICS INFLUENCE THE FATTY ACID PROFILE OF OLIVE OILS

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Abstract

Olive tree is a typical Mediterranean plant grown in Marmara, Aegean, Mediterranean and South East Anatolian regions of Turkey and important oil sources for Mediterranean countries, fulfilling 90 % of the world olive oil production. Turkey is one of the important producer and stakeholders of olive oils after Spain, Italy, Greece and Tunisia. Cultivated olive cultivars in Turkey represent high genetic diversity, which may result in a standardization problem in terms of olive production and their fatty acid composition because the constituents of the fruit and the composition of its oil mainly depend on several factors such as climate, maturity, index variety, etc. Hence, the present study was designed to investigate the optimal soil characteristics for the favourable oil quality. In the study, the olive oils from South-Eastern region in Turkey according to their fatty acid profiles using gas chromatography of their fatty acid methyl esters were characterized and compared using chemo-metrics techniques. In this context, fatty acid profiles characterization was determined on ten olive samples collected from ten different locations. For the statistical evaluation, principal component analysis, variance analysis and correlation analysis were used. Accordingly, physical properties of soils influence the chemical composition and subsequently the quality of olive oils.

Key words: *Soil, olive oil, fatty acid, chemo metrics*

INTRODUCTION

Traditionally, plants have been extensively used for medicinal, nutritional, flavouring, cosmetically and industrial purposes. Of those plants, *Olea europaea* L. (olive) belonging to the Oleaceae family is one of the most important crops especially in Mediterranean countries on which they cover around 8 million hectares on the worldwide (Guinda et al., 2004) and its fruit and oil have a major agricultural importance in Turkey. Besides its fruits as table olive, its fatty oil is characterized with distinguished fatty acid composition, of which sanitary importance has been proven by a number of studies (Leon et al. 2004; Matson and Grundy, 1985). The important property of olive oil, the odour, as well as flavours association with oil quality have been found to be correlated with fatty acid composition (Maestro and Borja, 1990; Leon et al., 2004). Moreover, the oil obtained from olive fruits have essential key roles of reactive oxygen species (ROS) which are associated with pathology of some diseases including cancer, diabetes, cardiovascular, age related, and neurological disorders has been well documented (Chacraborty et al., 2009; Ishii, 2007; Burhans and Weinberger, 2007; Polidori et al., 2007; Halliwell and Guteridge, 1999; Soholm, 1998).

The chemical and physical properties of the soil influence plant growth, development and subsequently main primary and secondary metabolite production, secretion and accumulation. It is worth to note that the produced metabolites transport among the organs of the plants is also significantly affected by soil properties.

Uptake of an element from soil to plant depends on not only on the structure of the element, but also on different physicochemical factors of soil. Herein, transfer factor presents important information with respect to the certain amount of element transport from soil to the plant. Physicochemical parameters such as pH, $CaCO_3$ content, conductivity, organic matter content and soil texture are important factors affecting the transport of elements from the soil to plant species and consequently influence the plant growth, development and subsequently biochemical and physiological aspects of plant (Adriano, 2001: Lindsay and

Norvell, 1978; Kabata-Pendias and Mukherjee, 2007).

Application of chemo-metric approach in characterization of experimental samples has been extensively applied to quantitative evaluation of discrimination of variable results. In the current study, olive oil samples collected from different ten locations were compared for their fatty acid profile using analysis of variance (ANOVA) followed by the multiple comparison test of Duncan using SPSS. Furthermore, some characteristics of the sampling soils including pH, ($CaCO_3$), total salt, P (P_2O_5), K (K_2O) and organic matter were also determined and subsequently correlated with the fatty acid components by Pearson correlation matrix in Excel. Due to the existence of different experimental factors, chemo- metric techniques including Principal Component Analysis (PCA) were applied for analytical evaluation of fatty acid components between locations.

MATERIALS AND METHODS

Experimental Material:

The olive fruits were sampled from the Kilis Yaglik cv. (approximately the same aged trees) from Kilis district of south-eastern part of Turkey. Fruits were also harvested in the same ripening period (mid-December 2015) from the same position on the sampled trees.

Analysis of soil characteristics:

Organic matter by a modification of the Walkley and Black, calcium carbonate ($CaCO_3$) contents by Scheibler Calcimeter, total dissolved salts by Saturation Extract Method, soil phosphorus content by Olsen method, soil potassium content by flame photometer were determined for each samplings locations (Ure, 1990; Kaçar, 1995; Falciani et. al., 2000; Kaçar and İnal, 2008; Marin et al., 2008) The measurements were done in three replicates.

Oil extraction and fatty acid composition analysis:

The oils were extracted from olive fruits (each 10 g sample) with n-hexane for four hours using a Soxhlet Extraction Apparatus (Thermal). Then the solvent was evaporated under reduced pressure and temperature using a Rotary Evaporator (Heidolph). 0.5 g of olive oil was added 10 ml n-heptanes into a screw-capped tube for esterification. The fatty acid analyses were conducted according to the official method COI/T.20/Doc.no.24 2001. 0.1 g of olive oil was taken into screw-capped tube. 2 ml n-heptanes were added to it and shaken. After 0.2 ml methanolic potassium hydroxide was added for esterification, tubes were vigorously shaken for 30 sec. after the vials were closed. The supernatant of the solution was taken followed after one hour of incubation at room temperature. Then, the supernatant was put in 2 ml vials for injection. Gas chromatography with flame ionization detector (GC-FID) analyses of fatty acids methyl esters was carried out on a Shimadzu gas chromatography (GC-2010 series) equipped with an Supelco SP 2380 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 µm film thickness). Helium was used as carrier gas, at a flow rate of 3 mL/min. The injection and detector temperature were 140 ºC and 240 ºC, respectively. The oven temperature was held isothermal at 140 ºC for 5 min, then raised to 240 ºC

Statistical analysis

SPSS statistical program was used to determine statistical significance levels by employing the independent one-way ANOVA followed by Duncan multiple range test and the differences between individual averages were considered to be statistically important at $p< 0.05$. The results were expressed as mean.

RESULTS AND DISCUSSIONS

The fatty acid composition is a quality indicator parameter of olive oils and hence the component profile of fatty acids should be monitored. For all the samples, 13 fatty acids were identified and quantified but the major fatty acid components including arachidic acid, behenic acid, linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid and stearic acid were compared using variance analysis and correlated with soil characteristics (Table 1-2). Accordingly, oleic and palmitic acid were the major fatty acids and ranged between 68.77–73.32% and 12.74–14.64%, respectively. No statistical differences were found in sampling locations for oleic acid and palmitic acid different for each location ($p< 0.05$).

Table 1. Fatty acid profile of the samples olive fruits and soil characteristics of the sampling locations

	(C20:0)	(C22:0)	(C18:2)	(C18:3)	(C18:1)	(C16:0)	(C16:1)	(C18:0)	pH	$CaCO_3$ (%)	Total salt (%)	P (P_2O5, %)	K (K20, (%)	Organic matter (%)
1	0.57 a	0.15 bc	8.94 b	0.86	69.71	14.02 ab	0.85 bcd	3.65 cd	7.85 bcd	19.10 e	0.017 bc	2.21 g	87.23 d	2.75 d
2	0.53 c	0.14 d	8.64 c	0.81	70.27	13.98 ab	0.92 abc	3.54 d	7.83 bcd	28.43 b	0.033 ab	2.29 g	75 f	3.50 b
3	0.50 c	0.13 d	8.60 c	0.89	69.67	14.81 a	1.08 a	3.20 e	7.87 bc	46.175 a	0.011 a	0.46 h	52.42 h	2.30 f
4	0.60 a	0.17 a	6.41 f	0.78	73.32	12.74 b	0.64 ef	3.88 ab	7.67 d	11.44 h	0.033 ab	3.30 e	92.5 b	0.99 h
5	0.59 a	0.16 bc	7.29 e	0.81	72.24	13.06 ab	0.69 def	3.86 ab	7.76 cd	13.35 g	0.034 ab	2.79 f	102 a	3.06 c
6	0.58 a	0.15 ab	9.30 a	0.90	68.77	14.64 ab	0.80 cde	3.63 cd	7.86 bcd	24.98 c	0.031 ab	5.51 c	63.15 g	2.56 e
7	0.60 a	0.16 ab	8.70 c	0.86	69.39	14.40 ab	0.84 cd	3.73 bc	7.86 bcd	23.11 d	0.037 a	4.52 d	21.5 j	2.57 e
8	0.60 a	0.17 a	7.43 e	0.94	71.19	13.54 ab	0.61 f	4.11 a	8.10 a	4.725 i	0.035 a	5.82 b	90.42 c	1.51 g
9	0.60 a	0.16 ab	7.41 e	0.81	72.31	12.78 b	0.66 ef	3.91 ab	7.99 ab	18.66 f	0.025 bc	6.88 a	78.32 e	4.00 a
10	0.43 c	0.11 e	7.88 d	0.78	72.06	13.40 ab	1.02 ab	3.18 e	7.76 cd	25.145 c	0.027 ab	2.195 g	43.5 i	2.16 f

C20: 0; Arachidic acid, **C22: 0;** Behenic acid, **C18:2;** Linoleic acid, **C18:3;** linolenic acid, **C18:1;** Oleic acid, **C16:0;** Palmitic acid, **C16:1;** Palmitoleic acid, **C18:0;** Stearic acid

Table 2**:** Correlation matrix (Pearson (n)) for the fatty acid components and soil properties

Variables	C20:0	C22:0	C18:2	C18:3	C18:1	C16:0	C16:1	C18:0	pH	$CaCO_3$ (%)	Total salt (%)	P (P_2O5, %)	K (K_20, (%)	Organic matter (%)
C20:0	**1**	**0.972**	-0.229	0.265	0.041	-0.226	**-0.838**	**0.898**	0.274	-0.602	0.437	**0.635**	0.421	0.023
C22:0	**0.972**	**1**	-0.405	0.227	0.196	-0.346	**-0.896**	**0.944**	0.258	**-0.699**	0.495	0.598	0.499	-0.139
C18:2	-0.229	-0.405	**1**	0.455	**-0.952**	**0.889**	0.606	-0.483	0.144	0.594	-0.325	-0.173	-0.511	0.356
C18:3	0.265	0.227	0.455	**1**	**-0.655**	0.623	-0.042	0.181	**0.700**	0.046	-0.115	0.242	-0.082	-0.187
C18:1	0.041	0.196	**-0.952**	**-0.655**	**1**	**-0.950**	-0.477	0.322	-0.251	-0.523	0.246	0.097	0.487	-0.196
C16:0	-0.226	-0.346	**0.889**	0.623	**-0.950**	**1**	**0.640**	-0.506	0.136	**0.682**	-0.334	-0.294	-0.576	0.034
C16:1	**-0.838**	**-0.896**	0.606	-0.042	-0.477	**0.640**	**1**	**-0.967**	-0.257	**0.883**	-0.576	**-0.721**	**-0.635**	0.121
C18:0	**0.898**	**0.944**	-0.483	0.181	0.322	-0.506	**-0.967**	**1**	0.384	**-0.850**	0.581	**0.713**	0.576	-0.065
pH	0.274	0.258	0.144	**0.700**	-0.251	0.136	-0.257	0.384	**1**	-0.165	-0.039	0.579	0.025	0.196
$CaCO_3$ (%)	-0.602	**-0.699**	0.594	0.046	-0.523	**0.682**	**0.883**	**-0.850**	-0.165	**1**	**-0.647**	-0.573	-0.581	0.260
Total salt (%)	0.437	0.495	-0.325	-0.115	0.246	-0.334	-0.576	0.581	-0.039	**-0.647**	**1**	0.495	0.084	-0.149
P (P_2O5, %)	**0.635**	0.598	-0.173	0.242	0.097	-0.294	**-0.721**	**0.713**	0.579	-0.573	0.495	**1**	0.096	0.148
K (K_20, (%)	0.421	0.499	-0.511	-0.082	0.487	-0.576	**-0.635**	0.576	0.025	-0.581	0.084	0.096	**1**	-0.030
Organic matter(%)	0.023	-0.139	0.356	-0.187	-0.196	0.034	0.121	-0.065	0.196	0.260	-0.149	0.148	-0.030	**1**

Values in bold are different from 0 with a significance level α= 0,05

C20: 0; Arachidic acid, **C22: 0;** Behenic acid, **C18:2;** Linoleic acid, **C18:3;** linolenic acid, **C18:1;** Oleic acid, **C16:0;** Palmitic acid, **C16:1;** Palmitoleic acid, **C18:0;** Stearic acid

The average linolenic acid level of olive oil samples ranged between 0.78-0.94% in south-eastern region of Turkey, below the maximum value fixed by the IOOC (1.0%) (International Olive Oil Council, 2003); but within the ranges proposed by the Turkish Codex (0.9%). However, it is worthy to note that no statistical differences were determined under different growing conditions.

Pearson correlation coefficients among fatty acid profiles are presented in Table 2. The maximum positive correlations were found

between C22:0 and C20:0 (r=.972), C18:0 and C22:0 (r=.944), C18:0 and C20:0 (r=.898), C16:0 and C18:2 (r=0.889) whereas strong and negative ones were observed between C18:1 and C18:2 (r=−.952), C18:1 and C16:0 (r=−.819). The major component, oleic acid (C18:1) was negatively correlated with pH (r=-.251), $CaCO_3$ (r=-.523) and organic matter (r=-.0196) but positively moderate associated with K content (r=.487).

On the other hand, salt content also positively-but weak-correlated with oleic acid content (r=.246). Of those major components, palmitic acid (C16:0) composition significantly varied with $CaCO_3$ content (r=.682) but negatively affected with salt content (r=.-334), P content (r=-.294) and K content (r=-.576). Linoleic acid (C18:2) displayed similar reaction with palmitic acid against $CaCO_3$ content (r=.594) and positive moderate relation with organic matter content (r=.356) but negative correlation with salt content (r=-.325), P content (r=-.173), K content (r=-.511) were found with linoleic acid.

Data of the fatty acids compositions corresponding to all olive oil samples were submitted to Principal Component Analysis (PCA) to transform a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components (PC). Only eigenvalues of greater than 1.0 are considered significant descriptors of data variance, according to Kaiser's rule (Kammoun and Zarrouk, 2012). Eigen analysis of the correlation matrix loadings of the significant principal components were summarized in Table 3.

Table 3. Eigen analysis of the correlation matrix loadings of the significant principal components

	PC1	PC2	PC3	PC4
Eigenvalue	6.701	3.294	1.247	1.073
Variability (%)	47.867	23.531	8.909	7.662
Cumulative (%)	47.867	71.398	80.308	87.970

The first four components (PC1, PC2, PC3, and PC4) had eigenvalues of 6.701, 3.294, 1.247 and 1.073, and accounted for 47.867 %, 23.531 %, 8,909 % and 7,662 % of the variance in the data, respectively.

Table 4. Correlations between variables and factors

	PC1	PC2	PC3	PC4
Arachidic	-0.771	0.463	0.007	0.043
Behenic	-0.858	0.348	-0.118	-0.005
Linoleic	0.707	0.595	0.120	0.138
Linolenic	0.068	0.873	-0.327	-0.283
Oleic	-0.591	-0.756	0.064	-0.111
Palmitic	0.733	0.613	-0.226	0.073
Palmitoleic	0.985	-0.130	-0.010	0.019
Stearic	-0.947	0.297	0.014	-0.017
pH	-0.201	0.710	0.229	-0.318
$CaCO_3$	0.910	0.024	0.116	-0.062
Total Salt	-0.612	0.039	-0.131	0.685
P	-0.657	0.473	0.283	0.241
K	-0.641	-0.186	0.047	-0.565
Organic matter	0.153	0.157	0.944	0.045

The first PC accounted for more 47.867 % of total explained variance. Linoleic acid. palmitic acid. $CaCO_3$ content were the most important factors in PC1 whereas linolenic acid. pH were the most important factor in PC2 (Table 3).

The ten sampling locations are successfully discriminated by their fatty acid compositions and physicochemical factors of soil. Oleic acid, palmitic acid and linoleic acid-major fatty acid components- were discriminated with K content, $CaCO_3$ content, organic matter content, respectively (Figure 1-3).

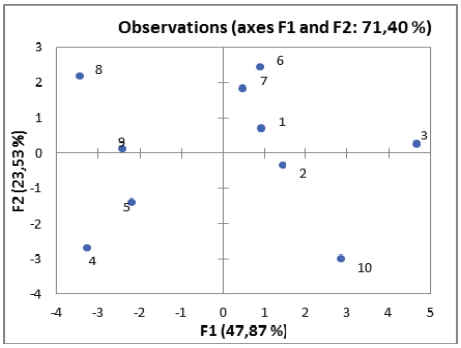

Figure 1. Observations (axes F1 and F2: 71.40 %)

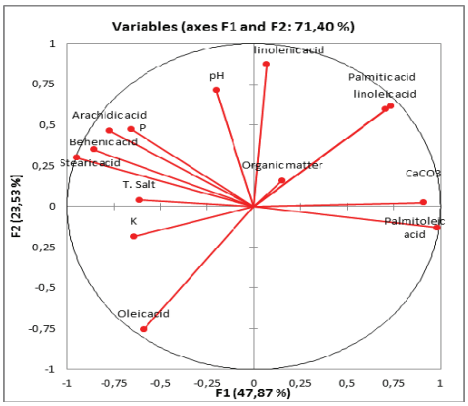

Figure 2. Variables (axes F1 and F2: 71.40 %)

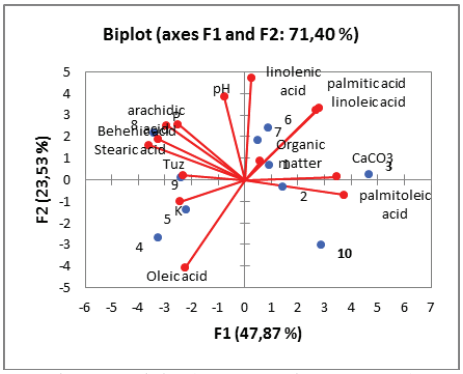

Figure 3. Biplot (axes F1 and F2: 71.40 %)

CONCLUSIONS

Of major fatty acids, oleic acid content was not affected in relation to the sampling locations. The possible effects of physicochemical characteristics of the sampling soils on the fatty acid profiles of olive oil were investigated by correlation test and then principal component analysis was performed to reduce the dimension of the experimental samples.

Oleic acid, palmitic acid and linoleic acid were more pronounced under K. $CaCO_3$ and organic matter content rich soils, respectively.

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