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Amino and fatty acids composition of olive stones for the discrimination of *Olea europaea* subsp. *europaea* varieties

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Abstract. Few studies have reported the relationship between wild (*Olea europaea* L. subsp. *europaea* var. *sylvestris*) and cultivated (*Olea europaea* L. subsp. *europaea* var. *europaea*) olive trees by using diverse markers. Herein, the amino and fatty acids composition of stones from wild and cultivated olives were assessed respectively using amino acids analyzer and gas chromatography coupled with mass spectrometry. Stones of 24 Tunisian olive samples including twelve cultivated trees and twelve wild trees were obtained from olives harvested at ripe stage. Results showed that 17 amino acids (with eight essential amino acids) and 15 fatty acids (eight saturated and seven unsaturated) were detected in the both olive taxa. Statistically, significant differences among wild and cultivated stones were observed for amino and fatty acids contents. Based on the major fatty acids and the essential amino acids, multivariate analyses classified olive varieties into three groups showing a close relationship between some wild and cultivated olive trees. Results were useful to distinguish some interest wild olive genotypes having stones richer in essential amino acids and monounsaturated fatty acids. Wild olive trees would constitute a genetic pool of interest criteria. These data would be used as complementary tool to morphological traits and molecular markers studies providing a relationship between the cultivated and wild olive trees.
Keywords: amino acids; fatty acids; olive stones; olive tree; cultivars; oleaster; multivariate analyses; Tunisia.

Contenido en aminoácidos y ácidos grasos de los huesos de aceitunas de variedades de *Olea europaea* L. subsp. *europaea*

Resumen. Para estudiar las relaciones entre los olivos silvestres (*Olea europaea* L. subsp. *europaea* var. *sylvestris*) y los cultivados (*Olea europaea* L. subsp. *europaea* var. *europaea*) se han usado diferentes marcadores. En el presente trabajo se ha evaluado el contenido en aminoácidos y ácidos grasos de los huesos de aceitunas procedentes de árboles silvestres y cultivados usando el analizador de aminoácidos y cromatografía de gases junto con espectrometría de masas, respectivamente. Se recogieron huesos de aceitunas cosechadas en la etapa madura de 24 olivos tunecinos, doce variedades cultivadas y doce silvestres. Se detectaron 17 aminoácidos (con ocho aminoácidos esenciales) y 15 ácidos grasos (ocho de ellos saturados y siete insaturados) en ambas variedades. Los análisis estadísticos revelaron diferencias significativas en el contenido de aminoácidos y ácidos grasos de los huesos de aceitunas entre las variedades silvestres y cultivadas. Basándose en el contenido de los principales ácidos grasos y los aminoácidos esenciales, las variedades fueron clasificadas en tres grupos en los que se observa una estrecha relación entre algunos olivos silvestres y cultivados. Los resultados fueron útiles para distinguir algunos genotipos de variedades silvestres que tienen huesos más ricos en aminoácidos esenciales y ácidos grasos monoinsaturados. Por lo tanto, estos olivos silvestres constituyen una reserva genética de interés. Estos datos se podrán utilizar como complementarios en los estudios de los caracteres morfológicos y de los marcadores moleculares que estudian relación entre los olivos silvestres y cultivados.
Palabras clave: aminoácidos; ácidos grasos; huesos de aceituna; olivo; acebuche; análisis multivariable; Túnez.

Introduction

There is a main number of wild or non-cultivated edible plant species as the olive trees. The wild olive trees namely oleaster (var. *sylvestris*) are non-cultivated and colonized natural ecosystem as park and forest or agro-ecosystem in olive verger borders. The cultivated olive trees (var. *europaea*) includes the olive cultivars. These two olive tree forms are the tow botanical varieties of the subsp. *Olea europaea* (Green, 2002). The cultivated one (var. *europaea*) is located mainly in the Mediterranean region and is one of the oldest agricultural trees crops worldwide. It is composed of two products, olive oil and table olives representing economic and industrial activities in the Mediterranean countries. Olive cultivation has traditionally played an important role in the human diet because of the nutritional value and beneficial

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properties of olive oil (Roche *et al.*, 2000; Kratz *et al.*, 2002; Hannachi *et al.*, 2013). The olive cultivars and oleasters were described using diverse markers as morphological traits (Idrissi & Ouazzani, 2003; Hannachi *et al.*, 2008a, b; Belaj *et al.*, 2011; Hannachi *et al.*, 2016), oil quality criteria (Krichene *et al.*, 2007; Dabbou *et al.*, 2011; Hannachi *et al.*, 2013) and molecular markers (Breton *et al.*, 2006a; Hannachi *et al.*, 2010; Belaj *et al.*, 2011). Close relationship between cultivated and wild olive trees has been demonstrated with different molecular markers such as isozymes, RAPD, SSR and chloroplast DNA (Breton *et al.*, 2006a; Hannachi *et al.*, 2010; Belaj *et al.*, 2011). In Tunisia, based on cytoplasmic and nuclear SSR markers it has been demonstrated the presence of native oleaster trees and feral olive tree forms (Breton *et al.*, 2006b; Hannachi *et al.*, 2008a, b; Hannachi *et al.*, 2010). The characterization and classification of olive oil have been reported according to its geographical origin using statistical analyses applied to fatty acids and sterol compositions (Hannachi *et al.*, 2007; Baccouri *et al.*, 2011; Hannachi *et al.*, 2013), volatile compounds (Kotti *et al.*, 2009; Ouni *et al.*, 2011) and minor components especially phenolic compounds (Tura *et al.*, 2007; Di Donna *et al.*, 2010; Hannachi *et al.*, 2013). Wild species would be a source of interest compounds as oil, protein and secondary metabolites (Kirillov *et al.*, 2019).

The objective of the current study was to evaluate the diversity between cultivated and wild olive trees based on biochemical criteria including amino and fatty acids composition from olive stones using univariate and multivariate analyses. The used criteria would highlight promote wild olive genotypes.

Materials and methods

Plant material

Stones from twelve cultivated olive or cultivars (*Olea europaea* L. subsp. *europaea* var. *europaea*) and twelve wild olive or oleasters (*Olea europaea* L. subsp. *europaea* var. *sylvestris*) trees from different geographical origins of Tunisia were used in this study (Table 1). Samples were identified at Tunisian Olive Institute (Tunis, Tunisia). Olives were harvested at ripe stage. The stones were separated from pulps, washed by distilled water, dried at room temperature and powdered. The obtained matter was conserved at glass bottles for future analyses.

Table 1. Localities of studied olive trees.

	Locality	Province	Latitude	Longitude
Cultivated olive trees (Cultivars)				
Sayali	Slouguia	Béja	36°36’37.81’’N	09°31’53.39’’E
Chemlali	Slouguia	Béja	36°36’37.81’’N	09°31’53.39’’E
Besbessi	Testour	Béja	36°33’06.92’’N	09°26’58.96’’E
Neb Jmel	Testour	Béja	36°33’06.92’’N	09°26’58.96’’E
Awam	Dougga	Béja	36°24’02.75’’N	09°14’14.75’’E
Gerboui	Dougga	Béja	36°24’02.75’’N	09°14’14.75’’E
Meski	Dougga	Béja	36°24’02.75’’N	09°14’14.75’’E
Zarras	Téboursouk	Béja	36°27’40.60’’N	09°14’47.48’’E
Limi	Téboursouk	Béja	36°27’40.60’’N	09°14’47.48’’E
Roumi	Téboursouk	Béja	36°27’40.60’’N	09°14’47.48’’E
Nib	Ras Jbel	Bizerte	37°11’59.56’’N	10°07’28.84’’E
Chétoui	Alia	Bizerte	37°10’38.70’’N	10°02’05.21’’E
Wild olive trees (Oleasters)				
Ozag	Zaghouan	Zaghouan	36°24’00.81’’N	10°09’29.77’’E
Omje	Mjez El Bab	Béja	36°38’44.87’’N	09°36’15.34’’E
Ojer	Jbel Abderrahman	Nabeul	36°44’21.79’’N	10°41’26.58’’E
Obel1	Belvédère	Tunis	36°49’12.99’’N	10°10’13.97’’E
Obel2	Belvédère	Tunis	36°49’21.27’’N	10°10’23.83’’E
Obel3	Belvédère	Tunis	36°49’21.27’’N	10°10’23.83’’E
Obel4	Belvédère	Tunis	36°49’21.27’’N	10°10’23.83’’E
Odou	Dougga	Béja	36°24’02.75’’N	09°14’14.75’’E
Orjb	Ras Jbel	Bizerte	37°11’59.56’’N	10°07’28.84’’E
Olaa	Laaroussa	Séliana	36°22’51.94’’N	09°27’16.37’’E
Oecr	Echraf	El Haouaria	36°59’57.92’’N	11°02’12.69’’E
Osej	Sejnen	Bizerte	37°03’07.55’’N	09°14’31.94’’E

Fatty acids composition

Oil extraction was conducted on dried powdered olive stones (10 g) by the Soxhlet method using hexane as extraction solvent. Each olive stone sample was extracted using 200 mL hexane during 6 h. The fatty acid methyl esters (FAME) were prepared before analysis by transesterification with methanolic potassium hydroxide. 0.2 mL of stone oil was added to 5 mL of NaOH 0.5 M and taken 15 min at 65°C. Three mL of BF_3 20% were added and taken for 5 min at 65°C. Ten mL of petroleum ether was added. Then, evaporation was conducted, and the obtained methyl esters were conserved in chloroform.

One µL of FAME were analyzed on a GC/MS apparatus type Agilent Technologies equipped with a flame ionizing detector (FID), a fused silica capillary column (60 m × 0.25 mm, length x inner diameter). The oven temperature was held 60°C for 1 min, increased to 90°C for 7 min, then to 240°C at a rate 5°C/min and then kept at 240°C for 15 min. Injector and the flame ionization detector temperatures were maintained at 260°C. The nitrogen is the carrier gas kept at flow rate of 1.51 mL/min. The fatty acid compositions were conducted in triplicate and results were reported as percentage (Anon., 2017).

Amino acids composition

Amino acids were determined after acid hydrolysis of the olive stone samples. Acid hydrolysis was performed in sealed glass tubes. Each tube containing 0.2 mg of each olive stone samples was added with 9 mL of 6.6 M HCl and kept under N_2 atmosphere for 24 h at 110°C. The hydrolyzed samples were mixed in 9 mL 6 M NaOH and adjusted to 100 mL with 0.02 M HCl. The sample solutions (1.5 mL) were filtered twice through a micropore filter (0.45 µm).

Twenty µL of the filtrate was injected into an amino acid analyzer (Hitachi L8800, Tokyo, Japan) to determine amino acids composition of olive stones. The determination was carried out at 38°C on flow rate of 1.0 mL/min. The detection wavelengths were respectively 570 nm for most amino acid residues and 440 nm for the ninhydrin–proline derivative. Chemical score was calculated, and results of amino acids composition were reported as mg per 100 g of protein. The amino acid compositions were conducted in triplicate. Tryptophan was not analyzed since it is degraded upon acid hydrolysis (Elfalleh *et al*., 2012).

Statistical analyses

A one-way analysis of variance (ANOVA) was used to compare amino and fatty acid contents in olive stones of wild and cultivated olive trees. In addition, multivariate analyses were conducted on essential amino acid contents and major fatty acid contents including the principal component (PCA) and cluster analyses (Di Donna *et al.*, 2010). The cluster analysis was conducted using unweighted pair-group method with arithmetic mean (UPGMA) based on Euclidian distance. Analyses were computed on the data using XLSTAT 2017 (www.xlstat.com).

Results

Fatty acid composition

Fifteen peaks were identified in the chromatograms of the 24 studied oils. None of the peaks was exclusive to cultivated or wild olive trees. The cultivated and wild olive trees had the same fatty acids profile of oil extracted from stone. The oleic acid (C18:1n-9) was the major fatty acid in all stone oils followed by the palmitic (C16:0) and stearic (C18:0) acids. The ANOVA analysis showed significant differences of fatty acids between olive stones oils, excepting tow fatty acids (C18:3n-6 and C14:0). Results were shown in Tables 2 and 3. By comparing olive genotypes, the oleic acid (18:1) content of Obel3 wild provenance was significantly higher than that of all other stone oils having an amount of 63.90%. Also, the palmitic acid (C16:0) content of Obel1 genotype was significantly higher than of all other genotypes with 18.8%. The mean of saturated fatty acids (SFA) has an amount of 21.20 and 20.99 % in cultivars and oleasters stone oils, respectively (Table 2). The mean of mono-unsaturated fatty acids (MUFA) of the oils extracted from stones of olive cultivars and oleasters have a percentage of 59.73 and 59.45, respectively. However, the poly-unsaturated fatty acid (PUFA) means have a percentage of 16.28 for cultivar stones and 18.60 for oleaster stones (Table 3).

Table 2. Saturated fatty acids of olive stone oils (percentage of the total fatty acids). Abbreviations are: SFA, saturated fatty acids; S, significant; NS, non-significant. Values are the mean of triplicate ± standard deviation.

	C9:0	C12:0	C14:0	C16:0	C18:0	C20:0	C22:0	C24:0	SFA
Cultivated olive trees (Cultivars)									
Sayali	0.18 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	14.19 ± 0.51	5.34 ± 0.11	1.16 ± 0.03	1.16 ± 0.02	0.12 ± 0.03	22.14 ± 0.14
Zarras	0.33 ± 0.01	0.15 ± 0.01	0.09 ± 0.03	15.18 ± 0.62	5.45 ± 0.16	1.26 ± 0.01	0.43 ± 0.01	0.28 ± 0.02	22.86 ± 0.51
Gerboui	0.25 ± 0.03	0.13 ± 0.01	0.07 ± 0.01	15.68 ± 0.50	4.77 ± 0.25	1.00 ± 0.01	0.88 ± 0.03	0.75 ± 0.30	22.76 ± 0.11
Chétoui	0.86 ± 0.14	0.04 ± 0.01	0.05 ± 0.01	15.69 ± 0.49	5.65 ± 0.14	1.14 ± 0.07	0.74 ± 0.01[c]	0.45 ± 0.05	24.16 ± 0.36
Awam	0.37 ± 0.01	0.51 ± 0.02	0.32 ± 0.03	12.92 ± 0.35	4.99 ± 0.35	1.20 ± 0.01	0.55 ± 0.02	0.39 ± 0.02	20.80 ± 0.29
Neb Jmel	0.10 ± 0.01	0.13 ± 0.01	0.18 ± 0.03	9.81 ± 0.14	4.35 ± 0.28	0.76 ± 0.02	0.36 ± 0.04	0.36 ± 0.01	15.68 ± 0.16

	C9:0	C12:0	C14:0	C16:0	C18:0	C20:0	C22:0	C24:0	SFA
Roumi	0.26 ± 0.01	0.15 ± 0.01	0.19 ± 0.01	15.84 ± 0.13	5.74 ± 0.10	1.07 ± 0.04	0.62 ± 0.04	0.39 ± 0.01	23.83 ± 0.07
Nib	0.13 ± 0.01	0.15 ± 0.01	0.09 ± 0.01	12.99 ± 0.28	5.03 ± 0.10	0.93 ± 0.09	0.41 ± 0.13	0.43 ± 0.04	19.71 ± 0.11
Limi	0.23 ± 0.01	0.54 ± 0.04	0.54 ± 0.06	13.52 ± 0.21	4.86 ± 0.17	1.12 ± 0.07	0.57 ± 0.01	0.38 ± 0.03	21.36 ± 0.47
Meski	0.12 ± 0.01	0.16 ± 0.01	0.10 ± 0.01	13.64 ± 0.12	3.90 ± 0.21	0.89 ± 0.03	0.46 ± 0.08	0.35 ± 0.01	19.24 ± 0.04
Besbessi	0.34 ± 0.014	0.13 ± 0.03	0.11 ± 0.01	14.05 ± 0.15	4.71 ± 0.70	1.45 ± 0.01	0.50 ± 0.02	0.38 ± 0.01	21.28 ± 0.04
Chemlali	0.09 ± 0.01	0.12 ± 0.02	0.09 ± 0.01	10.67 ± 0.24	3.61 ± 0.70	0.74 ± 0.03	0.50± 0.03	0.65 ± 0.06	15.81 ± 0.28
Mean cultivars	0.27 ± 0.12	0.19 ± 0.16	0.16 ± 0.14	13.66 ± 1.92	4.86 ± 0.66	1.06 ± 0.21	0.59 ± 0.23	0.40 ± 0.16	21.20 ± 2.77
Wild olive trees (Oleasters)									
Obel1	0.31 ± 0.01	0.135 ± 0.02	0.10 ± 0.01	18.80 ± 0.78	5.53 ± 0.14	1.37 ± 0.06	0.55 ± 0.07	0.31 ± 0.01	26.78 ± 0.22
Obel2	0.33 ± 0.04	0.195 ± 0.02	0.11 ± 0.01	16.17 ± 0.54	6.10 ± 0.41	1.57 ± 0.03	0.34 ± 0.06	0.35 ± 0.07	24.80 ± 0.75
Obel3	0.20 ± 0.08	0.095 ± 0.01	0.08 ± 0.01	11.03 ± 0.31	4.45 ± 0.49	0.47 ± 0.02	0.50 ± 0.14	0.25 ± 0.07	16.81 0.10
Obel4	0.21 ± 0.01	0.175 ± 0.01	0.10 ± 0.01	11.31 ± 0.21	4.55 ± 0.21	1.05 ± 0.01	0.61 ± 0.28	0.78 ±0.04	17.99 ± 0.53
Ozag	0.23 ± 0.02	0.115 ± 0.02	0.12 ± 0.02	13.14 ± 1.30	4.57 ± 0.46[j]	1.02 ± 0.03	1.38 ± 0.57	0.70 ± 0.13	20.56 ± 1.25
Omje	0.23 ± 0.01	0.145 ± 0.06	0.12 ± 0.02	11.83 ± 0.39	5.22 ± 0.30	1.14 ± 0.02	0.45 ± 0.02	0.27 ± 0.04	19.13 ± 0.66
Odou	0.19 ± 0.01	0.115 ± 0.01	0.14 ± 0.01	12.40 ± 0.64	5.54 ± 0.17	0.80 ± 0.01	0.35 ± 0.01	0.35 ± 0.14	19.52 ± 0.17
Orjb	0.30 ± 0.01	0.445 ± 0.06	0.15 ± 0.01	12.75 ± 0.42	4.80 ± 0.11	0.51 ± 0.02	0.45 ± 0.02	0.23 ± 0.01	19.39 ± 0.45
Olaa	0.19 ± 0.01	0.270 ± 0.01	0.17 ± 0.01	10.44 ± 0.77	4.91 ± 0.17	0.73 ± 0.01	0.27 ± 0.06	0.19 ± 0.04	16.97 ± 0.55
Oecr	0.20 ± 0.02	0.235 ± 0.06	0.06 ± 0.01	18.45 ± 0.70	5.82 ± 0.29	0.65 ± 0.03	0.29 ± 0.01	0.49 ± 0.01	25.69 ± 0.78
Ojer	0.25 ± 0.01	0.955 ± 0.08	0.24 ± 0.02	12.04 ± 0.85	4.37 ± 0.92	1.02 ± 0.01	0.60 ± 0.02	0.50 ± 0.10	19.47 ± 0.33
Osej	0.23 ± 0.01	0.250 ± 0.01	0.20 ± 0.01	12.97 ± 0.52	4.99 ± 0.28	0.82 ± 0.03	0.48 ± 0.02	0.45 ± 0.03	19.93 ± 0.57
Mean oleasters	0.24 ± 0.05	0.26 ± 0.24	0.13 ± 0.05	13.41 ± 2.81	5.07 ± 0.57	0.93 ± 0.33	0.52 ± 0.29	0.41 ± 0.19	20.99 ± 3.35
F-value	47.33	30.10	1.25	93.36	20.78	4.78	4.26	6.43	81.59
P-value	< 0.0001	< 0.0001	< 0.29	< 0.0001	< 0.0001	< 0.0002	< 0.0004	< 0.0001	< 0.0001
Effect	S	S	NS	S	S	S	S	S	S

Table 3. Unsaturated fatty acids of olive stone oils (percentage of the total fatty acids). Abbreviations are: PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; S, significant; NS, non-significant. Values are the mean of triplicate ± standard deviation.

	C16:1n-9	C16:1n-7	C18:1n-9	C20:1n-9	C18:2n-6	C18:3n-3	C18:3n-6	PUFA	MUFA
Cultivated olive trees (Cultivars)									
Sayali	0.125 ± 0.01	0.38 ± 0.0	61.03 ± 2.68	1.20 ± 0.08	13.98 ± 0.21	0.050 ± 0.01	0.59 ± 0.01	14.62 ± 0.10	62.72 ± 0.36
Zarras	0.11 ± 0.01	0.45 ± 0.02	51.96 ± 2.71	1.41 ± 0.02	18.20 ± 0.25	0.165 ± 0.01	0.305 ± 0.03	18.66 ± 0.51	53.93 ± 0.21
Gerboui	0.13 ± 0.01	1.03 ± 0.06	53.54 ± 1.25	1.14 ± 0.02[e]	17.78 ± 0.91	0.065 ± 0.02	0.13 ± 0.02	17.97 ± 1.00	55.83 ± 1.28
Chétoui	0.09 ± 0.02	0.22 ± 0.01	52.72 ± 1.48	0.95 ± 0.02	13.72 ± 0.28	0.165 ± 0.01	0.63 ± 0.01	14.52 ± 0.39	53.97 ± 0.30
Awam	0.13 ± 0.01	0.23 ± 0.01	58.79 ± 2.40	1.36 ± 0.02	13.46 ± 0.70	0.245 ± 0.01	0.39 ± 0.01	14.10 ± 0.33	60.50 ± 0.21
Neb Jmel	0.17 ± 0.02	0.30 ± 0.01	62.26 ± 1.84	0.85 ± 0.03	19.91 ± 0.35	0.055 ± 0.01	0.23 ± 0.01	20.19 ± 0.07	63.585 ± 0.50
Roumi	0.20 ± 0.01	0.33 ± 0.02	61.20 ± 3.96	0.49 ± 0.02	10.12 ± 0.38	0.190 ± 0.02	0.31 ± 0.02	10.61 ± 0.04	62.22 ± 0.41
Nib	0.12 ± 0.02	0.34 ± 0.01	59.01 ± 2.19	0.54 ± 0.01	19.03 ± 0.42	0.130 ± 0.01	0.24 ± 0.01	19.40 ± 0.08	60.01 ± 0.19
Limi	0.14 ± 0.01	0.22 ± 0.01	56.68 ± 1.71	1.12 ± 0.04	17.53 ± 0.35	0.135 ± 0.01	0.21 ± 0.01	17.87 ± 0.05	58.16 ± 0.01
Meski	0.22 ± 0.01	0.39 ± 0.02	58.65 ± 1.13	1.02 ± 0.01	15.22 ± 0.21	0.22 ± 0.02	0.86 ± 0.03	16.29 ± 3.10	60.27 ± 0.01
Besbessi	0.19 ± 0.02	0.36 ± 0.02	61.37 ± 1.27	0.93 ± 0.01	10.11 ± 0.28	0.22 ± 0.01	0.36 ± 0.02	10.69 ± 0.21	62.84 ± 0.25
Chemlali	0.18 ± 0.05	0.84 ± 0.02	60.69 ± 1.99	1.06 ± 0.03	19.88 ± 0.16	0.21 ± 0.01	0.36 ± 0.01	20.45 ± 0.01	62.76 ± 0.11
Mean cultivars	0.15 ± 0.04	0.42 ± 0.25	58.16 ± 3.62	1.00 ± 0.28	15.74 ± 3.50	0.15 ± 0.07	0.38 ± 0.21	16.28 ± 3.40	59.73 ± 3.49
Wild olive trees (Oleasters)									
Obel1	0.15 ± 0.01	0.27 ± 0.02	56.93 ± 1.64	0.84 ± 0.05	12.34 ± 0.21	0.19 ± 0.01	0.30 ± 0.01	12.83 ± 0.03	58.17 ± 0.10
Obel2	0.06 ± 0.02	0.52 ± 0.03	56.67 ± 1.06	1.11 ± 0.03	11.60 ± 0.67	0.20 ± 0.01	0.24 ± 0.01	12.03 ± 0.59	58.35 ± 0.98
Obel3	0.10 ± 0.01	0.39 ± 0.02	63.90 ± 2.02	0.84 ± 0.02	16.95 ± 0.17	0.15 ± 0.01	0.31 ± 0.01	17.40 ± 0.21	65.22 ± 0.39
Obel4	0.18 ± 0.01	0.50 ± 0.02	58.01 ± 2.52	1.04 ± 0.01	18.39 ± 0.28	0.24 ± 0.02	0.56 ± 0.01	19.19 ± 0.09	59.72 ± 0.93
Ozag	0.12 ± 0.02	0.50 ± 0.02	52.53 ± 2.55	1.00 ± 0.01	17.68 ± 0.19	0.11 ± 0.01	1.07 ± 0.03	18.86 ± 3.25	54.14 ± 2.64
Omje	0.12 ± 0.04	0.24 ± 0.02	56.65 ± 2.15	0.90 ± 0.02	15.21 ± 0.50	0.40 ± 0.02	1.29 ± 0.04	16.89 ± 1.05	57.91 ± 2.35

	C16:1n-9	C16:1n-7	C18:1n-9	C20:1n-9	C18:2n-6	C18:3n-3	C18:3n-6	PUFA	MUFA
Odou	0.22 ± 0.02	0.58 ± 0.02	58.15 ± 1.14	0.72 ± 0.01	19.11 ± 0.14	0.23 ± 0.01	0.39 ± 0.02	19.72 ± 0.11	59.66 ± 0.02
Orjb	0.17 ± 0.01	0.27 ± 0.01	59.69 ± 1.90	1.17 ± 0.02	16.51 ± 0.71	0.15 ± 0.01	0.23 ± 0.01	16.89 ± 0.01	61.30 ± 0.22
Olaa	0.19 ± 0.02	0.23 ± 0.02	61.63 ± 1.50[c]	0.65 ± 0.01	18.95 ± 0.92	0.18 ± 0.02	0.31 ± 0.01	19.44 ± 0.13	62.70 ± 0.27
Oecr	0.12 ± 0.03	0.16 ± 0.01	52.98 ± 2.76	0.63 ± 0.03	19.29 ± 0.70	0.12 ± 0.01	0.28 ± 0.01	19.68 ± 0.62	53.89 ± 0.05
Ojer	0.21 ± 0.02	0.74 ± 0.03	58.78 ± 2.38	1.48 ± 0.01	17.44 ± 0.49	0.43 ± 0.02	0.74 ± 0.03	18.60 ± 0.30	61.21 ± 0.05
Osej	0.14 ± 0.05	0.62 ± 0.02	59.98 ± 1.95	0.48 ± 0.04	16.10 ± 0.70	0.22 ± 0.01	0.52 ± 0.02	16.84 ± 0.15	61.21 ± 2.25
Mean oleasters	0.15 ± 0.05	0.42 ± 0.18	57.99± 3.24	0.90 ± 0.28	16.63 ± 2.51	0.22 ± 0.10	0.52 ± 0.34	17.36 ± 2.56	59.45 ± 3.27
F-value	7.91	4.79	24.71	12.66	17.97	3.71	1.35	18.13	6.10
P-value	< 0.0001	< 0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.24	< 0.0001	< 0.0001
Effect	S	S	S	S	S	S	NS	S	S

Amino acid composition

Table 4 showed the amino acids profile of stones of the 24 Tunisian wild and cultivated olives. Qualitatively, both forms of olive stones have the same profile of the amino acid composition with significant variation on their amounts as shown with ANOVA. Seventeen familiar amino acids including eight essential amino acids (EAAs) were identified in the olive stone.

Table 4. Amino acids composition of olive stones (mg/100g protein). Abbreviations are; % EAA, percentage of essential amino acids; S, significant. Amino acid contents expressed as mg per 100 g protein; values are the mean of triplicate ± standard deviation.

	Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met	Ile
Cultivated olive trees (Cultivars)										
Sayali	0.66 ± 0.00	0.59 ± 0.00	0.52 ± 0.00	0.73 ± 0.00	0.37 ± 0.00	0.44 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.74 ± 0.00	0.65 ± 0.00
Zarras	0.50 ± 0.00	0.22 ± 0.00	0.30 ± 0.00	0.85 ± 0.01	0.24 ± 0.00	0.25 ± 0.00	0.14 ± 0.00	0.50 ± 0.02	0.03 ± 0.00	0.25 ± 0.01
Gerboui	0.31 ± 0. 00	0.16 ± 0.00	0.20 ± 0.00	0.53 ± 0.00	0.15 ± 0.00	0.16 ± 0.00	0.12 ± 0.00	0.43 ± 0.01	0.01 ± 0.00	0.21 ± 0.01
Chétoui	0.51 ± 0.00	0.23 ± 0.00	0.31 ± 0.00	0.86 ± 0.00	0.24 ± 0.00	0.25 ± 0.00	0.13 ± 0.00	0.51 ± 0.01	0.02 ± 0.00	0.26 ± 0.01
Awam	0.27 ± 0.00	0.12 ± 0.00	0.16 ± 0.00	0.41 ± 0.00	0.13 ± 0.00	0.14 ± 0.00	0.04 ± 0.00	0.41 ± 0.01	0.01 ± 0.00	0.17 ± 0.02
Neb Jmel	0.45 ± 0.00	0.20 ± 0.00	0.29 ± 0.00	0.78 ± 0.00	0.25 ± 0.00	0.24 ± 0.00	0.13 ± 0.00	0.44 ± 0.00	0.01 ± 0.00	0.22 ± 0.00
Roumi	0.28 ± 0.00	0.12 ± 0.00	0.16 ± 0.00	0.39 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.05 ± 0.00	0.40 ± 0.00	0.01 ± 0.00	0.17 ± 0.01
Nib	0.30 ± 0. 00	0.14 ± 0.00	0.19 ± 0.00	0.49 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.11 ± 0.00	0.40 ± 0.00	0.02 ± 0.00	0.17 ± 0.01
Limi	0.33 ± 0.01	0.14 ± 0.00	0.20 ± 0.00	0.50 ± 0.00	0.16 ± 0.00	0.17 ± 0.00	0.10 ± 0.00	0.44 ± 0.00	0.01 ± 0.00	0.18 ± 0.01
Meski	0.22 ± 0.00	0.19 ± 0.00	0.15 ± 0.00	0.37 ± 0.01	0.12 ± 0.00	0.12 ± 0.00	0.10 ± 0.00	0.21 ± 0.00	0.20 ± 0.01	0.15 ± 0.01
Besbessi	0.26 ± 0.01	0.11 ± 0.00	0.15 ± 0.00	0.39 ± 0.00	0.11 ± 0.00	0.13 ± 0.00	0.06 ± 0.00	0.24 ± 0.01	0.14 ± 0.01	0.16 ± 0.00
Chemlali	0.31 ± 0.01	0.14 ± 0.00	0.19 ± 0.00	0.53 ± 0.00	0.15 ± 0.00	0.16 ± 0.00	0.11 ± 0.00	0.37 ± 0.01	0.01 ± 0.00	0.18 ± 0.02
Mean	0.36 ± 0.13	0.19 ± 0.13	0.24 ± 0.11	0.57 ± 0.19	0.18 ± 0.08	0.19 ± 0.09	0.13 ± 0.05	0.40 ± 0.10	0.10 ± 0.02	0.23 ± 0.14
Wild olive trees (Oleasters)										
Obel1	0.38 ± 0.00	0.16 ± 0.00	0.22 ± 0.00	0.54 ± 0.00	0.19 ± 0.00	0.20 ± 0.00	0.04 ± 0.00	0.41 ± 0.02	0.01 ± 0.00	0.18 ± 0.01
Obel2	0.39 ± 0.01	0.16 ± 0.00	0.22 ± 0.00	0.59 ± 0.01	0.17 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	0.43 ± 0.01	0.02 ±0.00	0.20 ± 0.01
Obel3	0.27 ± 0.00	0.13 ± 0.00	0.18 ± 0.00	0.45 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.10 ± 0.00	0.40 ± 0.04	0.01 ± 0.00	0.17 ± 0.00
Obel4	0.31 ± 0.02	0.15 ± 0.01	0.20 ± 0.01	0.48 ± 0.02	0.16 ± 0.02	0.16 ± 0.01	0.10 ± 0.00	0.35 ± 0.07	0.12 ± 0.14	0.18 ± 0.01
Ozag	0.31 ± 0.03	0.15 ± 0.02	0.20 ± 0.02	0.47 ± 0.07	0.15 ± 0.01	0.16 ± 0.02	0.11 ± 0.02	0.41 ± 0.04	0.01 ± 0.00	0.20 ± 0.02
Omje	0.37 ± 0.00	0.17 ± 0.00	0.23 ± 0.00	0.60 ± 0.01	0.19 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	0.39 ± 0.01	0.01 ± 0.00	0.20 ± 0.01
Odou	0.48 ± 0.00	0.21 ± 0.00	0.30 ± 0.00	0.85 ± 0.00	0.26 ± 0.00	0.25 ± 0.00	0.12 ± 0.00	0.45 ± 0.00	0.01 ± 0.00	0.23 ± 0.00
Orjb	0.32 ± 0.01	0.15 ± 0.00	0.20 ± 0.01	0.54 ± 0.01	0.17 ± 0.00	0.17 ± 0.00	0.14 ± 0.00	0.39 ± 0.01	0.01 ± 0.00	0.18 ± 0.01
Olaa	0.56 ± 0.00	0.20 ± 0.07	0.36 ± 0.00	0.97 ± 0.01	0.31 ± 0.00	0.30 ± 0.00	0. 15 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.23 ± 0.01
Oecr	0.32 ± 0.00	0.15 ± 0.00	0.21 ± 0.00	0.54 ± 0.01	0.17 ± 0.00	0.17 ± 0.00	0.11 ± 0.00	0.24 ± 0.00	0.20 ± 0.00	0.18 ± 0.01
Ojer	0.26 ± 0.00	0.12 ± 0.00	0.17 ± 0.00	0.36 ± 0.00	0.13 ± 0.00	0.14 ± 0.00	0.09 ± 0.00	0.41 ± 0.05	0.01 ± 0.00	0.20 ± 0.01
Osej	0.36 ± 0.00	0.17 ± 0.00	0.23 ± 0.00	0.55 ± 0.00	0.17 ± 0.00	0.18 ± 0.00	0.10 ± 0.00	0.43 ± 0.05	0.01 ± 0.00	0.20 ± 0.01
Mean	0.36 ± 0.09	0.16 ± 0.03	0.23 ± 0.05	0.58 ± 0.17	0.18 ± 0.05	0.19 ± 0.05	0.10 ± 0.02	0.40 ± 0.06	0.04 ± 0.01	0.19 ± 0.02
F-value	207.25	81.23	501.14	197.32	367.02	393.70	2.60	21.69	57.04	169.24
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.012	< 0.0001	< 0.0001	< 0.0001
Effect	S	S	S	S	S	S	S	S	S	S

Table 4. (cont.)

	Tyr	Leu	Phe	Lys	NH3	His	Arg	Pro	Total	% EAA
Cultivated olive trees (Cultivars)										
Sayali	0.90 ± 0.00	0.65 ± 0.00	0.82 ± 0.00	0.72 ± 0.00	0.08 ± 0.00	0.77 ± 0.00	0.86 ± 0.00	0.57 ± 0.00	11.24 ± 0.00	51.68 ± 0.10
Chétoui	0.14 ± 0.01	0.50 ± 0.00	0.32 ± 0.01	0.23 ± 0.00	0.09 ± 0.00	0.11 ± 0.01	0.29 ± 0.00	0.16 ± 0.00	5.14 ± 0.01	43.31 ± 0.30
Chemlali	0.09 ± 0.02	0.30 ± 0.00	0.19 ± 0.01	0.17 ± 0.00[e]	0.06 ± 0.00	0.08 ± 0.00	0.18 ± 0.00	0.08 ± 0.01	3.28 ± 0.02	49.10 ± 0.41
Gerboui	0.09 ± 0.01	0.32 ± 0.00	0.21 ± 0.01	0.12 ± 0.00[i]	0.07 ± 0.00	0.36 ± 0.41	0.16 ± 0.00	0.10 ± 0.01	3.40 ± 0.01	55.10 ± 3.23
Zarras	0.12 ± 0.00	0.46 ± 0.00	0.32 ± 0.01	0.25 ± 0.00	0.10 ± 0.00	0.11 ± 0.00	0.28 ± 0.01	0.13 ± 0.00	5.02 ± 0.02	43.32 ± 0.18
Awam	0.07 ± 0.00	0.25 ± 0.00	0.17 ± 0.00	0.09 ± 0.00	0.07 ± 0.00	0.06 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	2.75 ± 0.01	47.80 ± 0.20
Neb Jmel	0.11 ± 0.00	0.41 ± 0.01	0.25 ± 0.03	0.14 ± 0.00	0.10 ± 0.00	0.09 ± 0.00	0.22 ± 0.00	0.14 ± 0.00	4.50 ± 0.00	40.53 ± 0.76
Roumi	0.07 ± 0.00	0.25 ± 0.00	0.18 ± 0.00	0.08 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.13 ± 0.00	0.07 ± 0.00	2.71 ± 0.01	52.40 ± 0.58
Nib	0.09 ± 0.01	0.28 ± 0.00[l]	0.20 ± 0.01	0.17 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.15 ± 0.01	0.09 ± 0.01	3.22 ± 0.02	45.48 ± 0.53
Meski	0.06 ± 0.00	0.22 ± 0.00	0.15 ± 0.00	0.11 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.11 ± 0.01	0.07 ± 0.01	2.59 ± 0.00	48.10 ± 0.30
Limi	0.08 ± 0.00	0.29 ± 0.00	0.20 ± 0.01	0.07 ± 0.00	0.08 ± 0.00	0.06 ± 0.01	0.13 ± 0.01	0.09 ± 0.00	3.13 ± 0.01	46.03 ± 0.80
Besbessi	0.07 ± 0.00	0.25 ± 0.00	0.17 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.13 ± 0.00	0.06 ± 0.00	1.47 ± 0.82	46.87 ± 0.50
Mean	0.16 ± 0.03	0.35 ± 0.13	0.28 ± 0.08	019 ± 0.08	0.07 ± 0.02	0.16 ± 0.02	0.23 ± 0.02	0.14 ± 0.04	3.90 ± 0.38	47.48 ± 0.67
Wild olive trees (Oleasters)										
Ozag	0.09 ± 0.02	0.30 ± 0.04	0.21 ± 0.03	0.11 ± 0.02	0.07 ± 0.00	0.07 ± 0.01	0.13 ± 0.02	0.09 ± 0.01	3.21 ± 0.19	46.06 ± 0.27
Omje	0.09 ± 0.00	0.34 ± 0.00	0.22 ± 0.00	0.14 ± 0.00	0.09 ± 0.00	0.07 ± 0.00	0.17 ± 0.00	0.09 ± 0.01	3.67 ± 0.01	43.06 ± 0.21
Ojer	0.06 ± 0.00	0.25 ± 0.00	0.16 ± 0.00	0.09 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.10 ± 0.01	0.08 ± 0.00	2.63 ± 0.00	48.70 ± 1.97
Obel1	0.09 ± 0.00	0.32 ± 0.00	0.21 ± 0.00	0.11 ± 0.00	0.09 ± 0.00	0.06 ± 0.00	0.15 ± 0.00	0.12 ± 0.01	3.44 ± 0.01	43.95 ± 0.86
Obel2	0.10 ± 0.00	0.34 ± 0.01	0.23 ± 0.00	0.11 ± 0.00	0.08 ± 0.00	0.06 ± 0.00	0.17 ± 0.00	0.10 ± 0.00	3.54 ± 0.03	44.73 ± 0.11
Obel3	0.07 ± 0.00	0.26 ± 0.01	0.18 ± 0.01	0.13 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.12 ± 0.00	0.07 ± 0.00	2.90 ± 0.02	47.20 ± 2.02
Obel4	0.08 ± 0.00	0.30 ± 0.00	0.20 ± 0.00	0.11 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.15 ± 0.00	0.09 ± 0.01	3.23 ± 0.05	46.93 ± 3.39
Odou	0.10 ± 0.00	0.43 ± 0.00	0.28 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.24 ± 0.00	0.14 ± 0.00	4.74 ± 0.00	41.39 ± 0.70
Orjb	0.09 ± 0.01	0.30 ± 0.01	0.21 ± 0.02[d]	0.08 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.13 ± 0.01	0.09 ± 0.01	3.25 ± 0.06	43.53 ± 0.26
Olaa	0.15 ± 0.01	0.50 ± 0.00	0.32 ± 0.00	0.13 ± 0.00	0.11 ± 0.00	0.11 ± 0.01	0.27 ± 0.00	0.18 ± 0.01	5.37 ± 0.02	38.11 ± 1.63
Oecr	0.09 ± 0.00	0.30 ± 0.00	0.20 ± 0.01	0.13 ± 0.00	0.08 ± 0.00[f]	0.06 ± 0.00	0.17 ± 0.02	0.09 ± 0.00	3.39 ± 0.01	43.51 ± 0.40
Osej	0.10 ± 0.00	0.33 ± 0.00	0.23 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.05 ± 0.00	0.15 ± 0.00	0.10 ± 0.01	3.46 ± 0.02	44.41 ± 1.68
Mean	0.09 ± 0.02	0.33 ± 0.07	0.21 ± 0.04	0.12 ± 0.03	0.08 ± 0.02	0.07 ± 0.02	0.16 ± 0.05	0.10 ± 0.03	3.47 ± 0.73	44.30 ± 1.42
F-value	137.54	196.04	176.18	511.41	81.18	6.51	543.25	394.61	48.65	257.08
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Effect	S	S	S	S	S	S	S	S	S	S

In total amino acids, the EAAs in olive stones have an amount of 44.30% and 47.48%, respectively for wild and cultivated olive stones. The glutamic acid is the predominant amino acid followed by aspartate acid and leucin (Table 4). In olive cultivars stones, the total amino acids ranged between 1.47 (Besbessi) and 11.24 % (Sayali) with mean value of 3.9%. For the wild stones, the lower amino acids percentage was 2.63% (Ojer) and the highest was 5.37 % (Olaa) with mean value of 3.47%.

Multivariate analyses

The PCA was applied on wild and cultivated olive trees using major fatty acids (C16:0, C18:0, C18:1n-9, C18:2n-6) and essential amino acids (Thr, Val, Phe, Leu, Met, Lys, Ile, His) of olive stone (Figure 1). The two first components PC1 and PC2 explained 70% of variance. The PC1 explained 49.14% of variance and was correlated with major fatty acids. The PC2 explained 20.87% of variance and was correlated with essential amino acids. The PCA plot of wild and cultivated olive trees showed that the stones from the cultivars 'Chétoui', 'Zarras', 'Neb Jmel' and 'Gerboui' and the oleasters 'Odou' and 'Olaa' seemed to be richer in essential amino acids (Figure 1A). However, the stones from cultivars 'Roumi', 'Besbessi', 'Meski', 'Sayali', 'Awam', 'Limi' 'Chemlali' and the oleasters 'Orjb' 'Ojer', 'Obel4', 'Obel3' were richer in oleic acid (C18:1n-9). The plot obtained by PCA analysis showed close relationship between olive cultivars and oleasters (Figure 1A). The cluster analysis based on major fatty acids and essential amino acids from stone confirm the close relationship noted by PCA and showed three clusters (Figure 1B). The first cluster (I) comprised seven cultivars (Neb Jmel, Chemlali, Nib, Limi, Meski, Awam, Sayal,) and nine oleasters (Olaa, Obel3, Obel4, Ojer, Odou, Ozag, Osej, Orjb, Omje). The second cluster (II) grouped two cultivars (Zarras, Chétoui) and the oleaster 'Oecr'. The third cluster (III) comprised three cultivars (Chétoui, Roumi, Besbessi) and tow oleasters

(Obel1, Obel2). Stones including in cluster I have the high oleic acid (C18:1n-9) content (Table 2). Cluster II samples have high C16:0, C18:2n-6: Thr, Met, Leu, Phe, Lys, and Ile content. However, stones of cluster III were characterized by high C18:0 and Val contents (Figure 1B). Some cultivars shared close relationship with oleasters as 'Neb Jmel' cultivar with Olaa oleaster and Nib cultivar with Odou oleaster. Some cultivars are closely grouped as Sayali/Awam, Gerboui/Zarras, Besbessi/Roumi.

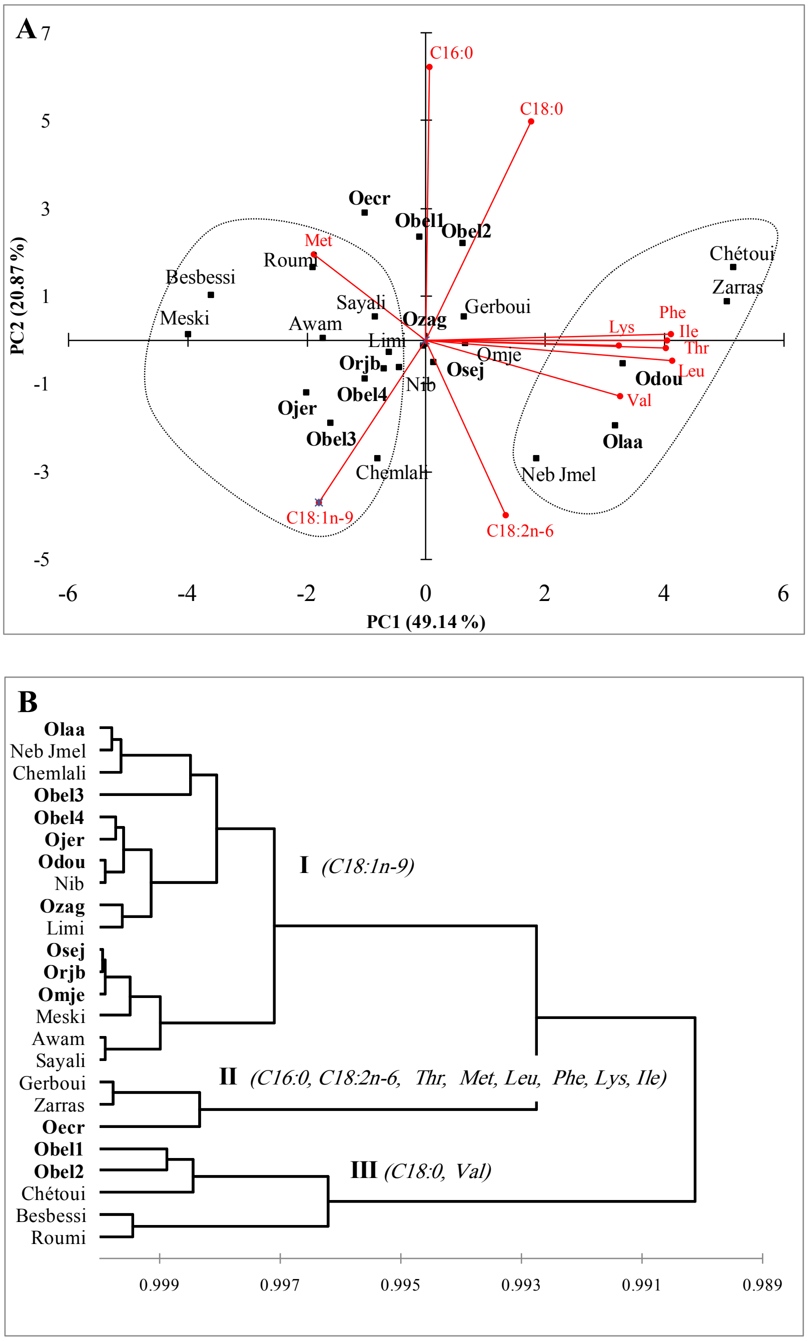

Figure 1. A, Principal component plot of wild (in bold) and cultivated olive trees based on major fatty acids and essential amino acids composition of stone oils; B, Hierarchical cluster analysis of wild (in bold) and cultivated olive trees based on major fatty acids and essential amino acids composition from stone oils. For each group the highly represented compounds are written in italic.

Discussion

Qualitatively, oil extracted from the 24 samples of wild and cultivated olive stones has the same fatty and amino acids profiles. However, quantitatively, significant differences were observed between the olive stone oils. This variation between genotypes could be explained by many factors. In fact, the whole olive oil composition could be influenced by genetic factor (Hannachi *et al.*, 2007; Tura *et al.*, 2007). Others factors as agronomic and technological factors (Hannachi *et al.*, 2007; Tura *et al.*, 2007), the climate (Cerretani *et al.*, 2006; Lazzez *et al.*, 2008) crop season (Rodney *et al.*, 2010) would influence the olive oil composition. In other hand, the geographical area is greatly responsible for the specific characteristics of olive oil (Hannachi *et al.*, 2007; Petrakis *et al.*, 2008). Therefore, herein, the significant variation of olive stone oils would be explained by genetic effect, technical practices and/or the geographic origin climate. Stone oils from two olive taxa would contribute on good fatty acids composition of olive oil because its richness on oleic acid followed by linoleic and palmitic acids characterizing the olive oil. It has been reported that about 90% of the oil of the olive drupe is produced by the mesocarp and 10% is produced by the seed containing lipid reserves and develops in parallel with the drupe (Salas *et al.*, 2000).

Seventeen familiar amino acids were detected on stones of both wild and cultivated olive trees. The amino acids extracted from olive stones would to be a source of EAAs since their percentage exceeded 40% in both variety types. Previous studies reported that sixteen amino acids were detected in olive including the EAAs (Lazovic *et al.*, 1999). Fernández (1960) reported the presence of all essential amino acid in olive seeds. The olive stone is a source rich of valuable components due its chemicals and physical properties in addition to its combustion heat. Likewise, the amino acids profiling was a complementary tool used to characterize seeds of pomegranate (Elfalleh *et al.*, 2012) and grapevine (Asensio *et al.*, 2002). The total amino acid content of grapevine seeds for example varied according to many factors as cultivars (Henscke & Jiranek, 1992), climate, nitrogen fertilization (Asensio, 2000) and grape ripeness (Asensio *et al.*, 2002). In general, the composition of chemical substances may vary according to the plant nutrition conditions, cultural practices and genetic factors (Vasconcelos *et al.*, 1997). In this study, we used two botanical olive varieties from the natural ecosystem and agro-ecosystem. In their natural ecosystems, wild olive does not have benefited to any technical practices contrary to olive cultivars. Consequently, the biochemical variation observed would be also explained by the technical practices added to the genetic and environmental factors.

In previous studies, morphological traits have been used for olive trees description and for assessment of existing diversity of olive cultivars and oleasters (Barranco *et al.*, 2000; Hannachi *et al.*, 2017). In order to complement the morphology descriptions, biochemical analyses has been used to characterized olive cultivars basing on isoenzyme (Trujillo *et al.*, 1995; Lumaret *et al.*, 2004) and molecular markers (Breton *et al.*, 2006 a, b; Hannachi *et al.*, 2009; Hannachi *et al.*, 2010; Belaj *et al.*, 2011). Herein, the fatty and amino acid compositions of olive stone from cultivars and oleasters were used as complementary tool to assess the relationship between wild and cultivated olive trees as noted by the plot obtained by multivariate analyses (Figure 1). Our results showed the gathering of some wild and cultivated olive trees on the same cluster basing on fatty and amino acids as previously testified using morphological or molecular markers (Angiolillo *et al.*, 1999; Bronzini de Caraffa *et al.*, 2002; Hannachi *et al.*, 2008a, b; Hannachı *et al.*, 2016). The close relationship between wild and cultivated olive trees would be explained by gene flow, which has been continued between these two olive forms (Lumaret & Ouazzani, 2001).

Fatty and amino acids extracted from stones of wild and cultivated olives showed close relationship between the two studied taxa. The wild olive stones were source of interest components. More attention is required for the special conservation of wild species. Therefore, these wild trees could serve as potential source of oil with interesting composition close to olive cultivars. Populations of wild olive trees are restricted to a few isolated areas of native forest from Mediterranean region, where pollen and or stones may be distributed by the wind and/or bird (Lumaret *et al.*, 2004). Compared to other crops, olive trees have been little modified by selective breeding. Olive cultivars are heterozygous clones selected from spontaneous, from uncontrolled crosses or are escaping from olive vergers constituting the feral forms. In addition, one of the potential solutions for the conservation measures is to convert the wild plant residues for industrial use (Benabderrahim *et al.*, 2019; Elfalleh *et al*., 2019).

Conclusion

The amino and fatty acid compositions of olive stone showed separation of some olive cultivars (cultivated olive trees) and oleasters (wild olive trees). However, close relationship between some oleasters and olive cultivars were found. In addition, olive stones of oleasters and cultivars seems to be source of valuables components. These data would be used as complementary metabolomic tool for the olive trees characterization. This description constitutes a crucial step in conservation strategy for maintaining genetic diversity within olive taxa and to avoid genetic erosion of native olive resources. This native gene pool reservoir could provide adaptation to specific conditions and would be a source of interest gene to build genetic improvement of olive cultivars.

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