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Quality index based on fatty acids for Syrian pistachio cultivars (*Pistacia vera* L.) grown in Mascara (North-West of Algeria)

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Abstract: Pistachio (*Pistacia vera* L.) is one of the most important hard-shelled nuts all over the world. Pistachio has a very high nutritional value with its quality index based on fatty acids composition, protein, mineral, vitamin-E, and antioxidant contents. In the current study, fatty acids values of the Syrian pistachio varieties cultivated in Algeria ('Adjmi', 'Bayadhi', 'Batouri', 'Achouri,' and 'Neb-djemel') were detected for the first time. Oil extraction of cultivars was performed using n-hexane in a Soxhlet apparatus, and the fatty acids composition of the oil was analyzed by gas chromatography coupled with a flame ionization detector (GC/FID) in the methyl ester form. The fatty acid composition of the pistachio cultivars was detected as: palmitic acid (8.23 % ± 0.36 to 9.49 % ± 0.07), palmitoleic acid (0.10 % ± 0.02 to 0.62 % ± 0.24), stearic acid (0.67 % ± 0.04 to 1.40 % ± 0.18), oleic acid (56.35 % ± 2.13 to 61.90 % ± 1.07), linoleic acid (19.48 % ± 0.27 to 26.76 ± 0.55) and linolenic acid (0.390 % ± 0, 03 to 0.59 % ± 0.01) in all samples. The results demonstrated that the five pistachio cultivars were rich in monounsaturated fatty acids (MUFA) (56.9 7% ± 1.88 to 62.10 % ± 1.02) and moderately low in saturated fatty acids (SFA) (9.90 % ± 0.04 to 10.37 % ± 0.23). 'Adjmi' has fatty acid composition lower than the other cultivars statically. Oleic acid value was determined higher than other fatty acid components. In the current study, the fat and fatty acid components of pistachio cultivars were determined and the results of this study can be used for future pistachio breeding programs.

Key words: kernels; pistachio; fatty acids composition

Na osnovi maščobnih kislin postavljen kakovostni indeks sirskih sort pistacije (*Pistacia vera* L.), ki se gojijo na območju Mascare (Severozahodna Alžirija)

Izvleček: Pistacija (*Pistacia vera* L.) je ena izmed najpomembnejših vrst oreškov, ki se goji širom po svetu. Ima zelo veliko hranilno vrednost, katere kakovostni indeks temelji na sestavi maščobnih kislin, beljakovin, mineralov, vitamina E in vsebnosti antioksidantov. V raziskavi je bila prvič preučena sestava maščobnih kislin v sortah sirske pistacije, ki se gojijo v Alžiri ('Adjmi', 'Bayadhi', 'Batouri', 'Achouri,' and 'Neb-djemel'). Ekstrakcija olja je bila narejena z n-heksanom v Soxhletovem aparatu, sestava maščobnih kislin v olju je bila analizirana s plinsko kromatografijo povezano s plamenskim ionizirajočim detektorjem (GC/FID) in metil estrom. V vseh vzorcih je bila ugotovljena naslednja sestava maščobnih kislin v olju pistacije: palmitinska kislina (8,23 % ± 0,36 do 9,49 % ± 0,07), palmitinsko-oleinska kislina (0,10 % ± 0,02 do 0,62 % ± 0,24), stearinska kislina (0,67 % ± 0,04 do 1,40 % ± 0,18), oleinska kislina (56,35 % ± 2,13 do 61,90 % ± 1,07), linoleijska kislina (19,48 % ± 0,27 do 26,76 ± 0,55) in linolenska kislina (0,390 % ± 0,03 do 0,59 % ± 0,01). Rezultati so pokazali, da je bilo olje vseh petih sort pistacije bogato na enkrat nenasičenih maščobnih kislinah (MUFA) (56,97 % ± 1,88 do 62,10 % ± 1,02) in sorazmerno revno na nasičenih maščobnih kislinah (SFA) (9,90 % ± 0,04 do 10,37 % ± 0,23). Sorta Adjmi je imela statistično slabšo sestavo maščobnih kislin, z večjo vsebnostjo oleinske kisline. Ugotovljeno je bilo, sa so oreški teh sort pistacije bogati na olju in maščobnih kislinah in da bi te sorte lahko uporabili v prihodnjih žlahtniteljskih programih.

Ključne besede: jedrca; pistacija; sestava maščobnih kislin

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1 INTRODUCTION

Pistacia genus has a dioecious flower habitat except for a few monoecious ones belonging to the order Sapindales, the family Anacardiaceae (Oukabli, 2005). It is included in more than 11 *Pistacia* species ranging from bush to tree form (Kafkas, 2006). *Pistacia vera* L. (pistachio) is the only *Pistacia* species in which nuts are edible and nutritional values are quite high since they are rich in fat and fatty acids ranging from 47.65 % to 63.31 % of the dry mass of the nuts (Agar et al., 1998., Yıldız et al., 1998; Küçüköner et al., 1998; Küçüköner and Yurt, 2001; Satil et al., 2003).

Several findings in the literature were reported regarding the biochemical composition of pistachio cultivars. Pistachio seeds contains 55.2-60.5 % oil, 15.0-21.2 % protein, and 14.9- 17.7 % carbohydrate and has a structure of fiber (10.3 g 100 g^{-1}), and 100 g of pistachio has 600 calories and it is one of the richest sources of energy (Krentz et al., 1994; Franz et al., 2002; Shahraki et al., 2014). The 100 g of pistachio nuts includes various micronutrients such as 4.0 mg sodium, 494-514.5 mg phosphorus, 120-150 mg calcium, 1,048-1,142 mg potassium, 494-514.5 mg phosphorus, 1.0-1.4 mg copper, 5.8-11.4 mg iron, 9.3 mcg selenium and 157.5-165.0 mg 100 g^{-1} magnesium (Franz et al., 2002; Shahraki et al., 2014). Pistachios are a serious nutritional source in terms of lutein and zeaxanthin (1,205 g 100 g^{-1}), vitamin B-6 (1.3 mg/100 g^{-1}), tocopherols (22.5 mg/100 g^{-1}), and carotenes (157g/100 g^{-1}), total phytosterols (279 mg /100 g^{-1}), sitosterol (210 mg/100 g^{-1}) (Kornsteiner et al., 2006; Bhagwat et al., 2008), and isoflavones (3.63 mg/100 mg) (Seeram et al., 2006; Gentile et al., 2007; Ballistreri et al., 2009).

The biggest producer countries all over the world were the US, Turkey, Iran, China, and Syria, respectively. Pistachio production in the world in 2020 was calculated as 1,205,532 Metric Tons (MTs). The highest production took place by the USA with 474,004 productions. Following, pistachio production was reported as 296,376 MT in Turkey, 190,000 MT in Iran, 80,000 MT in China, and 69,403 MT in Syria (Faostat 2022).

There are many cultivars that adapt well to the cultivated regions in pistachio (Karcı et al., 2022). There are a lot of pistachio cultivars in Iran, Turkey, and the US. Also, Syria is one of the oldest pistachio-growing countries and several cultivars grown in Syria were used commercially such as, 'Ajamy', 'Red Jalab', 'Adjmi', 'Bayadhi', 'Batouri', 'Neb-djemel', 'Bundouky', 'Marawhy', 'Lazwardy', 'White Oleimy', 'Nab Al-Djamal', 'White Jalab', 'Antaby', and 'Ein El-Tainah' (Kafkas, 2019). The oil composition of pistachio cultivars depends on environmental factors such as climate, geography, and soil type as well as cultivars (Chahed et al., 2007). There are serious differences in the biochemical traits of cultivars due to the climate conditions of the regions, because cultural application, rootstock, maturity at harvest, and moisture content also affect fat and fatty acid compositions.

Pistachio is a rich source of fixed oil and contains fatty acids such as oleic and linoleic acid, which are necessary for human nutrition (Garcia et al., 1992; Küçüköner and Yurt, 2003). The previous findings reported about positive effects on cardiovascular health, with significant reductions in total cholesterol (TC) and a dose-response improvement in TC/HDL, LDL/HDL, and non-HDL/HDL ratios (Sari et al., 2010; Koçyiğit et al., 2006; Gebauer et al., 2008). On the other hand, a pistachio-enriched diet is stated that to be an alternative drug agent and an effective hypoglycemic agent to protect against the prediabetic condition (Hernández-Alonso et al. 2014).

Several papers reported related to the detection of fat and fatty acids of pistachio cultivars exception of Syrian pistachio cultivars (Satil et al., 2003; Abdoshahi et al., 2011; Rabadan et al., 2018; Esteki et al., 2018; Pourian et al., 2019; Yahyavi et al., 2020). However, fat contents of Syrian pistachio cultivars have not been reported, to date. Thus, the results will be useful to provide a basis for the selection of parents and population construction, detection of the biochemical traits of all cultivars in the development of novel promising genotypes in terms of biochemical properties in future pistachio breeding.

Here, the objectives of this study were to identify the composition of the fat and fatty acids of Syrian pistachio cultivars. The fat and fatty acids results in Syrian cultivars can be used for future pistachio breeding programs.

2 MATERIALS AND METHODS

2.1 STUDY AREA

The study area is located at an altitude of 490 m above sea level with geographical landmarks (Latitude: 35°.22'N, Longitude: 00.11' 07"E). The climate conditions have a semi-arid Mediterranean climate which has a cool winter and hot summer. The annual rainfall was 380 mm irregularly distributed over the growing season. The soil of the plantation is sandy loam in texture with an alkaline pH (Figure 1).

2.2 PLANT MATERIAL

Pistachio samples ('Adjmi', 'Bayadhi', 'Batouri', 'Achouri' and 'Neb-djemel') were collected from experimental orchard of the Mascara University (Algeria) at

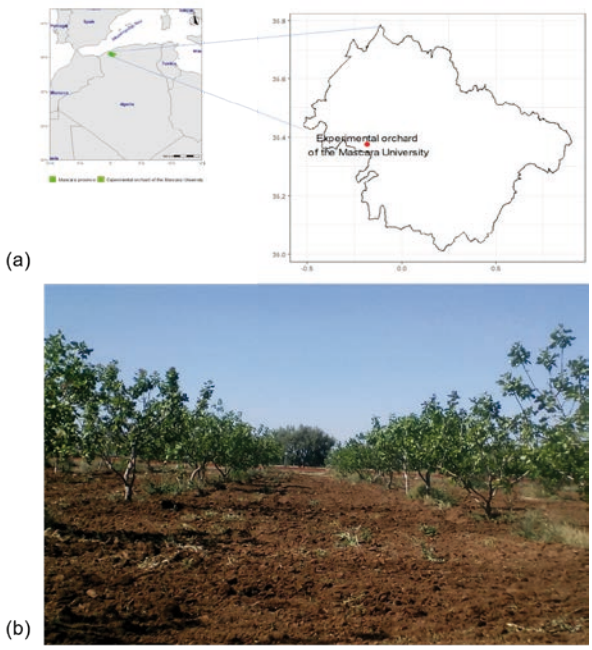

Figure 1: (a) Geographical location of pistachio cultivars of Syrian origin, (b) Pistachio orchard at the Mascara University experimental fields

harvest time. Pistachios' fruit exocarp tissues were removed and fruits were dried at room temperature condition. The samples were kept in a shell for one month at room temperature in the dark until analysis.

2.3 OIL EXTRACTION

Seed oil extraction was performed based on the Bligh and Dyer method (1959). Oils of 5 g nuts were extracted using hexane solvent for 2 h using automatic Soxhlet equipment (Gerhardt Soxtherm) and triplicate analysis was reported for each cultivar. The residue was dried until a constant mass was observed. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (FAMEs) (AOAC, 1990).

2.4 FATTY ACIDS ANALYSIS

Fatty acids were analyzed using a Clarus 500 Gas Chromatography with an autosampler (Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector and a fused-silica capillary SGE column (100 m × 0.32 mm, ID 0.25 µm, BP20 0.25 UM; Perkin Elmer, Austin, TX, USA). The oven temperature was held at 140 °C for 5 min, and then raised to 200 °C at a rate of 4 °C min^{-1} and then to 220 °C at a rate of 1 °C min^{-1}, while the injector and the detector temperatures were set to 220 and 280 °C respectively. The sample volume was 1 µl, and the carrier gas was controlled at 16 psi. The split ratio was 1: 100. Fatty acids were detected by comparing their retention indices of the FAMEs with a standard 37- component FAME mixture (Supelco, Bellefonte, PA, USA).

2.5 STATISTICAL ANALYSIS

The Shapiro-Wilk test, using the shapiro.test function (R Core Team, 2020), showed satisfied data normality (p = 0.14). Analysis of variance (ANOVA) calculations was done using the l m function in R (R core Team, 2020). Tukey's post hoc Honesty Significance Difference (HSDT) test was applied to detect the source of the differences, with the help of the agricolae R package (Mendiburu, 2020).

2.6 PRINCIPAL COMPONENT ANALYSIS (PCA)

Principal Component Analysis (PCA) is an extremely powerful tool for synthesizing information, very useful when there is a large amount of quantitative data to process and interpret. Experimental data can be identified by PCA, which plays an important role in reducing the dimensionality of a large number of interdependent variables into a new set of uncorrelated variables called principal components (PC). PCA is applied to series to construct several groups according to similar variance properties at different time scales. The first PC represents the highest variance in the original variable, followed by the second, third, and other components. This method provides useful information using a smaller set of variables and is relatively easy to interpret. The objectives of a PCA are as follows (Westra et al., 2007); (i) the individuals' graphic representation, in a 2-dimensional plane, showing the similarities between them, (ii) the variables' graphic representation, on the same level by explaining at best the initial connections between them. Principal component analysis (PCA) was applied to data using the FactoMineR R package (Le et al., 2008).

3 RESULTS AND DISCUSSION

3.1 OIL CONTENT

The detected oil content values of Syrian cultivars in this study were ranging from 50.23 % to 65.42 %, while the oil content of Iranian varieties such as 'Badami', 'Ohadi', and 'Mumtaz' varies from 58.96 % to 60.10 %

Table 1: The oil content (%) and fatty acid composition (%) of the five varieties of pistachio

Cultivars	Oil Content	Myristic C14:0	Palmitic C16:0	Stearic C18:0	Total SFA	Palmitoleic C16:1	Oleic C18:1	Total MUFAs	Linoleic C18:2	Linolenic C18:3	Total PUFAs
Adjmi	50.23 ± 0.43	0.43 ± 0.01	8.23 ± 0.36	1.40 ± 0.18	10.06 ± 0.17	0.62 ± 0.24	56.35 ± 2.13	56.97 ± 1.88	19.87 ± 1.53	0.57 ± 0.08	20.45 ± 1.46
Bayadhi	65.42 ± 1.05	0.3 ± 0.12	9.20 ± 0.04	0.79 ± 0.07	10.29 ± 0.14	0.20 ± 0.04	61.90 ± 1.07	62.10 ± 1.02	20.98 ± 0.12	0.47 ± 0.01	21.45 ± 0.12
Batouri	57.29 ± 1.94	0.24 ± 0.01	9.49 ± 0.07	0.64 ± 0.17	10.37 ± 0.23	0.45 ± 0.02	60.46 ± 0.66	60.46 ± 0.66	19.48 ± 0.27	0.48 ± 0.01	19.96 ± 0.26
Achouri	63.13 ± 2.09	0.28 ± 0.07	8.73 ± 0.07	0.97 ± 0.02	9.98 ± 0.16	0.21 ± 0.04	60.41 ± 0.61	60.41 ± 0.61	20.73 ± 0.28	0.59 ± 0.01	21.32 ± 0.29
Neb-djemel	55.92 ± 2.49	0.21 ± 0.07	9.01 ± 0.15	0.67 ± 0.04	9.90 ± 0.04	0.10 ± 0.02	57.24 ± 0.33	57.24 ± 0.33	26.76 ± 0.55	0.39 ± 0.03	27.15 ± 0.59

(Kamangar et al., 1975). On the other hand, the researchers reported that the fat contents of five Iranian cultivars were calculated ranged from 52.48 to 60.65 % (Abdoshahi et al., 2011). A total of 17 pistachio cultivars from different locations in Iran were characterized according to fatty contents and they were determined ranged from 49.9 to 58.5 % (Yahyavi et al., 2020). In addition to different traits of cultivars, the climatic conditions of the cultivation region and the maintenance of the pistachio orchards could be the causes of the difference in the fat amount (Chahed et al., 2008). The fat content and fatty acids compositions of five pistachio cultivars ('Adjmi', 'Bayadhi', 'Batouri', 'Achouri' and 'Neb-djemel') were given in Table 1.

3.2 FATTY ACIDS COMPOSITIONS

The kernels of pistachio are rich in monounsaturated fatty acids followed by polyunsaturated fatty acids and saturated fatty acids. Although the highest percentage of MUFA was detected as 56.97 % in 'Adjmi', the lowest percentage of MUFA was calculated as 62.10 % in 'Bayadhi'. The lowest and the highest amount of PUFA were identified as 19.96 % and 27.15 % in 'Batouri' and 'Neb-djemel', respectively. The average content of unsaturated fatty acids was determined as 76.93 % in this study, while this value was found as 81.5 % of the total fatty acids in Iranian cultivars (Kamangar et al., 1975). The percentage of SFA were ranged between 9.90 and 10.37 %, and found in 'Neb-djemel' and 'Batouri' cultivars, respectively. The ratio of unsaturated/saturated acids in the kernel of these pistachio cultivars varied from 7.69 to 8.52 with an average of 8.05 (Table 2). This ratio was determined as 7.90 in Turkish cultivars and was found to be similar to the results of this study (Agar et al., 1995).

Table 2: The total saturated and unsaturated fatty acids content of pistachio cultivars

Cultivars	Saturated fatty acids (%)	Unsaturated fatty acids (%)	Unsaturated/ saturated
Adjmi	10.06 ± 0.17	77.42 ± 3.34	7.69
Bayadhi	10.29 ± 0.14	83.55 ± 1.14	8.11
Batouri	10.37 ± 0.23	80.42 ± 0.92	7.75
Achouri	9.98 ± 0.16	81.73 ± 0.9	8.18
Neb-djemel	9.9 ± 0.04	84.39 ± 0.92	8.52

One of the main fatty acid components of pistachio cultivars, oleic acid, was found in ranging from 56.35 to 61.90 %. This value was detected as slightly lower in Iranian cultivars (48.96-55.24 %) (Abdoshahi et al., 2011). On the other hand, while linoleic acid values were calculated (19.48-26.76 %) significantly lower than Iranian cultivars (30.48- 36.88 %), palmitic acid values were identified (8.23–9.49 %) similar to another research (Abdoshahi et al., 2011). The previous report demonstrated that high oleic content and lower levels of linoleic acid content make nut oil more stable against oxidative changes (Küçüköner and Yurt 2003). Shakerardekani et al. (2015) reported that pistachio is highly susceptible to lipid oxidation due to its high oil content, however that high oleic and palmitic oil content increased the oxidation stability.

The myristic (0.21-0.43 %), stearic (0.64-1.40 %), palmitoleic (0.10-0.62 %) and linolenic (0.39-0.59 %) were detected in traces in the current study. The previous findings related to fatty acids supported that oleic acid was the most common monounsaturated fatty acid, while linoleic acid was the most common polyunsaturated fatty acid in pistachio (Shokraii, 1977; Garcia et al., 1992; Kafkas et al., 1995; Agar et al, 1995; Okay, 2002; Satil et al., 2003; Esteki et al., 2019) and the similar results were found in this study.

The two-factor ANOVA (Cultivar and Type) dem-

Table 3: The results of two-way ANOVA analysis

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Cultivars	4	8.51	2.13	7.71	0.0001
Type	6	28418.79	4736.47	17150.98	3.60E-59
Cultivar:Type	24	107.44	4.48	16.21	1.23E-12
Residuals	35	9.67	0.28		

onstrated that there was a significant difference between cultivars ($p < 0.001$), types ($p < 0.001$). And, this analysis showed the existence of a significant interaction between cultivar and type ($p < 0.001$) (Table 3).

To detect the source of the differences, Tukey's post hoc Honesty Signicance Difference (HSDT) test was applied with the help of the agricolae R package (Mendiburu, 2020). The 'Neb-djemel', 'Bayadhi', 'Achouri' and 'Batouri' pistachio cultivars generated close results, while "Adjmi" cultivar created relatively different result from the others (Table 4).

The Tukey test revealed that the highest fatty acid compositions were identified as O leic.C18:1 (59.12), while the lowest results were calculated in Stearic.C18:0, Linolenic.C18:3, Palmitoleic.C16:1, Myristic.C14:0, respectively. The most efficient interaction was determined between 'Bayadhi', 'Achouri', 'Batouri' pistachio cultivars. On the other hand, the highest linoleic fatty acid composition was calculated as 26.76 in 'Neb-djemel' and the lowest linoleic value was found in 'Batouri' pistachio cultivar. Although the highest and the lowest palmitic acid compositions were detected as 9.49 and 8.24 in 'Batouri' and 'Adjmi', respectively. Although the lowest linolenic, myristic and palmitoleic fatty acid values were calculated as 0.40, 0.22 and 0.11 in 'Neb-djemel', respectively, the highest myristic, palmitoleic and stearic fatty acids were produced as 0.43, 0.62 and 1.40 in 'Adjmi' pistachio cultivar, respectively (Table 5).

Table 4: The results of multiple comparisons between cultivars with Tukey's test

Cultivars	Value	Groups
Neb-djemel	13.47	a
Bayadhi	13.41	a
Achouri	13.10	a
Batouri	12.97	ab
Adjmi	12.50	b

3.3 PRINCIPAL COMPONENT ANALYSIS OF FATTY ACIDS

Principal Component Analysis (PCA) is very helpful for processing and interpretation large amounts of quantitative data. It is a very important analysis in reducing the dimensionality of a large number of interdependent variables. The PCA analysis can illustrate the individuals' values, in a 2-dimensional plane, using the similarities or differences between them. In the present study, PCA was performed using the FactoMineR package (Le et al., 2008). The percent 86.47 of the total variability was explained by axes 1 and 2. The projection of the variables demonstrated that Linolenic.C18:3, Palmitoleic.C16:1, Myristic.C14:0 and Stearic.C18:0 were correlated significantly with axis 1, while Oleic.C18:1 (Linoleic.C18:2) was correlated (inversely correlated) with axis 2 (Figure 2).

The average of fatty acids belonging to all cultivars were calculated and grouped according to letters. Oleic.C18:1, Linoleic.C18:2 and Palmitic.C16:0 fatty acids had different groups from others, a, b, c; respectively (Table 5, Figure 2). However, statistically significant?? differences were detected between cultivars in Oleic.C18.1 and Linoleic.C18:2 fatty acids. The Oleic.C18:1 values of the cultivars were classified in two groups such as a and b. These values were determined similar in Batouri, Achouri and Bayadhi pistachio cultivars, while Adjmi and Neb-djemel were replaced in same group according to Oleic.C18:1 values of them. The Linoleic.C18:2 fatty acid values of Neb-djemel were calculated as higher than other pistachio cultivars and found different statistically. Adjmi and Neb-djemel pistachio cultivars were separated from the others due to differences among all pistachio cultivars according to Oleic.C18:1 and Linoleic.C18:2 fatty acids values in the analysis of PCA (Figure 3).

4 CONCLUSION

In the present study, fatty acid compositions of Syrian pistachio cultivars were determined. The 'Bayadhi' and 'Achouri' cultivars have the highest oil content, while 'Adjmi' has the lowest oil content. It determined that pistachio is rich in monounsaturated fatty acids such as, oleic acid and quite poor in saturated fatty acids. The highest SFA, MUFA, and PUFA values were detected in 'Batouri', 'Bayadhi', and 'Neb-jemel' pistachio culti-

Table 5: The results of multiple comparisons between cultivars-type with the Tukey's test

Cultivars	The fatty acids	Values	Groups	Average Values	Groups
Batouri	Oleic.C18:1	60.01	a	59.12	a
Achouri		60.21	a		
Bayadhi		61.90	a		
Adjmi		56.36	b		
Neb-djemel		57.13	b		
Neb-djemel	Linoleic.C18:2	26.76	c	21.56	b
Batouri		19.49	d		
Adjmi		19.88	d		
Achouri		20.73	d		
Bayadhi		20.98	d		
Adjmi	Palmitic.C16:0	8.24	e	8.93	c
Achouri		8.73	e		
Neb-djemel		9.02	e		
Bayadhi		9.20	e		
Batouri		9.49	e		
Neb-djemel	Linolenic.C18:3	0.40	f	0.50	d
Bayadhi		0.47	f		
Batouri		0.48	f		
Adjmi		0.58	f		
Achouri		0.59	f		
Neb-djemel	Myristic.C14:0	0.22	f	0.29	d
Batouri		0.24	f		
Achouri		0.29	f		
Bayadhi		0.30	f		
Adjmi		0.43	f		
Neb-djemel	Palmitoleic.C16:1	0.11	f	0.32	d
Bayadhi		0.20	f		
Achouri		0.21	f		
Batouri		0.46	f		
Adjmi		0.62	f		
Batouri	Stearic.C18:0	0.64	f	0.89	d
Neb-djemel		0.67	f		
Bayadhi		0.80	f		
Achouri		0.97	f		
Adjmi		1.40	f		

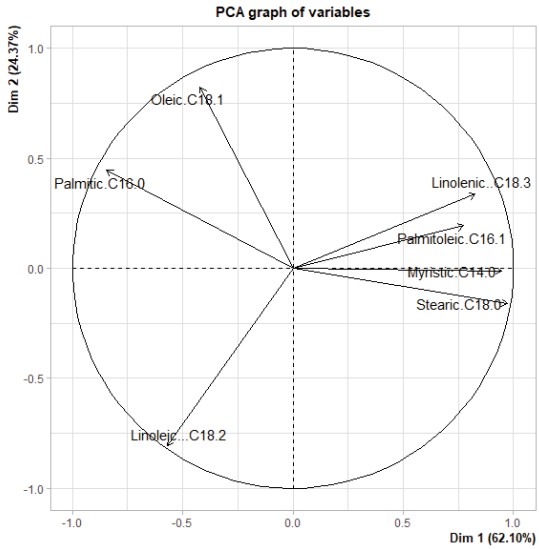

Figure 2: The projection of variables in the 1x2 plan

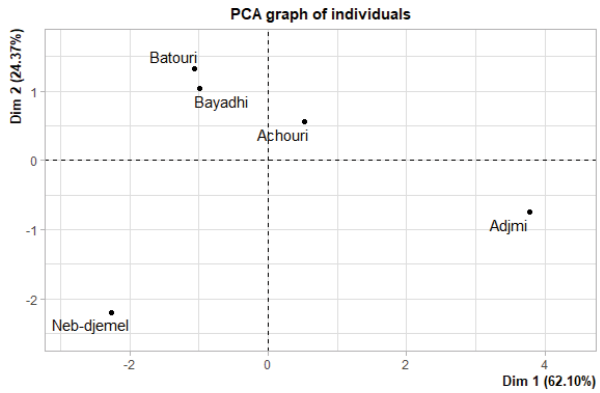

Figure 3: The projection of cultivars in the 1x2 plan

vars, respectively, and the lowest in 'Neb-jemel', 'Adjmi', and 'Batouri' cultivars. These results demonstrated that pistachio has a satisfying ratio of unsaturated and saturated fatty acids that provide superior nutritional value for consumers. As a result, the results obtained from this study are important in terms of using them for future pistachio breeding studies.

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