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Seed oil content and fatty acid profiles of endemic Phoenix theophrasti Greuter, *Phoenix roebelenii* O'Brien, *Phoenix caneriensis* Hort. Ex Chabaud, and *Phoenix dactylifera* L. grown in the same locality in Turkey

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Abstract: Plants-based oils and fats usually obtained from seeds have been indispensable substances for mankind, both in nutritional and industrial respect. This study aimed to determine the total amount of oil and the fatty acid compositions of *Phoenix theophrasti* Greuter, *P. roebelenii* O'Brien, *P. caneriensis* Hort. Ex Chabaud, and *P. dactylifera* L. The seeds of these four *Phoenix* species were collected from the same location, east Mediterranean agroecological conditions when the fruits were fully ripened, which means the environmental factors that may affect the oil content and the fatty acid compositions were controlled. The highest oil content of the samples was obtained from *P. caneriensis* (8.435 g/100 g) and followed by *P. dactylifera* (6.400 g/100 g), *P. roebelenii* (5.130 g/100 g), and *P. theophrasti* (4.730 g/100 g), respectively. The dominant fatty acids detected in the seed oils were oleic acid (C18:1n9c, 35.080–46.625%), lauric acid (C12:0, 26.160–18.055%), myristic acid (C14:0, 12.670–10.335%), linoleic acid (Cl8:2n6c, 13.295–7.990%), and palmitic acid (C16:0, 11.355–7.590%) for all four species. To the best of the knowledge, this study is the first to reveal the total oil amount of the seeds of *P. theophrasti* and *P. roebelenii*, and also the fatty acid composition of *P. roebelenii*.

Keywords: Phoenix, seed oils, fatty acids, agroecology

1. Introduction

Many of today's environmental challenges can be addressed through the use of naturally grown plants in different agroecological conditions around the world. Naturally grown plants hold soil in place, protect stream banks and shores, filter pollutants and offer food for livestock and cover for wildlife. They heal the land after wildfire and mining, floods, and drought. They beautify our surroundings. Sustainable agricultural practices are intended to protect the environment, expand the Earth's natural resource base, and maintain and improve soil fertility. Based on a multipronged goal, naturally grown plants gained more importance in sustainable agriculture (Kaskoniene et al., 2020; Subasi et al., 2020; Dogan et al., 2014a).

As a member of the Arecaceae family, the genus *Phoenix* contains 14 species (WCSP, 2015). The natural habitat of *Phoenix* reaches from West Africa to Hong Kong, including the Canary Islands, subtropical and tropical Africa, some regions of the Mediterranean Basin, the Arabian Peninsula, India, and Indochina. These areas may vary from sea level sandy scrublands to 2000 m asl pine forest underbrush, to semiarid areas, to mangrove forests (Barrow, 1998). This old World genus has found a large area of usage since the ancient ages. *Phoenix* palms have been utilized for purposes such as food, religion, construction, and ornamentation (Gros-Balthazard, 2013).

This study mainly aimed to determine the fatty acid compositions of *P. theophrasti* and *P. roebelenii*. Besides these, the fatty acid compositions of *P. dactylifera* and *P. caneriensis* were also determined for comparison. Among the *Phoenix* species, *P. dactylifera* is one of the most well-known and cultivated members of the family in Western Persia, Arabia, and North Africa. Its long life and high nutritional and medicinal value make *P. dactylifera* vital for the native populations in these areas. Moreover, it is also used as animal feed. In addition to all these, the survival ability of *P. dactylifera* in deserts makes it an ideal plant to counter the oncoming climate change effects (Fayadh and Al-Showiman., 1990; Akbari et al., 2012). *P. caneriensis* is native to the Canary Islands (Nehdi et al., 2010). Due to their high levels of minerals and carbohydrates, the seeds are used as animal feed. Additionally, the oil of fully-ripened date seeds contain desirable physicochemical features. It is edible cooking oil and it is also used for industrial, pharmacological (Nehdi et al., 2010) and

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ornamental purposes (Akbari et al., 2012). *P. theophrasti* was first reported in 1967 as distinct from *P. dactylifera* (Greuter, 1967). The main differences of *P. theophrasti* from *P. dactylifera* are its smaller fruits and erect fruit clusters (Garcia-Granero et al., 2020). It was considered as endemic to Crete, but this endemic area has extended, including some southwestern coastal areas of Turkey (Datça Peninsula, Kumluca-Karaöz, Gölköy, Eksera, and Patara) (Vardereli et al., 2019). Moreover, a new subspecies named, *P. theophrasti* Greuter ssp. *Gölköy* was discovered in Bodrum (Boydak, 1985; 1986; Parlak and Yigit, 2020). Due to its geographical and climate features, Turkey is rich in plant genetic resources (Gecer et al., 2020); however, the natural habitat of *Phoenix* is very narrow. Although it has been neglected for a long time, traditional populations used it for weaving, making spoons, and as a source of food (Garcia-Granero et al., 2020). *P. theophrasti* is a tertiary relict endemic (Vardareli et al., 2019). *P. roebelenii* naturally grows in northern Laos, Vietnam, and areas of Yunnan, in southern China (Barrow, 1994). It is also called Pygmy date palm. *P. roebelenii* has a good functional food potential (Amoros et al., 2014).

In plant cells, the lipids and fatty acids take an active role in structural and metabolic constituents. These lipids and fatty acids are vital components of cell membranes. In a functional fruit cell, they have substantial importance for the compartmental and orderly function of most physical and chemical reactions (Song and Bangerth, 2003). In addition to the internal importance, the lipids and fatty acids are also important due to their quality determining role in terms of health, nutrition, and economy. In other words, due to their nutritive features and their role in the oil industry, the oils obtained from the fruit of some of these species are important (Hirsinger, 1992).

In line with that, the determination of the seed fatty acids composition of the species of *Phoenix* is also necessary. Resulting from its economic and nutritional dominance among others, *P. dactylifera* is the most investigated species (Devshony et al., 1992; Nehdi et al., 2010; Akbari et al., 2012; Rivera et al., 2019; Lieb et al., 2020). On the other hand, the number of studies on *P. theophrasti* is insufficient. Moreover, to the best of the author's knowledge the seed fatty acids composition of *P. roebelenii* has not been investigated. Additionally, *P. caneriensis* was also determined.

In light of the information that the ratio of the saturated to unsaturated fatty acids in date seeds may change among individual varieties (Al-Hooti et al., 1998; Al-Shahib and Marshall, 2003), then factors such as maturity, cultivation standards, watering regime, and climate may also affect the fatty acids composition. The seed samples of four listed *Phoenix* members were collected from the same location (Cukurova University Ali Nihat Gökyiğit Botanical Garden, Adana/Turkey) when the fruits were fully ripened. This enabled control of the abovementioned factors. In other words, this study aimed to reveal the fatty acids composition differentiation among four *Phoenix* members (*P. roebelenii*, *P. theophrasti*, *P. caneriensis* and *P. dactylifera)* by focusing on *P. theophrasti* and also *P. roebelenii* and by controlling external factors that may affect the fatty acid composition.

2. Materials and methods

2.1. Plant material

The seeds of four *Phoenix* species, *P. theophrasti*, *P. roebelenii*, *P. dactylifera* and *P. caneriensis* were collected in 2020 from Çukurova University Ali Nihat Gökyiğit Botanical Garden, Adana, Turkey, when the fruits were fully ripened. The environmental factors that may affect the oil content and the fatty acid compositions were controlled by using the same plantation.

2.2. Oil extraction

An automatic Soxhlet device was used for the extraction of total lipids. Hexane was utilized as the solvent material. Through the extracted oil, the oil percentages of the samples were determined.

2.3. Determination of fatty acids

The fatty acids of *P. theophrasti*, *P. roebelenii*, *P. dactylifera* and *P. caneriensis* were determined through gas chromatography (GC) (Perkin Elmer, Shelton, USA). Chromatographic separation was done by a (30 m × 0.25 mm) column equipped with a flame ionization detector (FID). The oven temperature was 120 °C for 2 min, raised by 5 °C/min to 220 °C, which was then kept constant for 10 min, while the injector was set at 280 °C and the detector temperature was fixed at 260 °C. The results were expressed in GC area % as a mean value and ± standard deviation of triplicate measurements.

2.4. Statistical analysis

The statistics of fatty acids analyses were done using the Kruskal–Wallis H test, the cluster analysis, and the principal component analysis (PCA) methods. By employing the Kruskal–Wallis H test, the differences between *P. roebelenii*, *P. theophrasti*, *P. caneriensis* and *P. dactylifera* were investigated for total oil content, and each dominant fatty acid including total saturated fatty acids (SFA), total MUFA (monounsaturated fatty acids), and total PUFA (polyunsaturated fatty acids). This nonparametric median comparison test was employed due to the nonnormal distribution characteristics of the observations. Additionally, a pairwise comparison was made to observe the individual relationships among the investigated species. Secondly, hierarchical cluster analysis was used to group the observations (the four investigated species of *Phoenix*) through their similarities. Ward's

method was chosen as the linkage method. As an output of hierarchical cluster analysis, dendrograms enable us to see the groups. The third statistical method of this study was the PCA analysis. The biplot graph, which is an output of this analysis, is a practical tool to understand and visualize the interrelationships of observations and variables by generating a first principal component (PC1) and a second principal component (PC2). In biplots, the length of the lines gives the variances of the variables and longer lines point to higher variances. The cosine of the angle and the lines indicate the correlation between the variables. In detail, the closer the angle to 90, or 270 degrees, the lower a correlation is shown, and a correlation of 1 or −1 is approximated by an angle of 0 or 180 degrees (Kohler and Luniak, 2005) while SPSS software was employed for the Kruskal–Wallis H Test and cluster analysis, XLStat software was used for the PCA.

3. Results

The oil contents of the *Phoenix* seeds from the four species were reported in Table 1. The oil content of these species ranged from 4.730 g/100 g to 8.435 g/100 g. In detail, the oil content of the samples can be stated as *P. theophrasti* (4.730 g/100 g), *P. roebelenii* (5.130 g/100 g), *P. dactylifera* (6.400 g/100 g) and *P. caneriensis* (8.435 g/100 g) in ascending order. To the best of our knowledge, this study is the first to reveal the total fat amount of the seeds of *P. theophrasti* and *P. roebelenii*.

A range of saturated and unsaturated fatty acids is presented in all species of *Phoenix* seeds in Table 1. For the

Table 1. Total fat oil and fatty acids content of the seeds of four *Phoenix* species.

	P dactylifera		*P. caneriensis*		*P. theophrasti*		*P. roebelenii*	
Total fat	6.400	±0.080	8.435	±0.165	4.730	±0.165	5.130	±0.160
Caproic acid (C6:0)	0.010	±0.080	0.010	±0.030	0.020	±0.030	0.020	±0.160
Caprylic acid (C8:0)	0.295	±0.000	0.235	±0.000	0.415	±0.000	0.490	±0.000
Capric acid (C10:0)	0.370	±0.005	0.250	±0.015	0.505	±0.015	0.525	±0.000
Lauric acid (C12:0)	22.240	±0.000	18.055	±0.000	23.320	±0.005	26.160	±0.005
Tridecanoic acid (C13:0)	0.040	±0.000	0.040	±0.025	0.040	±0.050	0.050	±0.000
Myristic Acid (C14:0)	12.670	±0.000	11.160	±0.000	10.335	±0.000	12.570	±0.000
Pentadecanoic acid (C15:0)	0.040	±0.000	0.030	±0.010	0.080	±0.035	0.020	±0.020
Palmitic acid (C16:0)	11.355	±0.000	9.675	±0.000	9.980	±0.000	7.590	±0.000
Heptadecanoic acid (C17:0)	0.070	±0.005	0.080	±0.005	0.130	±0.010	0.070	±0.000
Stearic acid (C18:0)	3.030	±0.000	3.230	±0.000	1.920	±0.000	1.720	±0.000
Arachidic acid (C20:0)	0.330	±0.010	0.370	±0.010	0.170	±0.010	0.065	±0.000
Heneicosanoic acid (C21:0)	0.010	±0.000	0.015	±0.000	0.015	±0.000	0.000	±0.005
Behenic acid (C22:0)	0.170	±0.000	0.240	±0.005	0.110	±0.005	0.080	±0.000
Tricosanoic acid (C23:0)	0.030	±0.000	0.030	±0.000	0.030	±0.000	0.040	±0.000
Lignoceric acid (C24:0)	0.100	±0.000	0.140	±0.000	0.090	±0.000	0.100	±0.000
Σ SFA	57.160		48.290		51.890		54.630	
Palmitoleic acid (C16.1)ω−7	0.105	±0.060	0.070	±0.040	0.130	±0.050	0.110	±0.180
Elaidic acid (C18:1n9t)	0.045	±0.005	0.060	±0.000	0.040	±0.000	0.070	±0.000
Oleic acid (C18:1n9c)ω−9	40.390	±0.005	46.625	±0.000	39.735	±0.000	35.080	±0.000
cis-11-Eicosenoic acid (C20:1)	0.250	±0.000	0.370	±0.005	0.280	±0.035	0.225	±0.020
Erusic acid (C22:1n9)	0.000	±0.000	0.000	±0.000	0.000	±0.000	0.010	±0.005
Σ MUFA	40.790		47.125		40.185		35.495	
Linoleic acid (Cl8:2n6c) ω−6	7.990	±0.000	8.950	±0.000	11.940	±0.000	13.295	±0.000
a-Linolenic acid (C18:3n3) ω−3	0.020	±0.000	0.050	±0.020	0.075	±0.020	0.060	±0.005
Σ PUFA	8.010		9.000		12.015		13.355	

Results as mean ± standard deviations of triplicate measurements.

P. dactylifera, P. caneriensis, P. theophrasti and *P. roebelenii* species, the main fatty acids found in seed oil were oleic acid (C18:1n9c, 35.080%–46.625%), lauric acid (C12:0, 18.055%–26.160%), myristic acid (C14:0, 10.335%–12.670%), linoleic acid (Cl8:2n6c, 7.990%–13.295%), and palmitic acid (C16:0, 7.590%–11.355%).

Other fatty acids detected in *Phoenix* seed oil were stearic acid (C18:0), arachidic acid (C20:0), capric acid (C10:0), caprylic acid (C8:0), behenic acid (C22:0), and cis-11-eicosenoic acid (C20:1). The total unsaturated fatty acids (MUFA and PUFA) ranged from 48.80 to 56.125% in all four species. The level of SFA, MUFA and PUFA ranged between 48.290%–57.160%, 35.495%–47.125%, and 8.010%–13.355%, respectively.

The median comparison results of total oil content and for each dominant fatty acid including total SFA, total MUFA, and total PUFA of *P. roebelenii*, *P. theophrasti, P. caneriensis* and *P. dactylifera* are given in Table 2.

The first three columns are related to the results of the independent samples Kruskal–Wallis test. This test showed whether a difference existed among the species. In the case of when at least one difference occurs, the null hypothesis is rejected. But it does not give detail about the individual differences. As a one-step forward analysis, pairwise comparison was made to indicate the individual relationships for each parameter. The yellow lines represent statistically significant differentiation between the groups they connect. Thus, *P. theophrasti* and *P. caneriensis* seeds were differentiated significantly from each other by the means of total oil content. The lauric acid levels of *P. caneriensis* and *P. roebelenii* seeds were found to be significantly different from each other. Similarly, significant differences were detected for the myristic acids levels of *P. dactylifera* and *P. theophrasti*, the palmitic acids levels of *P. roebelenii* and *P. dactylifera*, the total SFA levels of *P. caneriensis* and *P. dactylifera*, the oleic acids and total MUFA levels of *P. caneriensis* and *P. roebelenii*, and the linoleic acids and total PUFA levels of *P. roebelenii* and *P. dactylifera*.

According to the hierarchical cluster analysis given in Figure 1, the four investigated species of *Phoenix* (*P. roebelenii*, *P. theophrasti*, *P. caneriensis* and *P. dactylifera*) were grouped through their similarities. At the first stage, *P. caneriensis* withdrew from the group and the three others created together with a cluster. In the second stage, *P. roebelenii* separated from the group as *P. caneriensis* had in the previous stage. So, the most similar species were *P. theophrasti* and *P. dactylifera*.

Before performing the PCA, the fatty acids observed as less than 1% in all *Phoenix* species were excluded to make the biplot graph clear. According to the PCA results of fatty acids of *P. roebelenii*, *P. theophrasti*, *P. caneriensis* and *P. dactylifera*. PC1 and PC2 could explain 66.24% and 26.02% of the total variance, respectively. 92.26% of the total variance was able to be explained by the first two principal components (Figure 2)

4. Discussion

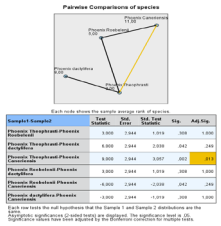
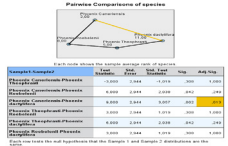
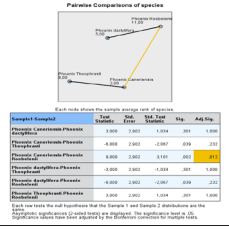
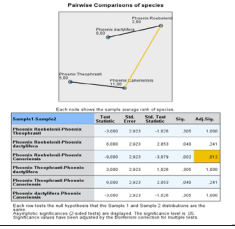
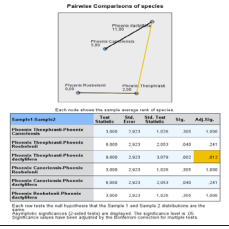
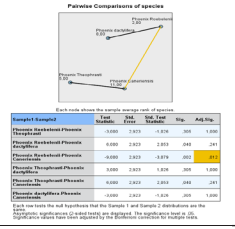
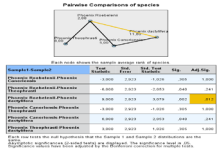
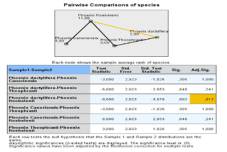
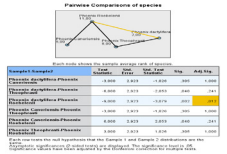
The findings of *P. dactylifera* were in accordance with Devshony et al. (1992); Al-Shahib and Marshall (2003); Akbari et al. (2012) and Lieb et al. (2020). In additions our findings show similarities in terms of total fat in *P. caneriensis* (Nehdi et al., 2010; 2011) with small deviations. The reason for these small differences might stem from extraction method differences (Szentmihályi et al., 2002), environmental conditions (Zia-Ul-Haq et al., 2013; Ilyasoglu, 2014) and ripening stage (Liolios et al., 2009; Dogan et al., 2014b).

Besides, the results of the dominant fatty acids were in line with the literature for *P. dactylifera* (Devshony et al., 1992; Al-Shahib and Marshall, 2003; Akbari et al., 2012; Lieb et al., 2020) and *P. caneriensis* (Nehdi et al., 2010; 2011). Although the findings of this study differed from the study of Liolios et al. (2009), which is the sole investigation made into the fatty acid composition of *P. theophrasti,* they were similar to other *Phoenix* species' results, as expected. The composition of fatty acids is not constant, and temperature, geographical location, genetic factors, and genotypes may affect them. Irrigation, sowing and harvest times, cultivation soil nature with N, P, K, and S nutrition variants, fertilizers, plant residues, photoperiod, light intensity, seed storage conditions, the process of seed maturation since seed formation, and quality may also influence fatty acid compositions (Ergun and Zarifikhosroshahi, 2021). The differences between the results of Liolios et al. (2009) and this study might be due to the abovementioned factors. Moreover, this sole investigation on *P. theophrasti* used the fruit, instead of the seeds.

Accordingly, the values of SFA (48.290%–57.160%), MUFA (35.495%–47.125%) and PUFA (8.010%–13.355%) of the *P. dactylifera* and *P. caneriensis* seed oil in this study were similar to those found in previous studies. For *P. dactylifera,* the SFA, MUFA and PUFA were found to be between 47% and 51%, 41.1% and 43.9%, and 7.8% and 9.1%, respectively (Lieb et al., 2020). For *P. caneriensis*, the SFA, MUFA and PUFA were found to be between 19.52% and 29.97%, 47.69% and 61.76%, and 18.72% and 25.59%, respectively (Nehdi et al., 2011).

It is a well-known fact that a diet rich in PUFAs is protective against many diseases and has a high nutritional value (Guney et al., 2015; Kafkas et al., 2020). The largest amount of PUFA was observed in the seed of *P. roebelenii* while the smallest amount was observed in *P. dactylifera.* Oleic acid is considered the most crucial fatty acid nutritionally among MUFAs (Reddy and Katan, 2004).

Table 2. Hypothesis test summary of independent-samples Kruskal–Wallis test and pairwise comparison results of total oil content for each dominant fatty acid including total SFA, total MUFA, and total PUFA.

Null Hypothesis	Sig.	Decision	Pairwise comparison	Null hypothesis	Sig.	Decision	Pairwise comparison
The distribution of Total fat is the same across all categories of species.	0.016	Reject the null hypothesis.		The distribution of ∑SFA is the same across all categories of species.	0.016	Reject the null hypothesis.	
The distribution of Lauric Acid is the same across all categories of species.	0.014	Reject the null hypothesis.		The distribution of Oleic Acid is the same across all categories of species.	0.015	Reject the null hypothesis.	
The distribution of Myristic Acid is the same across all categories of species.	0.015	Reject the null hypothesis.		The distribution of ∑MUFA is the same across all categories of species.	0.015	Reject the null hypothesis.	
The distribution of Palmitic Acid is the same across all categories of species.	0.015	Reject the null hypothesis.		The distribution of Linoleic Acid is the same across all categories of species.	0.015	Reject the null hypothesis.	
				The distribution of ∑PUFA is the same across all categories of species.	0.015	Reject the null hypothesis.	

Asymptotic significances are displayed. The significance level is 0.05.
Graphical representation of Dunn–Bonferroni's nonparametric pairwise comparison is for posthoc testing after a significant Kruskal–Wallis test.
Numerical values represent the mean rank of each group in the figures. Yellow lines represent statistically significant ($p < 0.05$) comparisons between the groups they connect. Black lines represent nonsignificant ($p \geq 0.05$) comparisons between the groups they connect.

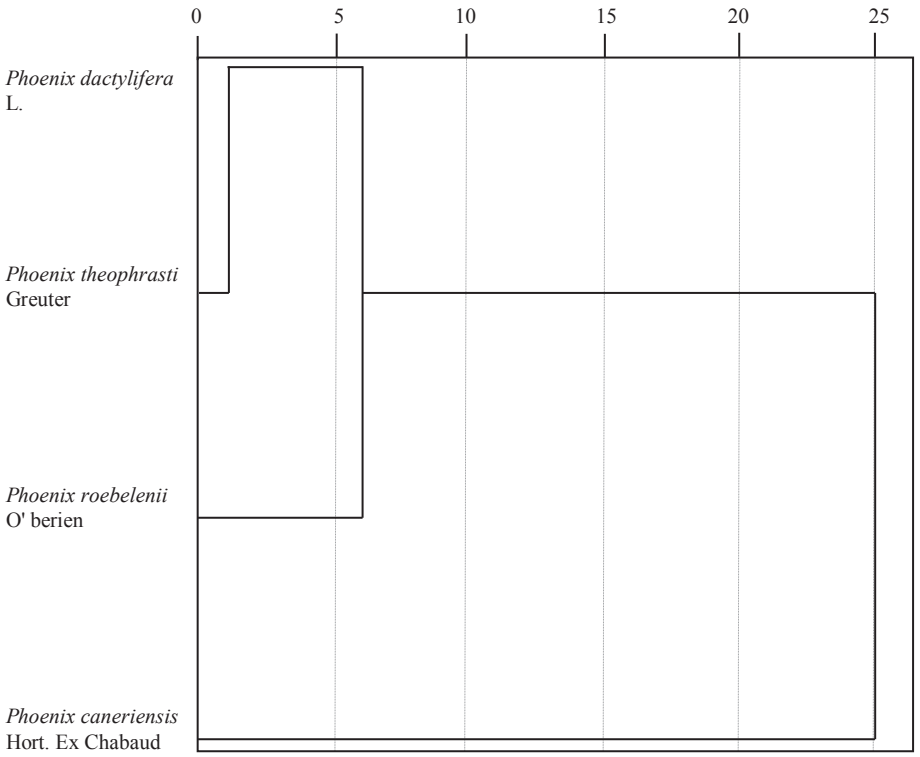


Figure 1. Hierarchical cluster analysis result (dendrogram using ward linkage, rescaled distance cluster combined).

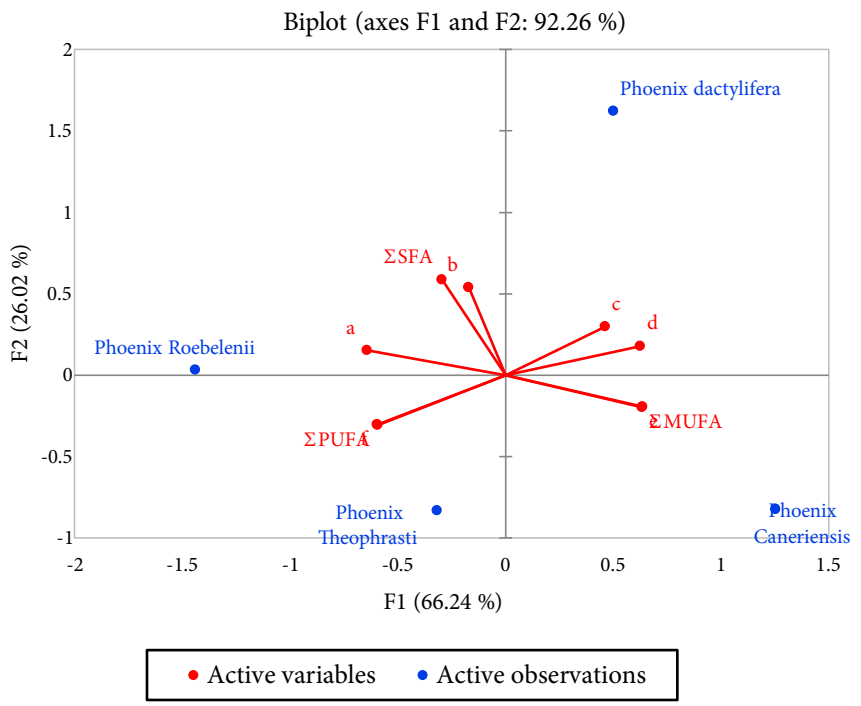


Figure 2. Biplot graph of fatty acids (scores and loading plots) obtained from principal component analysis (a: lauric acid (C12:0), b: myristic Acid (C14:0), c: palmitic acid (C16:0), d: stearic acid (C18:0), e: oleic acid (C18:1n9c)ω–9).

While the largest percentage of oleic acid was found in the seed of *P. caneriensis*, the smallest percentage was observed in the seeds of *P. roebelenii*.

The similarity result of *P. theophrasti* and *P. dactylifera*, shown in Figure 1, was parallel to the findings of Vardareli et al. (2019) in which the genetic characterization of *P. theophrasti* was investigated and the lowest genetic differentiation was found with *P. dactylifera*. To visualize the interrelations between variables and observations, given in Figure 2, was enabled through the biplot graph of fatty acids. Moreover, it can be understood which parameter was observed in which species and by how much.

5. Conclusion

The genus *Phoenix*, as a member of the Arecaceae family, has found a large area of usage, not only throughout history but also today. These species have been used for food, religion, construction, and ornamentation purposes especially in a large portion of the old world. Determination of the total oil content and fatty acid composition is a necessity for food, health, and economic aspects. In this study, the total oil content and fatty acid composition of *P. theophrasti*, *P. roebelenii*, *P. dactylifera* and *P. caneriensis* seeds were determined. The novelty of this study stems from three points. This study is the first that investigated the oil content of *P. theophrasti* and *P. roebelenii* seeds. Moreover, this study is the first which has determined the fatty acid composition of *P. roebelenii* seeds. Thirdly, using the same plantation for sample collection has enabled the control of environmental factors that may affect the investigated parameter. The oil content of the samples can be listed as *P. caneriensis*, *P. dactylifera*, *P. roebelenii*, and *P. theophrasti* in descending order. For all samples, the dominant fatty acids were oleic acid, lauric acid, myristic acid, linoleic acid, and palmitic acid. The results of the independent samples Kruskal–Wallis test and pairwise comparison performed for the oil content, dominant fatty acids, and total SFA, MUFA and PUFA levels, revealed significant differences between some of the samples. Cluster analysis showed that the *P. theophrasti* and *P. dactylifera* had the most similar observations.

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