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ORIGINAL ARTICLE

Comparative Study of the Fatty Acids and Tocopherol Profiles, Physical Properties, and Antioxidant Activities of *Zizyphus lotus* L. Seed Oils Based on the Geographical Origin

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Abstract

Zizyphus lotus L. seeds contain a high amount of lipids representing a rich source of health promoting components. However, the chemical composition is influenced by several factors for instance the geographic location of the plant. This study focused on the evaluation of the effect of the plant location on the fatty acids and tocopherols composition as well as the antioxidant activities of the cold-pressed oils extracted from *Zizyphus lotus* L. seeds. Physical properties of oil seeds were also investigated. The results revealed that the lipid fraction yield ranged from 20.13 to 24.57% (w/w) for samples harvested from Siliana and Sidi Bouzid, respectively. We identified ten fatty acids, among which oleic acid was the major component in all analysed samples, accounting for 60.45–70.36% of the total fatty acids, followed by linoleic acid (16.40–19.40%) and palmitic acid (5.02–12.60%). Besides, the total tocopherol amounts varied from 168.26 to 241.41 mg/100 g of oil, with β-tocopherol as the main component and compositional ratio differences between regions were noticed. Furthermore, remarkable values of DPPH and $ABTS^+$ radical scavenging activities were obtained. A cluster analysis highlighted differences attributed to the origin of the sample, which could be considered as an efficient tool for cultivar authenticity purposes and valorization. The obtained results are of great economic interest and could increase the demand for *Zizyphus lotus* L. seed oil for potential applications in the food, cosmetic and medicinal industries.

Keywords *Zizyphus lotus* L. · Fatty acids · Tocopherols · Tocotrineols · DPPH · $ABTS^+$

1 Introduction

Numerous fruit seeds have been recognized as rich sources for many edible oils that have been revealed to contain high amounts of essential fatty acids and other biologically active phytochemicals such as phenolic compounds, sterols, tocopherols and carotenoids [1, 2]. Most of these natural compounds have been proven to possess substantial health promoting properties and could be employed as natural preservatives for food applications. Although the most common use of synthetic antioxidants in the food industries as an inhibitor of lipid oxidation, it has been restricted because of their toxicity [3]. Hence, much attention has been drawn to the extraction of natural antioxidants with plant origins.

Zizyphus lotus L. (*Z. lotus*), commonly known as jujube, belongs to the botanic family of the "*Rhamanaceae*" which is native to China and became extensively cultivated in tropical and subtropical regions, especially in East Asia (China, India), North Africa, and Middle Eastern countries [4, 5]. In Tunisia, it is a well-adapted plant growing in arid and sub-arid climates mainly in the south of the country [6]. Different parts of jujube such as fruits and leaves are used as food and a source of components of some traditional medicines for curing several diseases [4]. Examples are digestive disorder, urinary troubles, fever and diarrhea to mention a few [7, 8]. The chemical analyses of *Z. lotus* fruits have demonstrated that they contain peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, betulinic acid and triterpenoidal saponin glycosides [7, 9–11].

Interestingly, seeds of *Z. lotus* are considered important due to the great amount of their lipid fraction, reaching

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about 33% of the dry weight and providing several health maintaining compounds [12]. Particularly, *Z. lotus* seeds oil is rich in unsaturated fatty acids, such as oleic and linoleic acids, offering thus many advantages for human consumption. It contains also high amounts of phytosterols and tocopherols [6]. Nonetheless, the aforementioned properties are dependent on abiotic and biotic factors. Many studies highlighted the effect of the plant species [13], the season of harvest and location [14] on the chemical composition of edible oils extracted from seeds. Some other factors were also investigated. Ghafoor et al. [15] studied the effect of roasting temperature on chia seed's oil quality and the obtained data indicated that heating at a temperature below or equal to 90 °C led to better preservation of the oil nutrients. Furthermore, the extraction methods affected the composition of total carotenoid and chlorophylls, phenolic compounds and fatty acids [16].

Apart from the medicinal and nutritional characters, and to the best of our knowledge, no research work pertaining to the evaluation of the effect of geographical origin of *Z. lotus* seed oils has been conducted so far.

Thus, this study's utmost objective is the evaluation of the fatty acids and tocopherol composition as well as the antioxidant activity of *Z. lotus* cold pressed seed oils from different geographic origins in Tunisia. Physical properties of oils, such as dynamic viscosity and interfacial tension were also investigated. Such information would be an important contribution to a further valorization of the seed oil extracted from this species in the food industry and a proof of the product authenticity.

2 Materials and Methods

2.1 Chemicals and Reagents

All the chemicals and solvents, used in this study, were of the highest commercial grade.

2.2 Plant Materials

The collection of *Z. lotus* fruits was performed at the same maturity stage on the basis of fruit brown color and mucilaginous aspect, during August 2019, from different Tunisian locations, namely: Nabeul, Zaghouan, Siliana, Sousse, Mehdia, Kairouan, Kasserine, Sidi Bouzid, Sfax, Gabes, Gafsa and Tozeur. The geographic positions and climatic data of these locations are shown in Fig. 1. After being botanically identified according to the protocol of Tunisian flora [17], each sample (500 g) was air-dried and blended in a Retsch AS 200 Basic blender (Retsch GmbH, Germany). Next, the seeds were manually isolated from the edible parts and stored in the dark at − 18 °C. Besides, voucher specimens of each sample were kept for future reference in our lab.

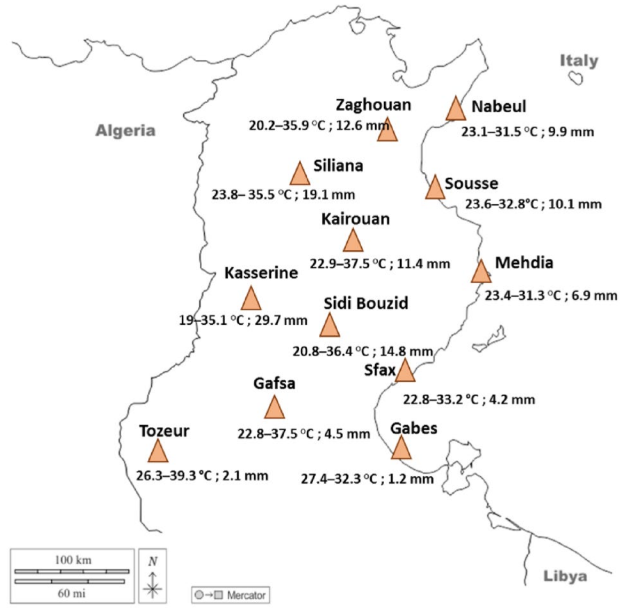


Fig. 1 Geographic position and climatic data (temperature (°C) and rainfall (mm)) of different locations for *Z. lotus* collection

2.3 Cold Pressed *Z. lotus* Seeds Oil Extraction

To extract oil, we made use of a cold-pressing apparatus (CA59G, German Monforts Group, Moenchengladbach, Germany) to press *Z. lotus* seeds at 20 °C. The obtained mixture was centrifuged (Avanti J-26 XP, Beckman Coulter Inc., Brea, CA, USA) at 2500 g for 5 min to separate the oil. The extracted oils were kept in vials under a nitrogen atmosphere and kept in the dark at − 18 °C, till assays performing [18].

2.4 Fatty Acids Analysis

The determination of the fatty acid composition was realized using Gas chromatography (PerkinElmer Wallac Auto System XL autoinjector, San Jose, CA, USA) coupled to flame ionization detection (GC-FID) after conversion to fatty acid methyl esters (FAME). The procedure used was the same as our previous study [19]. For the methylation purposes, 0.2 mL sodium methoxide solution (2 mol L^{-1} in anhydrous methanol) was mixed, for 1 min using a vortex, with 0.05 g of oil dissolved in 1 mL of hexane. After sodium glycerolate sedimentation, 1 µL of the clear supernatant was injected into a Supelco Sp™ 2340 fused-silica capillary column (60 m × 0.25 mm, 0.20 µm film thickness) under the following conditions: Injection and detection temperatures were set at 250 °C. The oven temperature program was as follows: heating from 130 to 170 °C at a rate of 20 °C. min^{-1}, then raised to 230 °C at the rate of 10 °C min^{-1}, hold at 230 °C for 10 min, and finally raised to 250 °C, at the rate of 30 °C min^{-1}. Nitrogen was used as a carrier gas at a flow rate of 50 mL min^{-1}. Peaks of FAME were identified according to the retention times of a standard mixture. The peak areas were computed and using the direct normalization method, the FAME contents were expressed as area percentages.

2.5 Tocopherols Analysis

The evaluation of the tocopherols analysis was realized by high-performance liquid chromatography according to AOCS Method [20], using a Hewlett-Packard apparatus, series 1100 system (Santa Clara, CA). This apparatus is equipped with a model 168 UV detector (Beckman Coulter, Inc., Fullerton, CA) at a wavelength of 292 nm and a Lichrosorb Si60 (Merck) column with a 7 mm silica particle size. An amount of 15 µL of a mixture made up of 2 g of oil sample dissolved in 10 mL of n-hexane was eluted with (1% 2-propanol/ hexane) as a mobile phase with a flow rate set at 0.65 mL min^{-1}.

Tocopherols were identified by comparing their retention times with those of standards.

The calculation of the conversion factors for vitamin E activities for each compound was carried out following the formulae below: α-tocopherol × 1.00; β-tocopherol × 0.40; γ-tocopherol × 0.10; δ-tocopherol × 0.01; α-tocotrienol × 0.30; β-tocotrienol × 0.05 and γ-tocotrienol × 0.01.

2.6 Physical Analysis

The physical properties such as dynamic viscosity and interfacial tension of *Z. lotus* seed oils were assessed according to the methods described by our previous study [19].

2.7 Antioxidant Activity

2.7.1 DPPH· Radical Scavenging Assay

The DPPH· radical scavenging activity of *Z. lotus* seed oil was evaluated following Essaidi et al. [21] procedure with a minor adjustment. 0.1 mL of seed oil, at different concentrations, was mixed with 2.2 mL DPPH· in ethanol (0.004%). The obtained mixture was thoroughly shaken and incubated for 60 min in the dark and at ambient temperature (± 20 °C); the absorbance was read at 515 nm. The radical scavenging activity was expressed as IC50 (mg/mL) which is the concentration providing the inhibition of 50% DPPH· radicals.

2.7.2 ABTS·+ Radical Scavenging Activity

The ABTS·+ Antiradical activity of *Z. lotus* seed oils was evaluated following the description of Zuleata et al. [22] on the basis of the capability of a sample to impede this radical (ABTS·+) in comparison with a reference antioxidant standard. First, the preparation of ABTS·+ radical was performed by the reaction of 25 mL of ABTS solution (7 mM) with 440 µl of potassium per sulphate ($K_2S_2O_8$; 140 mM). The obtained mixture was not only kept at ambient temperature in the dark for 12–16 h, allowing the monocation radical (ABTS·+) to form, but also diluted with ethanol to acquire an absorbance of 0.700 at 734 nm. Subsequently, 2 mL of obtained ABTS·+ solution was mixed with 100 µL of each *Z. lotus* seed oil, at different concentrations, separately and the absorbance was measured after 6 min of mixing. Hence, the radical scavenging activity was expressed as inhibition concentration (IC50, mg/mL) which reduces 50% of free radicals present in the ABTS solution.

2.8 Statistical Analysis

The obtained results for at least three analyses for each sample are expressed as mean ± standard deviation. As for the assessment of the statistical analyses, it was realized using SPSS (version 18). The determination of the variance analyses was achieved through an ANOVA procedure. Duncan's multiple range test was adopted to highlight significant differences ($p < 0.05$) between the means. To join *Z. lotus* origins in homogeneous groups, a cluster analysis was conducted taking into consideration the fatty acid composition and vitamin E composition [tocopherols (α, γ, δ) and tocotrienols (α, β, γ, δ)]. After performing a Hierarchical Cluster Analysis, using the nearest neighbor method and the squared Euclidean distance, the subjects were grouped. The total of clusters to retain was set up by the R-squared criteria, as explained by Maroco [23].

3 Results and Discussion

3.1 Oils Yield and Fatty Acids Composition

The harvest location significantly influenced the yield of the lipid fraction of *Z. lotus* seed. The obtained results showed that the percentage of oil extracted varied from 20.13 to 24.57% based on dry matter weight (Fig. 2).

Nonetheless, it is worthy to state that the highest and lowest oil yield values were found in samples collected from Sidi Bouzid and Siliana, respectively ($p < 0.05$). Nevertheless, no significant differences ($p > 0.05$) between samples collected from Sidi Bouzid and Kasserine were detected in terms of lipid amount.

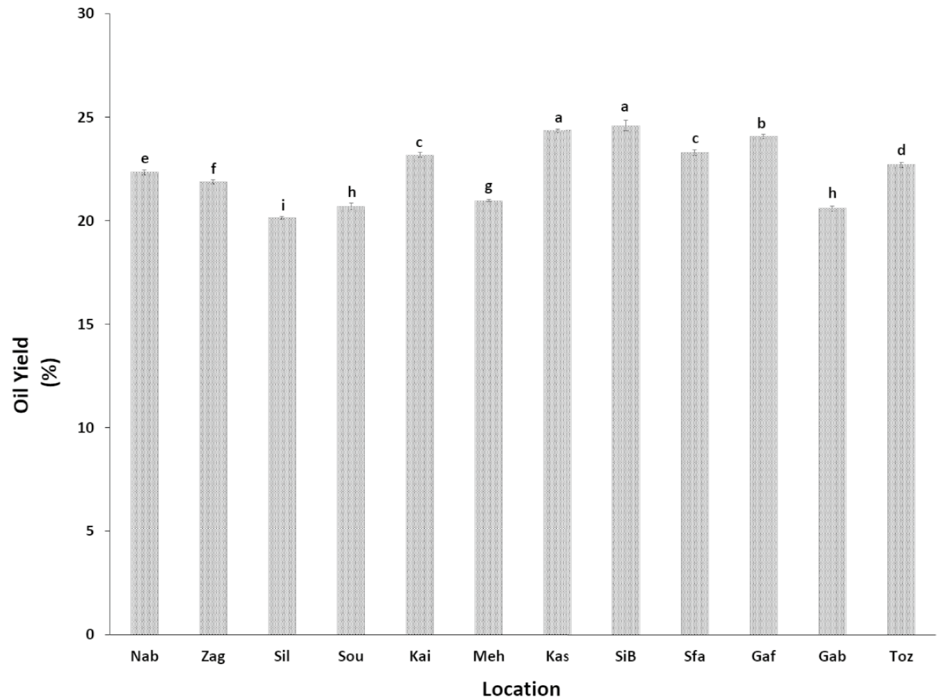


Fig. 2 Oil contents (%. v/m dry basis) of cold pressed *Z. lotus* seed oils from different origins. Nab, Zag, Sil, Sou, Kai, Meh, Kas, SiB, Sfa, Gaf, Gab and Toz: Oils extracted fromNabeul, Zaghouan, Siliana, Sousse, Mehdia, Kairouan, Kasserine, Sidi Bouzid, Sfax, Gabes,Gafsa and Tozeur, respectively. Each value is the mean of 3 independent trials ± SE

The study conducted by El Aloui et al. [24] focused on the lipid fraction of *Zizyphus jujube* Mill. leaves from different locations in Tunisia. Their results showed differences in oil yields depending on the growing location. The highest yield was obtained for leaves collected in the region of Sfax and it did not exceed 10.31%. In the present study, the lowest oil seed yield was observed for the sample collected from Siliana and it was about twice higher than oil leaves content (20.13%). This difference is mainly attributed to the studied part of the plant.

The obtained oils were analyzed using GC-FID for the determination of their composition and results for fatty acids contents, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) are summarized in Table 1.

Regardless of the location, MUFA (64.10–73.46%) predominated over SFA (6.14–17.96%) and PUFA (17.90–20.40%) due to the significant contribution of oleic acid. In all the samples, it was found that oleic acid was the predominant (60.45–70.36%), followed by linoleic acid (16.40–19.40%), two dominant acids accounting for 76.90–79.76% of the total fatty acids. The highest level in oleic acid was found in the sample harvested from Siliana followed by those from Zaghouan, Nabeul and Mehdia. Variations in oleic and linoleic acid contents observed in *Z. lotus* seed oil samples obtained from different Tunisian locations (Table 1) are probably related to region environmental interaction during the development and the maturity of the fruits. Furthermore, minor amounts of odd-chain fatty acids such as stearic, linolenic, and gadoleic acids were also observed in our study.

These results are in excellent agreement with those demonstrating that oleic acid is the most abundant fatty acid in *Z. lotus* seed oils [25]. Oleic acid was also found to be the most abundant acid in the lipid fraction of other parts of *Z. Lotus*. For instance, oils extracted from whole fruit [26] peel and pulp [27] were rich in C18:1. Oleic acid is characterized by its health benefits and its possible capacity to decrease blood pressure [28]. As a member of the family of main mono-unsaturated fatty acids of olive oil, oleic acid destroys the gene expression of breast cancer cells [29]. Hence, the edible oil industry's main concern is the high quality of oleic vegetable oils. Indeed, since oil rich in unsaturated fatty acids are assumed to be advantageous agents, they are included into infant formula and several food products. Besides, they are available as nutraceutical supplements in many countries [30, 31]. Considering the elevated amount of unsaturated fatty acids, the seed oils of the twelve *Z. lotus* seeds under investigation are exceedingly advocated for human consumption, indicating a more beneficial fatty acid profile than other vegetable oils. Moreover, it is proven that trans fatty acids are likely to elevate LDL cholesterol and lower HDL cholesterol concentrations. It was demonstrated, however, that the analyzed oils are characterized by extremely low amounts in these fatty acids. Therefore, *Z. lotus* seed oil may be judged as a precious source of essential fatty acids.

The obtained results were similar to those obtained by Ghazghazi et al. (2014) [32] who studied the fatty acid

Table 1 Fatty acid composition (%) of cold pressed *Z. lotus* seed oils from different origins

Fatty acid (%)	Origin											
	Nab	Zag	Sil	Sou	Kai	Meh	Kas	SiB	Sfa	Gaf	Gab	Toz
C14:0	0.02±0.01b	0.06±0.01a	0.03±0.01b	0.09±0.01c	0.20±0.01e	0.08±0.00d	0.10±0.02g	0.06±0.01af	0.07±0.02acdfg	0.08±0.02acdfg	0.10±0.01cg	0.10±0.03acdfg
C16:0	6.23±0.16a,c	6.20±0.12a	5.02±0.20b	8.04±0.18d	12.50±0.30f	6.04±0.36ace	10.08±0.20g	10.14±0.11g	9.98±0.18g	10.69±0.10h	10.03±0.20g	12.60±0.20f
C16:1	0.08±0.00c	0.06±0.01a	0.04±0.00b	0.10±0.01d	0.10±0.01d	0.10±0.01d	0.08±0.00c	0.10±0.01d	0.07±0.00c	0.09±0.01d	0.07±0.00e	0.10±0.02d
C18:0	3.10±0.04c	2.58±0.04a	1.05±0.03b	3.70±0.12d	4.96±0.07e	3.20±0.08c	4.65±0.12f	4.53±0.08f	4.59±0.14f	3.19±0.14c	4.89±0.08e	4.72±0.10ef
C18:1	68.40±0.60b	68.84±1.03a	70.36±0.92a	64.82±0.45c	61.60±0.80d	67.70±0.56b	62.40±0.61d	62.20±0.42d	63.49±0.54e	64.20±0.46ce	61.30±0.49f	60.45±0.35a
C18:2	18.01±0.22a	18.22±0.21a	19.20±0.10b	18.38±0.14a	16.40±0.12d	18.40±0.11c	18.10±0.16a	18.02±0.14a	17.63±0.21e	17.59±0.12e	18.80±0.11f	17.11±0.15g
C18:3	1.02±0.12a	1.15±0.05a	1.20±0.13a	1.40±0.09b	1.15±0.04a	1.28±0.10ab	1.25±0.06ab	1.20±0.04a	1.35±0.03b	1.49±0.10c	1.38±0.05b	1.10±0.10a
C20:0	0.02±0.00b	0.01±0.00a	0.01±0.00a	0.10±0.01c	0.20±0.03d	0.10±0.01c	0.12±0.01c	0.15±0.02d	0.10±0.01c	0.09±0.00c	0.15±0.02d	0.15±0.02d
C20:1	3.10±0.09b	2.86±0.08a	3.06±0.06b	3.30±0.15c	2.79±0.10d	3.05±0.05b	3.10±0.07bc	3.50±0.04e	2.74±0.09d	2.49±0.10c	3.20±0.10bc	3.55±0.31ce
C22:0	0.02±0.00a	0.02±0.00a	0.03±0.00b	0.07±0.00c	0.10±0.01e	0.05±0.00d	0.12±0.01f	0.10±0.00e	0.07±0.00g	0.09±0.00e	0.08±0.00g	0.12±0.02ef
ΣSFA	9.39±0.40c	8.87±0.25a	6.14±0.14b	12.00±0.09d	17.96±0.35 e	9.47±0.19c	14.40±0.18g	14.98±0.20f	14.72±0.11f	14.19±0.12i	15.25±0.14h	17.69±0.43e
ΣMUFA	71.58±0.98a	71.76±0.86a	73.46±1.08b	68.22±0.72c	64.49±0.75d	70.85±0.60ac	67.70±0.68c	65.80±0.50c	66.30±0.74d	66.78±0.82e	64.56±0.78d	64.10±0.45d
ΣPUFA	19.03±0.17a	19.37±0.32a	20.40±0.11b	19.78±0.13c	17.55±0.31e	19.68±0.19d	17.90±0.15f	19.22±0.21e	18.92±0.14g	19.08±0.22ag	20.18±0.12h	18.21±0.30f

Nab, Zag, Sil, Sou, Kai, Meh, Kas, SiB, Sfa, Gaf, Gab and Toz: Oils extracted from Nabeul, Zaghouan, Siliana, Sousse, Kairouan, Mehdia, Kasserine, Sidi Bouzid, Sfax, Gafsa, Gabes and Tozeur, respectively. Each value is the mean of 3 independent trials ± SE. Data sharing different letter below are significantly different at p < 0.05

composition of *Ziziphus lotus* L. fruits and they showed that oleic acid which was the dominant fatty acid presenting more than 88% of total fatty acids. Other studies focused on the identification and quantification of fatty acids from seed oils. Most of them indicated that seeds oil exhibit a common composition that includes both saturated and unsaturated fatty acids. Matthaus and Ozcan [33] studied the effect of habitat on the fatty acid composition of prickly pear seed oils. They reported that palmitic, oleic, and linoleic acids were found the major fatty acids. Their amounts ranged between 10.60 and 12.80; 13.00 and 23.50% and 49.30–62.10%, respectively.

A cluster method was used to classify the various *Z. lotus* seed oils according to their fatty acid compositions (Fig. 3).

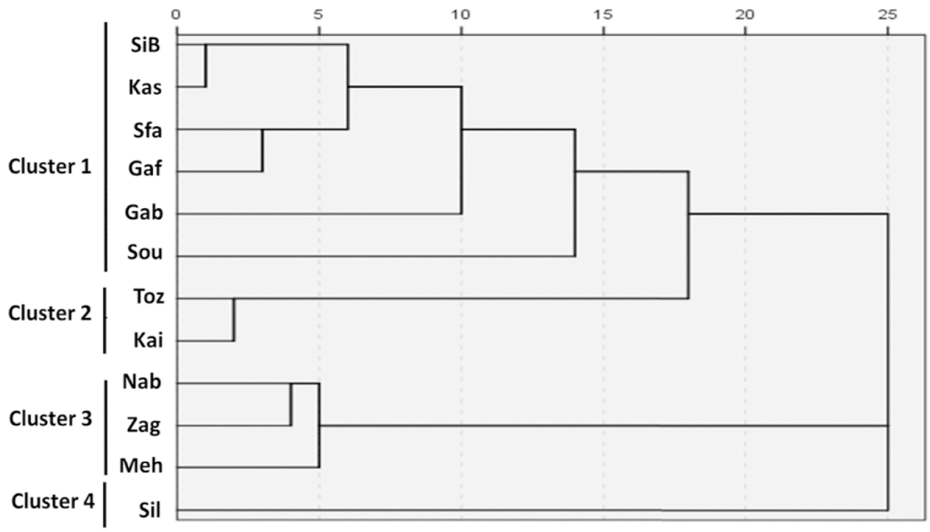
Four clusters were found with an *R*-squared of 0.92. As far as the first cluster is concerned, it includes six regions, namely, Sidi Bouzid, Kasserine, Sfax, Gabes, Sousse, and Gafsa, on the basis of similarity in fatty acid composition. Concerning the second and third clusters, they included Tozeur and Kairouan, and Mehdia, Nabeul and Zaghouan, respectively. While the second cluster (Tozeur and Kairouan) demonstrated the highest levels of SFA and MUFA, the third cluster encompassing Mehdia, Nabeul and Zaghouan has the lowest levels of SFA and MUFA. Finally, the fourth cluster consists of Siliana region presenting the highest PUFA and MUFA levels.

3.2 Tocopherols Composition

As displayed in Table 2, the tocopherols composition of the twelve Tunisian *Z. lotus* seed oils was analyzed. Eight compounds were detected in almost all seed oils, comprising four tocopherols (α-, β-, δ- and γ) and four tocotrienols (α-, β-, δ-and γ). It is trusty to mention that all seed oils were richer in tocopherols than tocotrienols, with β-tocopherol as the most abundant, followed by δ -tocopherol and δ-tocotrienol.

Generally, our findings are in good agreement with those reported by other studies found in the literature which have proven β-tocopherol as the most abundant tocopherol in *Z. lotus* seed oils [34]. However, α- and γ-tocopherols were only detected in lower amounts in all *Z. lotus* seed oils. Yet, *Z. lotus* seeds collected from Siliana and Mehdia, particularly comprised a considerable amount of total tocols and vitamin E, which were present in low amounts in all other samples collected from Gabes and Tozeur. However, there were no significant differences observed between all *Z. lotus* seed oils regarding δ-tocopherol amounts ($p > 0.05$). *Z. lotus* seed oils of the twelve Tunisian locations are likely to be utilized to postpone the body's aging process and to avoid the existence of many chronic diseases. Indeed, vitamin E is known for its numerous health benefits, given its power as a lipid-soluble antioxidant, and its ability to

Fig. 3 Dendrogram of cold pressed *Z. lotus* seed oils from different origins according to their fatty acid compositions. Nab, Zag, Sil, Sou, Meh, Kai, Kas, SiB, Sfa, Gaf, Gab, and Toz: Oils extracted fromNabeul, Zaghouan, Siliana, Sousse, Mehdia, Kairouan, Kasserine, Sidi Bouzid, Sfax, Gabes,Gafsa and Tozeur, respectively



protect the human body's cells against the damage caused by free radicals.

After completing a dendrogram to the vitamin E amounts of the twelve seed oils samples used in the present investigation (Fig. 4), we detected four clusters with R-squared equal to 0.89, through which a substantial proportion of the total variation was maintained.

Concerning the first identified cluster, it constituted seven *Z. lotus* seed oils, collected from Sidi Bouzid, Kasserine, Sfax, Kairouan, Sousse, Nabeul and Zaghouan regions, because all of them had similar tocopherols amounts. Oils extracted from seeds collected from Mehdia and Siliana were included in cluster 2 since both of them had relatively higher α- and β-tocopherols amounts. The *Z. lotus* seed oils of Gabes and Tozeur regions were included in cluster 3 due to their low α- and β-tocopherol amounts. The last cluster was represented by Gafsa region. This seed oil presented the lowest amount of δ-tocopherol.

3.3 Physical Properties

The physical properties of the twelve *Z. lotus* seed oils are presented in Table 3.

The dynamic viscosity varied from 67.23 to 82.45 Pa.s. The highest values were observed in the samples collected from Nabeul and Siliana. While the lowest was found in the sample of Gabes. Also, the findings revealed that there are no significant variations observed in the samples collected from Sidi Bouzid and Sfax ($p > 0.05$). In this context, all *Z. lotus* seed oils demonstrated Newtonian behavior characterized by constant dynamic viscosity, which was estimated from the plotting shear stress as function of shear rate [19]. These obtained data were similar to those of *Pistacia lentiscus* seed oils [35].

Regarding the interfacial tension, the values ranged from 13.79 to 17.02 mN/m and the highest value was observed in the sample collected in Mehdia region. However, there was no significant differences between samples collected from Sidi Bouzid, Kasserine, and Sfax ($p > 0.05$). Some authors declared that the interfacial tension depends on the chemical composition of studied oil like free fatty acids, tocopherols, phospholipids as well as the polyphenol contents [36]. *Z. lotus* seed oil is known to possess high levels of carotenoids, polyphenols and tocopherols [34].

Besides, other compounds, generated by several chemical reactions during the extraction, storage and processing of vegetable oils could affect the interfacial properties [19, 34]. For instance, it has been reported that the oxidation of frying oils led to the formation of aldehydes, ketones and alcohols responsible of a decrease of the interfacial properties [37]. The interfacial film is progressively reinforced by fractions of higher molecular weight compounds that will diffuse more slowly, and contribute to a further decrease in the interfacial tension [36].These properties are highly used in certain food applications such as the formulation of emulsions [38].

3.4 Antioxidant Activities

Table 4 shows the DPPH$^{\cdot}$ and ABTS^{+} radical scavenging activities of the *Z. lotus* seed oil samples which are expressed by IC50 referring to the lowest concentration of antioxidants necessary for 50% of radicals inhibition. Thus, the lower the IC50 value the more reactive the studied oil.

Hence, the present study reveals that the DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radical scavenging activities of *Z. lotus* seed oils obtained from Siliana are significantly higher (2.87 and 4.08 mg/mL) than all other seed oil samples ($p < 0.05$).

Table 2 Tocopherol, tocotrienol and vitamin E amounts (mg/kg of oil) of cold pressed *Z. lotus* seed oils from different origins

	Origin											
	Nab	Zag	Sil	Sou	Kai	Meh	Kas	SiB	Sfa	Gaf	Gab	Toz
Tocopherol amounts(mg/kg of oil)												
α-tocol	14.69 ± 1.03efg	17.42 ± 2.01a	15.61 ± 2.00fg	9.59 ± 1.03bcd	10.21 ± 1.02bcd	18.42 ± 0.97g	10.45 ± 1.98bcd	12.50 ± 1.03def	11.91 ± 1.02cde	7.58 ± 1.02k	6.46 ± 1.01ab	4.89 ± 0.99a
β-tocol	137.71 ± 10.02abc	128.92 ± 9.02abc	145.67 ± 5.03c	125.68 ± 9.99abc	140.85 ± 10.97bc	142.21 ± 4.03bc	136.86 ± 5.94abc	125.60 ± 1.05abc	120.57 ± 8.03abc	119.45 ± 9.98abc	112.32 ± 6.02a	115.42 ± 10.03ab
δ-tocol	29.73 ± 4.06a	27.64 ± 3.03a	30.10 ± 3.07a	24.9 ± 1.97a	26.72 ± 3.01a	31.72 ± 4.94a	25.57 ± 1.97a	28.80 ± 1.8a	22.35 ± 2.00a	22.79 ± 2.02a	24.57 ± 2.01a	21.45 ± 4.48a
γ-tocol	2.96 ± 0.02c	2.53 ± 0.02a	2.61 ± 0.02b	0.09 ± 0.02d	1.52 ± 0.02f	2.77 ± 0.01e	0.03 ± 0.00h	1.18 ± 0.02g	2.66 ± 0.02i	0.28 ± 0.02k	0.19 ± 0.01j	0.45 ± 0.03l
Tocotrienol amounts (mg/kg of oil)												
α-totr	9.89 ± 0.00c	8.42 ± 0.07a	10.52 ± 0.10b	8.43 ± 0.05a	6.93 ± 0.07e	10.38 ± 0.10d	5.36 ± 0.08g	9.34 ± 0.02f	7.72 ± 0.09h	6.91 ± 0.09j	5.28 ± 0.08i	4.34 ± 0.06k
β-totr	14.81 ± 0.98cd	13.67 ± 1.02cd	16.74 ± 1.01d	11.14 ± 0.12ab	12.65 ± 0.98bcd	15.21 ± 0.52cd	12.29 ± 0.99ab	14.67 ± 1.08cd	14.23 ± 1.98cd	11.88 ± 1.01ab	9.47 ± 0.97ab	8.43 ± 1.02a
δ-toctr	19.52 ± 1.97b	16.20 ± 1.48ab	19.50 ± 0.98b	15.42 ± 0.98ab	18.98 ± 0.98ab	18.98 ± 1.00b	16.47 ± 1.02ab	18.90 ± 2.04b	17.90 ± 1.01ab	15.51 ± 0.98ab	15.65 ± 1.97ab	13.22 ± 1.02a
γ-toctr	0.72 ± 0.05h	0.50 ± 0.01f	0.65 ± 0.02g	0.04 ± 0.01ab	0.13 ± 0.01be	0.64 ± 0.04g	n.d	0.11 ± 0.01de	0.09 ± 0.01cd	0.07 ± 0.01bcd	0.03 ± 0.00ab	0.05 ± 0.01c
Vitamin E amounts (mg/kg of oil)												
Vit E	74.08 ± 4.93cde	72.54 ± 6.03cde	78.45 ± 3.95de	63.20 ± 3.07abc	69.68 ± 3.44bcde	79.78 ± 2.56e	67.67 ± 0.32bcd	66.68 ± 0.54bcd	63.65 ± 5.11abc	58.28 ± 2.93ab	53.72 ± 3.44a	53.04 ± 3.01a

Nab, Zag, Sil, Sou, Kai, Meh, Kas, SiB, Sfa, Gaf, Gab and Toz: Oils extracted from Nabeul, Zaghouan, Siliana, Sousse, Kairouan, Mehdia, Kasserine, Sidi Bouzid, Sfax, Gafsa, Gabes and Tozeur, respectively. Each value is the mean of 3 independent trials ± SE. Data sharing different letter below are significantly different at $p < 0.05$

However, *Z. lotus* seed oils obtained from Gafsa, Gabes and Tozeur had a significantly higher ($p < 0.05$) value of IC50 than those of studied seed oil samples, indicating thus a lower antioxidant potential for oils from these locations (Gafsa, Gabes and Tozeur). Nevertheless, high negative correlations were noticed between IC50 of $ABTS^{+}$ radical scavenging activity ($r = -0.903$, $p < 0.01$), between IC50 of $DPPH^{\cdot}$ radical scavenging activity ($r = -0.922$, $p < 0.01$) and the total tocols amounts in all studied oils. Negative correlations between IC50 of $ABTS^{\cdot+}$ radical scavenging activity ($r = -0.918$, $p < 0.01$), and between IC50 of DPPH radical scavenging activity (-0.922, $p < 0.01$) and vitamin E contents were also observed. Indeed, vitamin E is a well-known lipid-soluble antioxidant conferring increased oxidative stability to vegetable oils, particularly those rich in unsaturated fatty acids [39]. Thus, higher contents of Vitamin E in oils entail higher antioxidant activity and consequently lower IC_{50}. Negative correlations between vitamin E content and methods determining the antioxidant activity were observed in currant and gooseberry fruits [40]. However, it is worth mentioning that studies focusing on correlations between vitamin E and methods for the evaluation of the antioxidant activity from plants, fruits or extracts have been very rare.

The antioxidant potential is attributed to different chemical compounds whose the amounts could be further affected by many factors such as the cultivar type, plant part, maturity and harvest period and environmental conditions of the locality. Though, synergistic or antagonistic effects of these compounds play a crucial role in the resulting antioxidant properties [41].

4 Conclusions

The present study undertakes for the first time the investigation of *Z. lotus* seeds oil, collected from different locations, for their fatty acids and tocopherols profiles, as well as their antioxidant activities. The obtained results revealed that *Z. lotus* seed oils were characterized by oleic acid as the predominant fatty acid. Such data represent great economic benefits due to the numerous potential applications of this component in the food, cosmetic and pharmaceutical industries. Beta-tocopherol was the main component. However, different compositional ratios between locations were observed. Moreover, *Z. lotus* seed oil collected from different locations are endowed with a promising antioxidant activities against $DPPH^{\cdot}$ and $ABTS^{+}$ radicals. The use of this oil may also serve in improving human health and the prevention of chronic diseases.

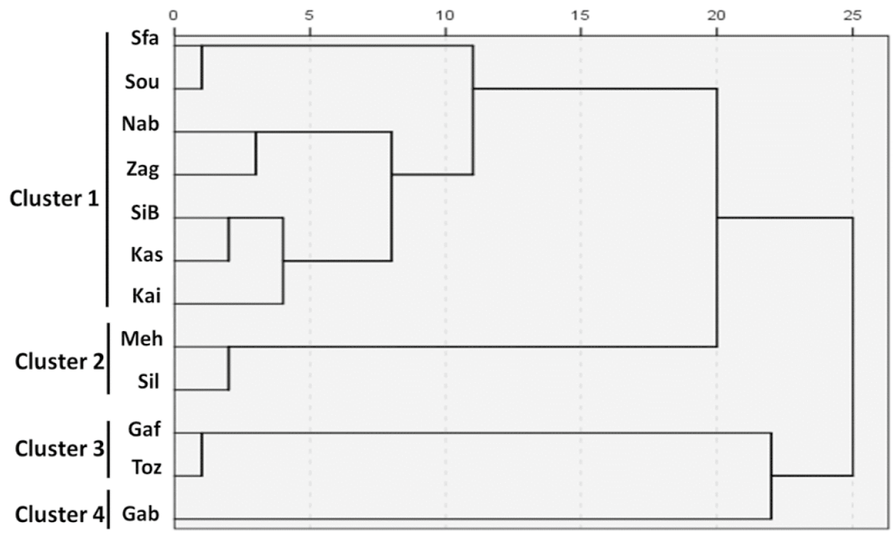


Fig. 4 Dendrogram of cold pressed *Z. lotus* seed oils from different origins according to their vitamin E amounts. Nab, Zag, Sil, Sou, Meh, Kai, Kas, SiB, Sfa, Gaf, Gab and Toz: Oils extracted fromNabeul, Zaghouan, Siliana, Sousse, Mehdia, Kairouan, Kasserine, Sidi Bouzid, Sfax, Gabes,Gafsa and Tozeur, respectively

Table 3 Physical properties of cold pressed *Z. lotus* seed oils from different origins

Location	Viscosity (mPa.s)	Interfacial tension (mN/m)
Nab	82.45 ± 0.10^{j}	16.30 ± 0.05^{g}
Zag	71.33 ± 0.08^{d}	16.85 ± 0.06^{i}
Sil	82.40 ± 0.12^{j}	16.71 ± 0.08^{h}
Sou	74.66 ± 0.05^{g}	15.68 ± .0.06^{e}
Kai	70.93 ± 0.10^{c}	15.53 ± 0.04^{d}
Meh	78.17 ± 0.09^{i}	17.02 ± 0.04^{j}
Kas	71.73 ± 0.13^{e}	13.83 ± 0.09^{a}
SiB	70.32 ± 0.14^{b}	13.79 ± 0.04^{a}
Sfa	70.20 ± 0.15^{b}	13.85 ± 0.06^{a}
Gaf	76.24 ± 0.12^{h}	15.90 ± 0.05^{f}
Gab	67.23 ± 0.10^{a}	14.25 ± 0.13^{b}
Toz	74.09 ± 0.05^{f}	15.32 ± 0.06^{c}

Nab, Zag, Sil, Sou, Kai, Meh, Kas, SiB, Sfa, Gaf, Gab and Toz: Oils extracted from Nabeul, Zaghouan, Siliana, Sousse, Kairouan, Mehdia, Kasserine, Sidi Bouzid, Sfax, Gafsa, Gabes and Tozeur, respectively. Each value is the mean of 3 independent trials ± S.E. Data sharing different letter are significantly different at $p < 0.05$

Table 4 Inhibitory concentrations (IC50 (mg/mL)) of DPPH· and $ABTS^{+}$ radical scavenging activities of cold pressed *Z. lotus* seed oils from different origins

Location	DPPH IC50 (mg/mL)	$ABTS^{+}$ IC50 (mg/mL)
Nab	4.78 ± 0.12^{g}	3.16 ± 0.11^{e}
Zag	4.80 ± 0.03^{g}	3.08 ± 0.08^{e}
Sil	4.08 ± 0.05^{h}	2.87 ± 0.12^{f}
Sou	4.92 ± 0.06^{a}	3.35 ± 0.10^{a}
Kai	5.60 ± 0.10^{b}	4.10 ± 0.15^{b}
Meh	4.52 ± 0.09^{f}	2.98 ± 0.07^{e}
Kas	5.28 ± 0.07^{e}	3.70 ± 0.10^{a}
SiB	5.02 ± 0.16^{a}	3.56 ± 0.12^{a}
Sfa	6.63 ± 0.11^{d}	4.93 ± 0.20^{d}
Gaf	6.10 ± 0.10^{i}	4.62 ± 0.14^{d}
Gab	6.46 ± 0.20^{d}	4.87 ± 0.16^{d}
Toz	5.95 ± 0.10^{c}	4.33 ± 0.11^{c}

Nab, Zag, Sil, Sou, Kai, Meh, Kas, SiB, Sfa, Gaf, Gab and Toz: Oils extracted from Nabeul, Zaghouan, Siliana, Sousse, Kairouan, Mehdia, Kasserine, Sidi Bouzid, Sfax, Gafsa, Gabes and Tozeur, respectively. Each value is the mean of 3 independent trials ± S.E. Data sharing different letter are significantly different at $p < 0.05$

Declarations

Conflict of Interest The authors declare that there is no conflict of interest.

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