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Fatty Acid Profiling and Oil Content Variation among Iranian Fennel (*Foeniculum vulgare* Mill. var. *vulgare*) Landraces

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ABSTRACT

In this study, 50 Iranian fennel landraces from different phenological types were evaluated for their oil content and fatty acid profile. Landraces were categorized into three phenological groups: late (180 days), medium (140 days) and early maturing habits (110 days). The highest fatty acid content among the early, medium, and late maturity types were detected in Hamedan (19.5%), Marvdasht (23%), and Sari (21%), respectively. The highest yield of oil per square meter among the early, medium, and late maturing types were detected in Fasa (65.3 ml/m^2), Meshkin Shahr (92.5 ml/m^2), and Sari (71.4 ml/m^2), respectively. The main components of fatty acid profile were petroseli nic/oleic acid (52-64%), linoleic acid (26-39%), palmitic acid (0.3-4.1 %), stearic acid (1.3-2.4%), linolenic acid (0.6-3.6%), and myristic acid (0.35-1.07%). It was observed that landraces with high petroselinic and oleic acid content originated from areas with dry and warm environments, while landraces with high linoleic acid content originate d from regions with a humid and cool climate. This pattern shows potential evolutionary adaption of biochemical pathways to the enviro -nmental condition. Our data showed that fennel oil contains a lower ratio of omega-6 to omega-3 fatty acids, and a higher ratio of monouns aturated to polyunsaturated and saturated fatty acids. In conclusion, our results indicated that bitter fennel, as a medicinal plant, has a high potential for oil production and a high percentage of unsaturated fatty acids.

Introduction

The oldest evidence of medicinal plant usage goes back to 60,000 years ago in Shanidar cave, Kurdistan (Lietava, 1992). Fennel is one of the oldest medicinal plants, because of its specific metabolites, and is used as a flavoring agent in food and beverages. Also, it serves as a curative agent in pharmaceutical products (Bahmani et al., 2016).

Bitter fennel (*Foeniculum* vulgare Mill. var. *vulgare*), hereafter “fennel”, is a subspecies and source of fennel-derived drugs. Though it originated from Mediterranean regions, it has been naturalized in many other regions. Fennel produces several valuable phytochemicals in the seeds. One group of these compounds is fatty acids, also called fixed oils, or just oil (Hornok, 1992).

Plant-based oils are considered healthier than animal-based oils, due to a lower ratio of omega-6 to omega-3 fatty acids, and a higher ratio of monounsaturated to polyunsaturated and saturated fatty acids (Vidrih et al., 2009). Exploring new crops as complementary or substituting sources of fatty acids to the current main oil crops, including soybeans, sunflower, canola, is a valuable approach for meeting market

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demand, and diversifying our oil production sources. Currently, species within the family Apiaceae are gaining a lot of attention as potential sources of fatty acids. Among Apiaceae species, fennel is a potential source and a new reservoir of fatty acids, due to its suitability for mechanized mass production, high-yielding seed potential (400-3000 kg/ha), and high fatty acid content (12-20% of seed mass) (Matthaus and Ozcan 2015; He and Huang, 2011; Cosge et al., 2008; Gupta et al., 1995; Reiter et al., 1998; Msanne, 2020).

Seeds are the main storage location of fatty acids in fennel, as they can contain from 3 to 20% oil, with the major fatty acids being C18:1 isomer (25-83%), C18:2 or linoleic acid (1-17%), C16:0, or palmitic acid (0-13%), C14:0 or myristic acid (0-6.5%), C18:3(N3) or linolenic acid (0.3-4%), C18:0 or stearic (0.8-1.9%), and C20:0 or arachidic acid (0-0.4%) (Rezaei Chiyaneh et al., 2020; Hayat et al., 2019; Sayed-Ahmad et al., 2018; Nguyen et al., 2015). Petroselinic acid (18:1-cis6), oleic acid (18:1-9cis), and vaccenic acid (18:1-11cis) are three positional isomers of C18:1 which can make separation and quantification a difficult task, but petroselinic acid is the major isomer among them (Reiter et al., 1998). Both oleic acid and linoleic acid are essential fatty acids with many health benefits in the human nutrition, and with numerous usages in various industries (Sales Campos et al., 2013; Simopoulos, 2008).

High expenses of farm establishment and low-yielding cultivars are discouraging to fennel growers. In turn, this has caused a disequilibrium between fennel demand and supply in current markets. Nowadays, there is a need to develop high-yielding fennel for higher seed yield and essential oil content, associated with shorter maturity habits that could reduce expenses and increase profits. According to a previous study, there is a significant genetic diversity (Izadi-Darbandi et al., 2013), agro-morphological and essential oil variation among Iranian fennel landraces (Bahmani et al., 2015, 2016). In terms of fixed oil, so far, only two studies have concluded that two fennel populations were suitable for fixed oil production and suitable oil compositions (Rezaei Chiyaneh et al., 2020; Sayed Ahmad et al., 2018). Our knowledge on fixed oils in Iranian fennels is still insufficient. With the least incentive to address this issue, the current research was conducted to 1) evaluate potential fixed oil production in 50 Iranian fennel landraces, 2) evaluate fixed oil compositions in 12 of these landraces, and 3) identify highly potential landraces.

Materials and Methods

Plant materials and field experiment

Seeds of 50 fennel landraces (*Foeniculum* vulgare var. *vulgare*) were provided by the seed bank at College of Aburaihan, University of Tehran (Izadi-Darbandi et al., 2013). These were planted in a research field located in Pakdasht, Tehran, under a randomized complete block design with three replications. The seeds of each landrace was sown in a 1.5 m^2 plot in sandy–clay soil. The weeds were eliminated manually and regular irrigation was performed once in every 7 days. During the growing season, no diseases or pests were observed. After seedling emergence, seedlings were thinned to a final plant density of 10 plants per m^2 for each landrace in each plot (Khorshidi et al., 2010; Falzari et al., 2006; El-Gengaihi and Abdallah, 1978). Wheat had grown on this field, two years before the current study. No supplemental fertilizer was applied. In terms of their maturity habit, these Iranian fennel landraces were classified into three different maturity habit groups, i.e. early, medium, and late maturing (with 110, 140, and 180 days to harvest time, respectively).

Extraction of oils

Fatty acids were extracted from the seeds by hexane in an accelerated solvent extraction system (ASE) (Richter et al., 1996, Alameldin, et al., 2017). Seeds from three replications were milled and kept overnight in the oven at 105 °C to reduce moisture content and make it reach below 10%. Then, about 1.3 g of the dried milled seeds from each landrace were placed in an extraction cell. During the extraction process, the conditions were set to 105 °C as the oven temperature, with 10 min static time, 70% flush volume, 60 s purge time, two static cycles, and 6.89 MPa pressure. The extracted oils were air-dried overnight, and then the dry mass and oil percentage were calculated.

Fatty acids methylation

For methyl esterification of fatty acids, the collected oil samples were dried again by evaporating the hexane, followed by the addition of 1 ml methanol: H2SO4 (5:1 by volume) in each sample. After mixing them for a few minutes, the samples were maintained overnight at room temperature. The following day, 1 ml chloroform and 5 ml deionized water were added to each sample and the supernatant phase, which contained fatty acid methyl esters (FAMEs), was separated (Alameldin, et al., 2017).

Gas chromatography

These fatty acids and methyl esters were identified and quantified using Thermo TRACE gas chromatography, coupled with a DSQII mass spectrometry (GC-MS). C19:0 methyl ester served as an internal standard, and four concentrations (0.4 to 400 ng/ml) of a 37-component FAME standard mixture were used as external standards. In GC, a DB-23 column (30 m × 0.25 mm i.d. × 0.25 mm film thickness) was used. Syringe washes were done by ethyl acetate and hexane, where the injection volume was 1 ml, inlet temperature was 250 °C, and helium flow rate was 1.3 ml/min. The MS system had an electron ionization source which operated at 70 eV, and a single quadrupole mass analyzer operated with a 3 min solvent delay. The ion source temperature was set at 250 °C. The raw GC-MS results were processed in the MassLynx program to obtain the final data. The protocols for oil extraction and GC-MS analysis were described in detail (Alameldin, et al., 2017).

Fixed oil contents and fatty acid concentrations were reported as a percentage (%), whereby fixed oil contents were expressed as ml of fixed oil per 100 g of dry ripened seeds (seed mass). For fatty acid concentrations, the measurement was expressed as ml of fatty acids per 100 ml of total fixed oil. Analyses of variance were performed using SAS 9.4 software, according to a randomized complete block design.

Results

There were significant differences among the landraces in terms of oil content (Table 1). Among all landraces, the oil content ranged from 9.5 to 23% and the comparison of mean values, by Duncan's Multiple Range Test, showed their significant differences (Fig 1). Average oil content among early-maturing landraces was 13.45±0.44%, among medium-maturing ones 17±0.74%, and among late-maturing ones 16.7±1.08%. The highest oil contents among early-maturing landraces belonged to Hamedan, Arak, and Mahalat landraces, with 19.55±1.15%, 18.5±0.86%, and 17.5±0.26%, respectively. Marvdasht, Kohn, and Meshkin Shahr landraces were among the medium-maturing landraces, with the highest oil contents, 23±1.7%, 20.5±0.58%, and 19±0.27%, respectively. Among the late-maturing fennels, the Sari landrace had the highest oil content (21±0.57%).

Table 1. Analysis of variance (mean squares) for oil content in the fennel landraces of the second year of growth

SOV	DF	Oil content (%)
Genotype	49	27.54**
Block	2	8.34**
Error	98	1.581
Mean	-	14.66
CV (%)	-	8.7

Oil compositions of the twelve landraces were quantified by GC-MS (Table 2). Sixteen different fatty acids were identified, which constituted 99.5% of the total oil content in the samples. Among these, the most abundant fatty acids were petroselinic and oleic acid (52-64%), linoleic acid (26-39%), palmitic acid (0.3-4.1%), linolenic acid (0.6-3.6%), stearic acid (1.3-2.4%), myristic acid (0.35-1.07%), arachidic acid (0.12-0.83%) and lauric acid (0.15-0.58%). The highest amounts of petroselinic and oleic acid belonged to the Khash, Fozveh, and Arak landraces, whereas the highest amounts of linoleic acid were observed in the Meshkin Shahr and Sari landraces.

The mean percentage of unsaturated fatty acids among the twelve landraces was 91.55±0.54% of the total oil content (Table 2), including 59.43±1.33% monounsaturated fatty acids, primarily petroselinic and oleic acid, and 32.12±1.37% polyunsaturated fatty acid, primarily linoleic acid). The mean value of saturated fatty acids was 7.67±0.63%, which was mainly composed of palmitic acid and stearic acid. Among the twelve fennel landraces, Qazvin, Sari, Rafsanjan, Meshkin Shahr, and Chahestan landraces had the highest ratios of omega-3 to omega-6 fatty acids. The comparison of mean values for oil yield (ml/m^2) among the 50 landraces (Fig. 2) was made by measuring oil volume (with an average density of 0.97 g/ml) and by dividing it with the seed performance in the second year. Among late-maturing fennels, the Sari landrace (71.4 ml/m^2) was prominent. Among medium-maturing landraces, there were Meshkin Shahr and Moqhan (92.5, and 85.4 ml/m^2, respectively), and among early-maturing landraces, there were Fasa, Saqez, and Rafsanjan (65.3, 49.8, and 44 ml/m2, respectively) which had the highest oil yields. For the twelve landraces, the percentages of petroselinic, oleic acid and linoleic acids were the major fatty acids (Table 3).

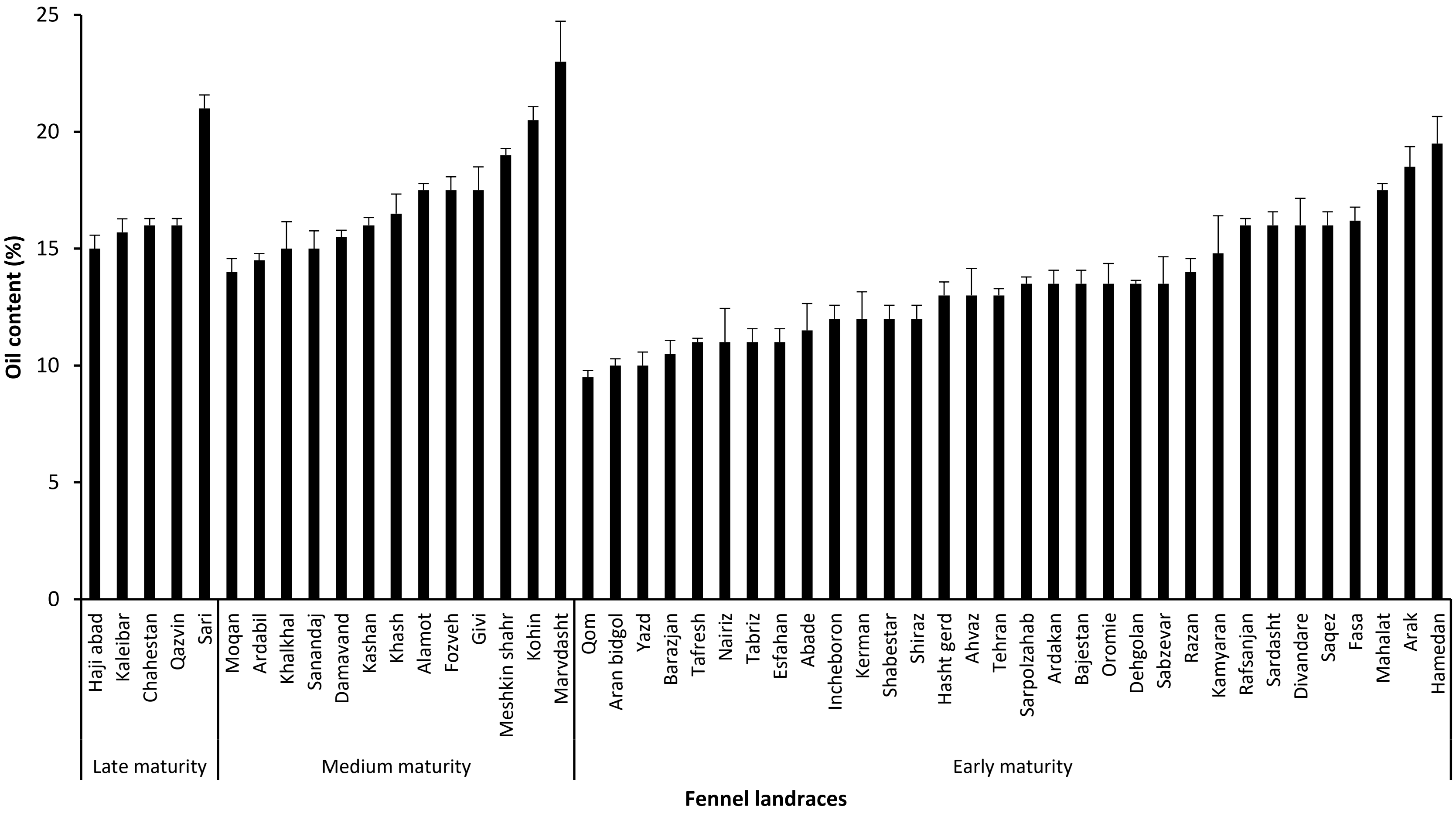

Fig. 1. Total oil content (%) of 50 Iranian fennel landraces in the second year of growth. Error bars represent the standard error of means.

Table 2. The results of GCMS for fatty acids (% of total fatty acids) in 12 select fennel landraces

Composition		Retention time (min)	Late maturity			Medium maturity				Early maturity				
			Sari	**Qazvin**	**Chahestan**	**Meshkin shahr**	**Khash**	**Alamot**	**Fozveh**	**Sanandaj**	**Fasa**	**Rafsanjan**	**Sabzevar**	**Arak**
Caprylic acid	C8:0	3.95	0.204	0.321	0.515	0.247	0.134	0.231	0.171	0.122	0.176	0.502	0.172	0.296
Capric acid	C10:0	4.63	0.133	0.066	0.254	0.278	0.083	0.049	0.077	0.162	0.104	0.166	0.116	0.105
Lauric acid	C12:0	5.46	0.246	0.263	0.579	0.294	0.277	0.308	0.281	0.151	0.337	0.554	0.416	0.348
Myristic acid	C14:0	6.7	0.568	0.729	1.069	0.471	0.359	0.624	0.609	0.577	0.613	1.069	1.019	0.636
Palmitic acid	C16:0	8.6	3.803	2.806	3.914	2.58	3.203	2.774	2.856	2.681	3.112	3.778	2.913	2.913
Margaric acid	C17:0	9.82	0.183	0.36	0.531	0.113	0.221	0.139	0.256	0.096	0.266	0.524	0.311	0.251
Stearic acid	C18:0	11.26	1.386	1.368	2.374	1.104	1.878	1.247	1.679	1.201	2.103	2.097	1.527	1.739
Petroselinic aand Oleic acid	**C18:1**	**11.75**	**52.264**	**54.335**	**58.709**	**52.098**	**64.523**	**57.415**	**64.641**	**58.061**	**60.127**	**60.562**	**62.566**	**64.984**
Linoleic acid	**C18:2**	**12.36**	**35.268**	**32.135**	**27.352**	**39.447**	**27.02**	**33.651**	**27.123**	**34.257**	**29.249**	**26.091**	**28.505**	**26.467**
Linolenic acid	C18:3(N3)	12.82	2.321	3.624	1.601	2.184	0.675	1.352	1.025	1.174	1.427	1.658	1.01	0.881
Arachidic acid	C20:0	14.5	0.19	0.351	0.557	0.131	0.382	0.163	0.321	0.125	0.833	0.559	0.318	0.308
Paullinic acid	C20:1(N9)	14.9	0.164	0.219	0.452	0.149	0.287	0.236	0.203	0.097	0.392	0.16	0.282	0.253
Heneicosylic acid	C21:0	16.24	0.095	0.15	0.221	0.069	0.067	0.095	0.041	0.082	0.1	0.082	0.084	0.044
Behenic acid	C22:0	18.54	0.322	0.815	0.766	0.117	0.307	0.179	0.248	0.12	0.337	0.954	0.317	0.363
Tricosylic acid	C23:0	19.82	0.284	0.453	0.392	0.226	0.297	0.273	0.165	0.211	0.108	0.562	0.095	0.062
Lignoceric acid	C24:0	21.04	0.377	0.514	0.63	0.291	0.28	0.463	0.302	0.305	0.377	0.679	0.339	0.346
Sum			98.308	98.509	99.916	99.799	99.993	99.199	99.998	99.422	99.661	99.997	99.99	99.996
SFA[z]	-	-	4.291	8.196	11.802	5.921	7.488	6.545	7.006	5.833	8.466	11.526	7.627	7.411
UFA	-	-	90.017	90.313	88.114	93.878	92.505	92.654	92.992	93.589	91.195	88.471	92.363	92.585
MUSA	-	-	52.428	54.554	59.161	52.247	64.81	57.651	64.844	58.158	60.519	60.722	62.848	65.237
PUFA	-	-	37.589	35.759	28.953	41.631	27.695	35.003	28.148	35.431	30.676	27.749	29.515	27.348
UFA/SFA	-	-	20.978	11.019	7.466	15.855	12.353	14.156	13.273	16.044	10.771	7.675	12.110	12.492
Omega3/omega6			0.065	0.112	0.058	0.055	0.024	0.040	0.037	0.034	0.048	0.063	0.035	0.033

zSFA: saturated fatty acid, UFA: unsaturated fatty acid, MUSA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid

Table 3. Fatty acid compositions in group 1 (petroselinic and oleic acid-rich landraces) and group 2 (linoleic acid-rich landraces)

Group	Climate (precipitation and annual temperature)	petroselinic and Oleic acid	Linoleic acid	Linolenic acid	Stearic acid	Arachidic acid	Palmetic acid
1	Dry/warm (154mm±24 and 16.7ºC±0.6)	62.4%±0.95	27.34%±0.42	1.18%±0.14	1.91%±0.11	0.47%±0.07	3.24% ±0.16
2	Humid/cool (465mm±60 and 10.5ºC±0.7)	54.6%±1.25	34.95%±1.23	2.13%±0.38	1.26%±0.05	0.19%±0.04	2.22%±0.48

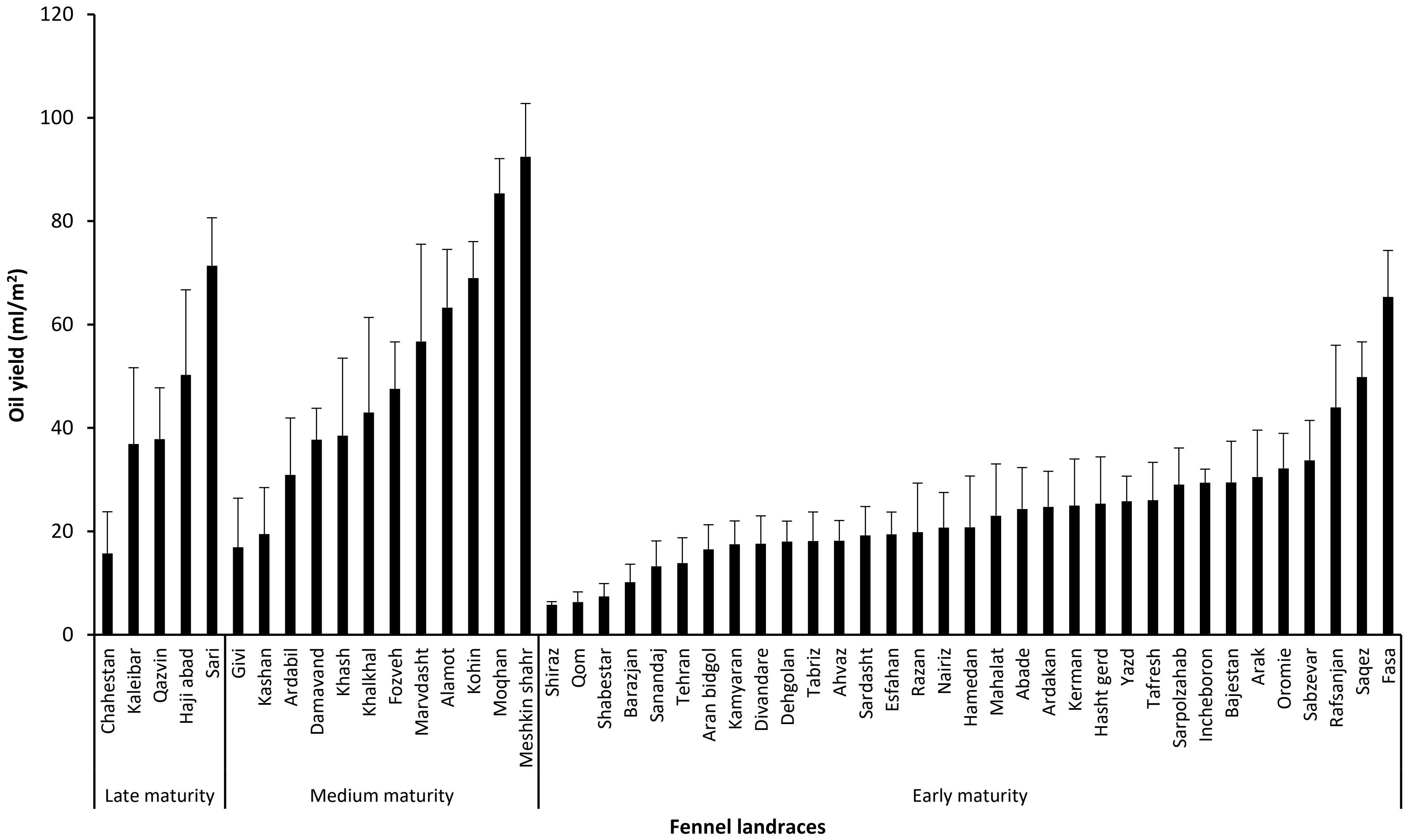

Fig. 2. Total oil yield (ml/m2) of 50 Iranian fennel landraces in the second year of growth. Error bars represent the standard error of means.

The twelve fennel landraces were grouped according to their fatty acid profiles (Fig. 3, Table 3), which yielded two groups. Group 1 contained higher contents of petroselinic and oleic acid (62.3±0.95%), stearic acid (1.91±0.11%), arachidic acid (0.47±0.070%), and palmitic acid (3.24±0.16%), compared to group 2. Meanwhile, group 2 contained higher contents of linoleic acid (34.95±1.23%) and linolenic acid (2.13±0.38%). The landraces in group 1 (petroselinic and oleic acid chemotypes) originated in regions with dry and warm climates (eastern Zagros Mountains, and southern Alborz Mountains), while the landraces in group 2 (linoleic acid chemotypes) originated in regions with humid and cool climates (the western Zagros Mountains, and northern Alborz Mountains). These results (Table 3) showed that the amounts of linoleic and petroselinic acid were higher in humid/cool and dry/warm conditions, respectively.

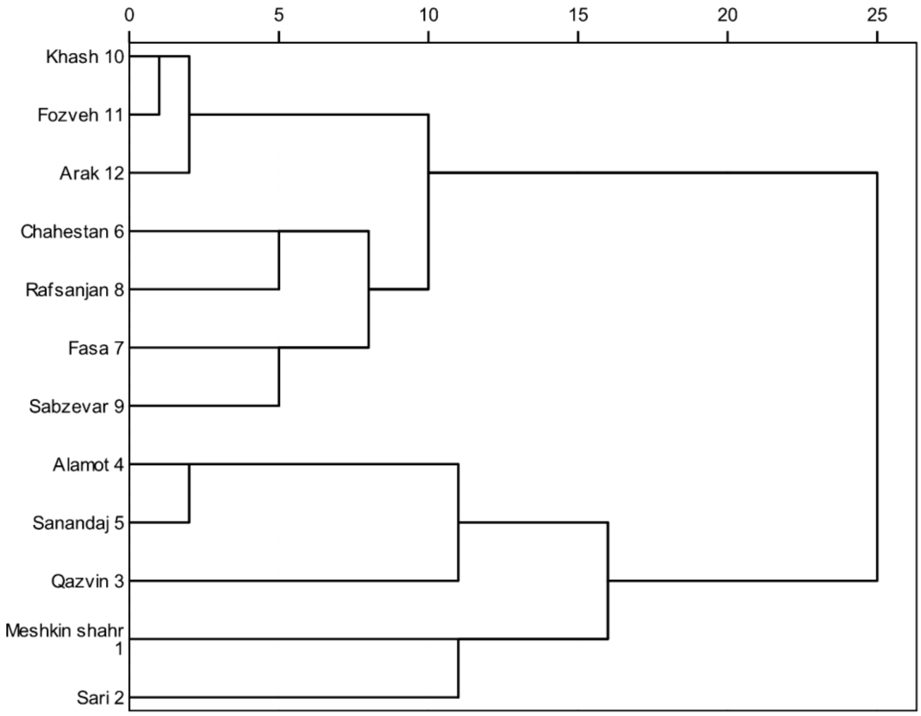

Fig. 3. Dendrogram of the landraces based on fatty acid compositions by the Euclidean coefficient and WARD methods.

Discussion

The 50 Iranian fennel landraces exhibited considerable diversity in terms of oil yield. Specifically, higher oil yields were observed in Sari, Haji Abad, Meshkin Shahr, Moqhan, Kohin, Alamot, Marvdasht, Fasa, Saqez, and Rafsanjan landraces. The main oil compositions in the twelve fennel landraces were petroselinic and oleic acid, which were similar to the results of previous studies on other non-Iranian fennels (Hayat et al., 2019; Agarwal et al., 2018). The results were also similar to the two previously studied Iranian fennel populations (Rezaei Chiyaneh et al., 2020; Sayed Ahmad et al., 2018). It was reported that petroselinic and oleic acids were two of the major fatty acids in other Apiaceae species, such as dill, celery, cumin, coriander, and carrot (Gao et al., 2016; Uitterhaegen et al., 2016; Sowbhagya, 2014; Amin et al., 2010; Saleh et al., 2009).

It seems that the landraces with higher petroselinic and oleic acids, all originated in regions with dry/warm climates (eastern Zagros Mountains, and southern Alborz Mountains), while landraces with higher linoleic acid (linoleic acid chemotypes) all originated in regions with humid/cool climates (the western Zagros Mountains, and northern Alborz Mountains) (Table 3). This pattern showed the potential evolutionary adaption of biochemical pathways to environmental conditions, as experienced by plant ancestors over a long period. Changes in fatty acid profiles by factors related to the climate were reportedly observed in many plant species

(Mustiga et al., 2019; Raziei et al., 2018). One reason for such a pattern could be the partially shared biosynthetic pathway for petroselinic acid, oleic acid and linoleic acid, which may be affected by environmental factors. These factors may shift the pathway more toward one of the components and reduce the production of others (i.e. a negative correlation between oleic and linoleic acids). A pattern like what we found here can help breeders in high, throughput preliminary screening programs.

It has been reported that temperature is positively associated with palmitic, arachidic, and stearic acid concentrations, while increasing the temperature negatively impacted linoleic and oleic acid concentrations (Mustiga et al., 2019). Also, Raziei et al. (2018) reported that lower temperatures can increase the production of unsaturated fatty acids, such as petroselinic and oleic acids. Hixson and Arts (2016) reported that phytoplankton temperature is negatively associated with omega-3 fatty acids, such as linolenic acid, while it was positively associated with omega-6 fatty acids, such as linoleic acid. For the most part, our results are compatible with these previous studies, except for oleic acid, which was similar to what Hixson and Arts (2016) reported, but contradictory to what Mustiga et al. (2019) and Raziei et al. (2018) reported. Analyzing a higher number of samples from different climates could clarify potential relationships between temperature, petroselinic and oleic acids.

The petroselinic and oleic acid chemotypes, compared to the linoleic acid chemotypes, had higher amounts of monounsaturated fatty acids (62.6±0.9% vs 55±1.2%) and saturated fatty acids (8.8%±0.7 vs 6.1%±0.6), whereas linoleic acid chemotypes had more polyunsaturated acids (25.1%±1.2 vs. 16.5%±0.4). Compared to petroselinic and oleic acid chemotypes, the linoleic acid chemotypes had a higher ratio of unsaturated to saturated fatty acids (15.6±1.6 vs 10.8±0.8), which makes them healthier sources of oil for human use. Petroselinic and oleic acid chemotypes also contained higher concentrations of stearic acid (1.91±0.11%), arachidic acid (0.47±0.070%), and palmitic acid (3.24±0.16%), compared to linoleic acid chemotypes, whereas linoleic acid chemotypes contained higher contents of linolenic acid (2.13±0.38%). Our data (Table 2) showed that fennel oil contained a lower ratio of omega-3 to omega-6 fatty acids (less than 0.6), and a higher ratio of monounsaturated to polyunsaturated and saturated fatty acids. These two features can be used as screening indicators or quality characteristics for edible oils (Vidrih et al., 2009; Alameldin et al., 2017).

Conclusion

Fatty acid profiling by GCMS analysis indicated that some of the fennel landraces had the potential to be complementary sources of certain fatty acids, such as petroselinic and oleic acids or linoleic acid. It was observed that landraces with high amounts of petroselinic and oleic acids originated in areas with dry and warm environments, while landraces with high linoleic acid content originated in regions with humid and cool climates. This pattern showed a potential evolutionary adaption of biochemical pathways to the environmental condition. A pattern of diversity like this can make the search for specific fennel chemotypes faster. The Meshkin Shahr landrace was classified as a linoleic-rich chemotype and could be introduced as a superior ecotype because of its highest oil content and seed-related performance. Furthermore, it had high ratios of unsaturated to saturated fatty acids (15.85), and a low ratio of omega-3 to omega-6 (0.05) which are important health-related indicators in edible oils. In conclusion, our results indicated that the bitter fennel, as a medicinal plant, has a high potential for oil production while having a high percentage of unsaturated fatty acids.

Conflict of interest

There are no conflicts of interest to declare.

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