Erwerbs-Obstbau
https://doi.org/10.1007/s10341-017-0351-9

ORIGINAL ARTICLE

Seed Oil Quantity and Fatty Acid Composition of Different Sea Buckthorn (*Hippophae Rhamnoides* L.) Wild Populations in Iran

Ali Kuhkheil[1] · Ali Mehrafarin[2] · Vahid Abdossi[1] · Hassanali Naghdi Badi[2]

Received: 4 December 2016 / Accepted: 15 September 2017

Abstract Sea buckthorn (*Hippophae rhamnoides* L.) is a valuable multipurpose medicinal plant belonging to Elaeagnaceae family. In this study, oil content and fatty acid composition of seed oil from 20 sea buckthorn wild populations of Iran were evaluated in 2014 and 2015. Oil extraction was done using *n*-hexane solvent in a Soxhlet apparatus and then GC analysis of fatty acid methyl esters was performed. The highest and lowest amount of seed oil content in two years were obtained from Sarein and Kejel populations, respectively (3.88 to 8.63% in 2014 and from 4.07 to 9.66% in 2015). Importance of seed oil is related to it's highly amount of unsaturated fatty acids. Maximum content of oleic acid cis were existed in Kelavenga (21.19%) on 2014. The highest value of oleic acid trans were related to Shahrestanak (6.34%) in 2015. Maximum content of linoleic acid in 2014 were existed in Baladeh (42.03%) on 2015. The highest linolenic acid content were obtained from Dehdar (30.73%) in 2014. Factor analysis based on PCA revealed, first three components (PC1–PC3) explained 63.86% of the total variation. The first component (PC1) was contributed by some traits such as seed oil percentage, Palmitoleic acid, oleic acid trance and linoleic acid contents with about 29% of total variation. Hierarchical cluster analysis divided the populations into four main groups with high diversity. Wide range of variation across the sea buckthorn populations in seed oil traits could be exploited for selection of suitable genotypes to improvement and commercial exploitation of this plant.

Keywords Hippophae rhamnoides · Hierarchical cluster · Populations · Seed oil · Fatty acids

Kernölmenge und Fettsäurezusammensetzung verschiedener, im Iran wild wachsender, Sanddorn-Herkünften (*Hippophae rhamnoides* L.)

Schlüsselwörter Hippophae rhamnoides · Hierarchisches Cluster · Bestände · Kernöl · Fettsäuren

Introduction

Hippophae rhamnoides L., known as sea buckthorn is a deciduous shrub or tree belonging to Elaeagnaceae family. It is a thorny and nitrogen fixing plant with high nutraceutical and therapeutical properties. It is widely distributed in temperate zone of Asia, Europe and North America. It also grows in a distinct area from the Elburz Mountains in Persia to Caucasia and eastern Turkey (Rousi 1971). Its flower, seed, leaf and fruit possesses an exclusive composition of vitamins, nutrients and essential fatty acids. Growth and performance of plants are under influence of different factors such as genetic characteristics, regional climate and geographical location, etc. Furthermore, previous studies have demonstrated that medicinal plants produce various contents of secondary metabolites in different environments, resulting in differences in their medicinal qualities (Dong et al. 2011).

Oil of sea buckthorn seeds is the most valuable product of this plant. Extracted oil from pulp and seed of sea buck-

✉ Hassanali Naghdi Badi
Naghdibadi@yahoo.com

[1] Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Medicinal Plants Research Centre, Institute of Medicinal Plants, ACECR, Karaj, Iran

Published online: 10 October 2017

thorn presented different fatty acid composition according to the subspecies, origins, cultivation activities, harvesting time of the berries, and the extraction method (Yang and Kallio 2002b). There are two sources of oil in sea buckthorn fruit, the seed which contains 6.47–20.2% (w/w) oil, and the oil held in the pulpy fruit parts surrounding the seed which is termed pulp oil (Li and Beveridge 2003). Of course, its pulp oil contains more saturated fatty acids while seed oil contains highly amount of unsaturated fatty acids. So the seed oil has more important therapeutic effects such as preventing heart disease and arthritis (Yang and Kallio 2002a). Solvent extraction using hexane, chloroform, acetone and methanol in a soxhelt apparatus and also supercritical CO_2 extraction are the main methods used for sea buckthorn oil extraction, both in industrial and laboratory scale (Gutierrez et al. 2008). Hexane has the advantage of low toxicity and easy removal from the extracted oil with distillation procedures (Li and Beveridge 2003).

The sea buckthorn grown widely in central and Northern provinces of Iran and have used in Iranian folk medicine. In several studies, oil content and major fatty acid composition of the oils extracted from seeds of sea buckthorns populations growing in different regions of the world were investigated. According to most studies, the predominant fatty acids in seed oil are polyunsaturated fatty acids (65–72%) such as linoleic and linolenic acids (Cenkowski et al. 2006; Fatima et al. 2012; Gutierrez et al. 2008; Li and Beveridge 2003). The human body absolutely requires these polyunsaturated fatty acids. Both fatty acids repair the cell membrane after oxidation due to attack of free radicals. Sea buckthorn seed oil is very high in these two essential fatty acids. Seed oil accumulates at a very rapid rate with the onset of maturation to a maximum and thereafter is constant or decreases as the fruit becomes mature and ripens (Li and Beveridge 2003). The seed oil from different fruit origins vary in linoleic (37–44%), linolenic (27–31%), palmitic (7–9%), stearic (2.5–3%), and vaccenic acids (2.2–2.8%) (Yang and Kallio 2001).

Despite important of sea buckthorn seed oil, the study about sea buckthorn seed oil quantity and quality of Iran aren't well documented. The present study was carried to determine the variations of seed oil content and fatty acid composition in wild populations of sea buckthorn growing in Iran. The results of this study are useful to identify suitable sea buckthorn populations when organizing the berry breeding programs and also provides important information for food and pharmaceutical industry.

Material and Methods

Plant Material and Collection

20 sea buckthorn populations were collected and evaluated from their different natural habitat in Iran in middle October 2014 and 2015. Voucher specimens have been deposited in the herbarium of Medicinal Plants Institute (MPI), ACECR,

Table 1 Geographical origins of *H. rhamnoides* populations

Population No	Herbarium No	Region originated	Latitude (N)	Longitude (E)	Altitude (m)
1	MPIH-4529	Ardebil-Sarein	38° 05′ 42″ N	48° 07 39″ E	1474
2	MPIH-4528	Ardebil-Kejel	37° 25′ 25″ N	48° 10′ 48″ E	1703
3	MPIH-4515	Alborz-Bozaj	36° 12′ 42″ N	50° 48′ 35″ E	2298
4	MPIH-4511	Alborz-Parachan	36° 14′ 45″ N	50° 56′ 49″ E	2339
5	MPIH-4507	Alborz-Khodkavand	36° 08′ 35″ N	50° 49′ 59″ E	2231
6	MPIH-4510	Alborz-Dehdar	36° 11′ 22″ N	50° 03′ 06″ E	2328
7	MPIH-4516	Alborz-Shahrestanak	35° 58′ 23″ N	51° 21′ 28″ E	2220
8	MPIH-4512	Alborz-Shahrak	36° 10′ 32″ N	50° 46′ 47″ E	1830
9	MPIH-4517	Tehran-Jajrood	35° 45′ 53″ N	51° 41′ 35″ E	1481
10	MPIH-4519	Tehran-Dizin	36° 06′ 18″ N	51° 21′ 18″ E	2380
11	MPIH-4530	Zanjan-Zanjan	36° 38′ 13″ N	48° 30′ 33″ E	1675
12	MPIH-4518	Semnan-Shahmirzad	35° 47′ 08″ N	53° 18′ 54″ E	2191
13	MPIH-4525	Qazvin-Zarabad	36° 29′ 36″ N	50° 26′ 14″ E	1802
14	MPIH-4526	Qazvin-MoallemKelaye	36° 27′ 13″ N	50° 28′ 44″ E	1615
15	MPIH-4527	Qazvin-Razmian	36° 32′ 16″ N	50° 12′ 57″ E	942
16	MPIH-4524	Gilan-Astara	38° 26′ 52″ N	48° 48′ 22″ E	10
17	MPIH-4520	Mazandaran-Baladeh	36° 11′ 24″ N	51° 47′ 35″ E	2070
18	MPIH-4521	Mazandaran-Kelavenga	36° 11′ 19″ N	51° 26′ 11″ E	2817
19	MPIH-4522	Mazandaran-Gachsar	36° 06′ 54″ N	51° 19′ 32″ E	2293
20	MPIH-4523	Mazandaran-Yoosh	36° 11′ 27″ N	51° 42′ 48″ E	2180

Karaj, Iran. geographical origins of the 20 sea buckthorn populations and their GPS coordinates are shown in Table 1.

Oil Extraction

10 g of the dried seeds were milled and placed in an extraction thimble and extracted with organic solvent *n*-hexane using a 250 ml capacity soxhlet apparatus for 8 h (60 °C) in 3 replications (AOCS 1989). The oil was then separated by rotary-evaporated under reduced pressure at 35 °C.

GC Analysis of Fatty Acid Methyl Esters

Determination of the fatty acids was done by gas chromatographic measurement of the prepared samples. We used a Unicam 4600 GC instrument was equipped with a flame ionization detector and a split/splitless injector. A fused-silica capillary column BPX70 (SGE, Melbourne, Australia) with 30 m length, 0.22 mm internal diameter and 0.25 µm thickness was used for analysis. Injector and detector temperatures were 230 and 250 °C, respectively. Oven conditions were 180 °C increased to 220 °C at a rate of 2 °C/min and maintained for 5 min. Helium was the carrier gas and nitrogen used as the make-up gas at a flow rate of 30 ml/min. The quantification of fatty acid methyl esters composition was realized by integration of the FID peak area and comparing their retention times with standards methyl esters to be expressed by percentage (El-Adawy et al. 1999). All the GC analyses were run in triplicate and the average values were reported in the work. All of chemical materials and solvents used in this study has prepared from Merck Company, Germany.

Data Analysis

Analysis of variance was performed for all traits by SPSS statistics (ver. 22) software. ANOVA analysis and mean comparison of the seed oil content and fatty acid composition were done by using Duncan multiple range tests at $p \leq 0.05$ significant level. In order to determine the most variable characters among the populations, factor analysis based on principal component analysis (PCA) was performed. Hierarchical cluster analysis of studied populations was based on the Euclidean distances of traits using Wards method. The simple correlation coefficient was calculated to determine the relationships between the studied traits using the Pearson correlation coefficient.

Results and Discussion

Seed oil content and all of fatty acid composition (including myristic acid ($P < 0.01$), palmitic acid ($P < 0.01$), Palmitoleic acid ($P < 0.01$), stearic acid ($P < 0.01$), oleic acid cis ($P < 0.01$), oleic acid trance ($P < 0.05$) and linoleic acid ($P < 0.01$)) except linolenic acid changed significantly in the experimental years. Also, variance analyses showed that the various populations had significantly differences in respect of seed oil content and fatty acid composition ($P < 0.01$) in two studied years (Table 2). The average seed oil content was 6.09% and 6.89% in 2014 and 2015 year, respectively.

Table 2 Analysis of variance for effect of sea buckthorn populations and experimental years on seed oil content fatty acid composition and their interaction

Source of Variation	Year	Degree of Freedom (df)	Mean Squares								
			Seed Oil (%)	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid cis	Oleic acid Trance	Linoleic acid	Linolenic acid
Population (Treat)	2014	19	5.729^{**}	0.005^{**}	13.697^{**}	38.884^{**}	3.541^{**}	23.566^{**}	1.625^{**}	52.304^{**}	25.839^{**}
	2015	19	7.459^{**}	0.017^{**}	23.342^{**}	24.09^{**}	6.376^{**}	15.247^{**}	2.75^{**}	36.606^{**}	23.111^{**}
	Means	19	12.706^{**}	0.012^{**}	27.947^{**}	36.661^{**}	7.243^{**}	27.654^{**}	2.016^{**}	57.231^{**}	29.448^{**}
Year (Y)	–	1	18.965^{**}	0.014^{**}	46.635^{**}	47.511^{**}	34.563^{**}	989.674^{**}	0.683^{*}	112.162^{**}	0.104^{ns}
Block (REP)	2014	2	0.067^{ns}	0.00002^{ns}	0.025^{ns}	1.123^{ns}	0.027^{ns}	0.081^{ns}	0.002^{ns}	0.298^{ns}	3.330^{ns}
	2015	2	0.23^{ns}	0.002^{ns}	0.484^{ns}	0.753^{ns}	0.032^{ns}	0.055^{ns}	0.097^{ns}	0.051^{ns}	1.948^{ns}
	Means	2	0.095^{ns}	0.0008^{ns}	0.365^{ns}	1.846^{ns}	0.0001^{ns}	0.134^{ns}	0.063^{ns}	0.062^{ns}	5.129^{ns}
$P \times Y$	–	19	0.481^{ns}	0.011^{**}	9.093^{**}	26.312^{**}	2.675^{**}	11.159^{**}	2.359^{**}	31.679^{**}	19.501^{**}
Error	2014	38	0.709	0.0004	0.980	2.316	0.295	1.620	0.135	3.304	2.514
	2015	38	0.719	0.0013	1.64	1.815	0.511	1.429	0.229	3.989	1.396
	Means	78	0.701	0.0009	1.28	2.013	0.394	1.486	0.179	3.56	1.909
CV (%)	2014	–	13.824	7.284	6.281	23.561	19.505	8.255	9.233	5.761	6.673
	2015	–	12.314	12.088	7.530	17.457	18.529	12.355	11.586	5.964	4.960
	Means	–	12.903	10.197	6.906	20.016	18.899	9.715	10.434	5.802	5.808

ns non-significant, *significant at 0.05, **significant at 0.01 probability level

Table 3 Results of means comparisons for seed oil traits among studied Hippophae rhamnoides populations during 2014 and 2015 harvesting season

Treatment (Population)																					
Traits	Year	Sarein	Kejel	Bozaj	Parachan	Khod-kavand	Dehdar	Shahres-tanak	Shahrak	Jajrood	Dizin	Zanjan	Shah-mirzad	Zarabad	Moallem kelaye	Razmian	Astara	Baladeh	Kelavenga	Gachsar	Yoosh	Mean
Seed Oil (%)	2014	8.63^{a}	3.88^{g}	5.94^{def}	5.28^{efg}	5.27^{efg}	4.69^{fg}	8.20^{ab}	6.75^{bcde}	7.55^{abc}	5.2^{8efg}	5.31^{efg}	7.84^{abc}	5.8^{9def}	5.30^{efg}	7.65^{abc}	7.22^{abcd}	6.47^{cde}	4.44^{fg}	4.35^{fg}	5.89^{def}	6.09^{b}
	2015	9.66^{a}	4.07^{g}	6.59^{cde}	7.38^{bc}	5.78^{cdef}	5.52^{defg}	9.29^{a}	7.01^{bcd}	8.28^{ab}	5.56^{defg}	6.13^{cdef}	8.64^{ab}	5.66^{def}	7.30^{bc}	8.28^{ab}	8.56^{ab}	7.31^{bc}	5.01^{efg}	4.67^{fg}	7.03^{bcd}	6.89^{a}
	Mean	9.14^{a}	3.97^{h}	6.26^{de}	6.33^{de}	5.52^{efg}	5.11^{fg}	8.75^{ab}	6.88^{cd}	7.91^{bc}	5.42^{efg}	5.72^{ef}	8.24^{ab}	5.78^{ef}	6.30^{de}	7.97^{b}	7.89^{bc}	6.89^{cd}	4.72^{fgh}	4.51^{gh}	6.46^{de}	6.49
Myristic acid	2014	0.23^{jk}	0.26^{fghi}	0.28^{defgh}	0.32^{abc}	0.23^{ijk}	0.29^{cdefg}	0.27^{efgh}	0.26^{fghij}	0.20^{k}	0.32^{abc}	0.33^{abc}	0.30^{cdef}	0.36^{a}	0.29^{cdefg}	0.25^{ghij}	0.27^{efgh}	0.32^{abcd}	0.25^{hij}	0.34^{ab}	0.31^{bcde}	0.28^{b}
	2015	0.25^{efg}	0.18^{h}	0.28^{defg}	0.33^{bcd}	0.29^{def}	0.31^{cdef}	0.39^{b}	0.36^{bc}	0.36^{bc}	0.37^{bc}	0.25^{fg}	0.22^{gh}	0.29^{def}	0.32^{bcde}	0.18^{h}	0.33^{bcd}	0.24^{fgh}	0.35^{bcd}	0.32^{bcde}	0.50^{a}	0.31^{a}
	Mean	0.24^{gh}	0.22^{gh}	0.28^{ef}	0.33^{bc}	0.26^{fg}	0.30^{cdef}	0.33^{bc}	0.31^{bcde}	0.28^{ef}	0.35^{b}	0.29^{def}	0.26^{fg}	0.32^{bcd}	0.31^{bcde}	0.22^{h}	0.30^{cde}	0.28^{ef}	0.30^{cdef}	0.33^{bc}	0.40^{a}	0.29
Palmitic acid	2014	15.36^{de}	17.04^{abcde}	15.96^{cde}	17.32^{abc}	11.68^{f}	15.17^{e}	17.22^{abcd}	12.39^{f}	11.69^{f}	15.74^{cde}	18.09^{ab}	15.35^{de}	16.25^{bcde}	18.09^{ab}	18.31^{a}	18.82^{a}	16.43^{bcde}	16.01^{cde}	13.02^{f}	15.26^{e}	15.76^{b}
	2015	18.09^{bcde}	18.14^{bcde}	12.23^{ij}	16.40^{efg}	13.76^{hi}	17.81^{cdef}	20.74^{a}	13.53^{hi}	15.45^{fgh}	15.17^{gh}	21.32^{a}	16.84^{defg}	17.51^{defg}	17.86^{cdef}	20.14^{abc}	20.26^{ab}	11.11^{j}	17.34^{defg}	17.41^{defg}	19.05^{abcd}	17.01^{a}
	Mean	16.73^{cde}	17.59^{cd}	14.10^{gh}	16.86^{cde}	12.72^{h}	16.49^{cdef}	18.98^{ab}	12.96^{h}	13.57^{h}	15.46^{efg}	19.70^{a}	16.10^{def}	16.88^{cde}	17.97^{bc}	19.22^{ab}	19.54^{a}	13.77^{h}	16.67^{cde}	15.22^{fg}	17.15^{cd}	16.38
Palmi-toleic acid	2014	3.86^{ef}	9.47^{abc}	8.94^{bcd}	3.90^{ef}	1.73^{f}	2.82^{f}	3.80^{ef}	3.83^{ef}	1.73^{f}	10.18^{ab}	11.44^{ab}	6.21^{de}	9.26^{abc}	12.11^{a}	2.50^{f}	8.73^{bcd}	9.62^{abc}	1.73^{f}	10.16^{ab}	7.19^{cd}	6.46b
	2015	6.54^{cd}	10.98^{ab}	6.28^{de}	6.45^{cde}	10.40^{ab}	9.93^{ab}	4.70^{def}	8.87^{bc}	3.95^{ef}	10.36^{ab}	11.11^{ab}	$5.14d^{ef}$	10.80^{ab}	4.89^{def}	6.74^{cd}	8.92^{bc}	3.94^{ef}	9.45^{ab}	11.79^{a}	3.11^{f}	7.72^{a}
	Mean	5.20^{efg}	10.22^{ab}	7.61^{cd}	5.18^{efg}	6.06^{defg}	6.37^{def}	4.25^{gh}	6.35^{def}	2.83^{h}	10.27^{ab}	11.28^{a}	5.68^{efg}	10.03^{ab}	8.50^{bc}	4.62^{fg}	8.82^{bc}	6.78^{de}	5.59^{efg}	10.97^{a}	5.15^{efg}	7.09
Stearic acid	2014	4.90^{a}	1.73^{c}	2.54^{bc}	2.14^{bc}	2.39^{bc}	2.27^{bc}	5.64^{a}	2.59^{bc}	3.07^{b}	2.53^{bc}	2.30^{bc}	4.88^{a}	2.86^{b}	2.30^{bc}	2.65^{bc}	2.08^{bc}	2.17^{bc}	2.00^{bc}	1.69^{c}	2.98^{b}	2.79^{b}
	2015	4.76^{cd}	3.90^{de}	3.23^{efg}	2.97^{efg}	2.52^{efg}	3.46^{ef}	5.94^{abc}	5.64^{bc}	2.58^{efg}	2.98^{efg}	1.99^{g}	6.46^{ab}	3.26^{efg}	6.99^{a}	3.07^{efg}	2.49^{fg}	2.80^{efg}	5.29^{bc}	3.51^{ef}	3.33^{efg}	3.86^{a}
	Mean	4.83^{b}	2.81^{defg}	2.89^{defg}	2.55^{efg}	2.45^{efg}	2.86^{defg}	5.79^{a}	4.12^{bc}	2.82^{defg}	2.76^{efg}	2.15^{g}	75.67^{a}	3.06^{def}	4.64^{b}	2.86^{defg}	2.28^{fg}	2.48^{efg}	3.65^{cd}	2.60^{efg}	3.15^{de}	3.32
Oleic acid cis	2014	14.18^{ef}	19.67^{ab}	10.03^{g}	13.51^{ef}	21.07^{a}	14.20^{ef}	16.66^{cd}	14.93^{def}	18.05^{bc}	14.09^{ef}	15.85^{cde}	13.52^{ef}	14.69^{def}	15.99^{cde}	15.91^{cde}	12.68^{f}	12.96^{f}	21.19^{a}	14.61^{def}	14.59^{def}	15.42^{a}
	2015	9.70^{defg}	12.53^{abc}	8.58^{efg}	13.24^{a}	11.35^{abcd}	8.62^{efg}	11.63^{abcd}	8.59^{efg}	13.09^{ab}	9.96^{def}	7.48^{gh}	4.86^{i}	10.87^{bcde}	5.93^{hi}	9.42^{defg}	7.65^{gh}	8.01^{fgh}	10.66^{cde}	11.17^{abcd}	10.19^{def}	9.68^{b}
	Mean	11.94^{cdef}	16.10^{a}	9.30^{h}	13.37^{bc}	16.21^{a}	11.41^{defg}	14.14^{b}	11.76^{cdef}	15.57^{a}	12.02^{cdef}	11.66^{defg}	9.19^{h}	12.78^{bcd}	10.96^{fg}	12.66^{bcd}	10.16^{gh}	10.49^{fgh}	15.93^{a}	12.89^{bcd}	12.39^{cde}	12.55
Oleic acid Trance	2014	2.89^{gh}	3.44^{efg}	3.64^{ef}	4.35^{bcd}	5.15^{a}	4.91^{ab}	3.38^{efg}	4.87^{ab}	3.28^{fgh}	3.73^{def}	3.87^{def}	4.05^{cde}	2.67^{h}	3.79^{def}	5.21^{a}	3.82^{def}	3.51^{efg}	4.56^{abc}	3.68^{def}	4.79^{ab}	3.98^{b}
	2015	3.86^{efghi}	4.29^{cdefg}	3.40^{ghijk}	4.04^{defgh}	3.77^{fghij}	2.54^{k}	6.34^{a}	4.42^{cdef}	4.70^{bcde}	3.24^{hijk}	3.06^{ijk}	5.46^{b}	2.91^{jk}	5.39^{b}	4.04^{defgh}	4.02^{defgh}	4.77^{bcd}	3.39^{ghijk}	3.85^{efghi}	5.13^{bc}	4.13^{a}
	Mean	3.37^{f}	3.86^{def}	3.52^{ef}	4.20^{bcd}	4.46^{abc}	3.73^{def}	4.86^{a}	4.64^{ab}	3.99^{cde}	3.48^{ef}	3.46^{ef}	4.76^{a}	2.79^{g}	4.59^{ab}	4.63^{ab}	3.92^{cdef}	4.14^{bcd}	3.97^{cde}	3.76^{def}	4.96^{a}	4.06
Linoleic acid	2014	32.51^{cd}	26.99^{fg}	34.65^{bc}	33.18^{c}	37.74^{ab}	29.62^{def}	31.38^{cde}	34.58^{bc}	39.72^{a}	31.26^{cde}	22.21^{h}	32.54^{cd}	28.86^{ef}	26.90^{fg}	32.21^{cd}	32.43^{cd}	32.86^{cd}	24.39^{gh}	33.58^{c}	33.50^{c}	31.55^{b}
	2015	34.00^{bcd}	34.23^{bcd}	40.87^{a}	34.24^{bcd}	34.89^{b}	31.52^{bcdef}	29.54^{ef}	34.63^{bc}	30.98^{cdef}	32.58^{bcde}	30.75^{def}	34.82^{bc}	28.57^{f}	35.33^{b}	34.10^{bcd}	34.97^{b}	42.03^{a}	29.51^{ef}	29.29^{ef}	32.90^{bcde}	33.49^{a}
	Mean	33.25^{def}	30.61^{gh}	37.76^{a}	33.71^{de}	36.31^{abc}	30.57^{gh}	30.46^{gh}	34.61^{cd}	35.35^{bcd}	31.92^{efg}	26.48^{i}	33.68^{de}	28.71^{hi}	31.11^{fgh}	33.16^{def}	33.70^{de}	37.44^{ab}	26.95^{i}	31.43^{efg}	33.20^{def}	32.52
Linolenic acid	2014	26.07^{bc}	21.39^{fgh}	23.96^{bcdef}	25.28^{bcd}	20.02^{h}	30.73^{a}	21.65^{fgh}	26.55^{b}	22.28^{efgh}	22.14^{efgh}	25.91^{bcd}	23.16^{cdefg}	25.05^{bcde}	20.53^{gh}	22.96^{defgh}	21.19^{fgh}	22.14^{efgh}	29.88^{a}	22.93^{defgh}	21.39^{fgh}	23.76
	2015	22.79^{fgh}	15.77^{i}	25.13^{bcde}	22.33^{fgh}	23.02^{efg}	25.83^{bc}	20.73^{h}	23.95^{cdef}	28.90^{a}	25.35^{bcd}	24.05^{cdef}	26.20^{bc}	25.79^{bc}	23.31^{defg}	22.31^{fgh}	21.36^{gh}	27.11^{ab}	24.02^{cdef}	22.66^{fgh}	25.80^{bc}	23.82
	Mean	24.43^{cdef}	18.58^{i}	24.54^{cde}	23.80^{cdef}	21.52^{h}	28.28^{a}	21.19^{h}	25.25^{bcd}	25.59^{bc}	23.74^{cdef}	24.98^{cd}	24.68^{cd}	25.42^{bcd}	21.92^{gh}	22.64^{fgh}	21.27^{h}	24.62^{cd}	26.95^{ab}	22.79^{efgh}	23.59^{defg}	23.79

a–h means in each column followed by the same letter (a–h) are not significantly different according to Duncan's multiple range test at the 5% level of probability.

Based on results of mean comparison, the highest amount of seed oil content was obtained from Sarein population in two years as 8.63% and 9.66% for 2014 and 2015 year, respectively. The lowest amount of oil content was obtained from the Kejel population as 3.88% and 4.07% for 2014 and 2015 year, respectively (Table 3). The rate of oil accumulation in the pulp and seed varies depending on environmental (climatic and meteorological) factors (Li and Beveridge 2003). In accordance to our study results on sea buckthorn seeds, Sabir et al. (2005) were found the oil content ranging from 7.69 to 13.7% between studied populations from different areas of northern Pakistan.

It was found that the myristic acid content (0.31%) in second year was more than first year (0.28%). Regarding with this parameter, the highest (0.36%) and lowest (0.20%) value of myristic acid in 2014 year were related to Zarabad and Jajrood populations, respectively. Also, the highest (0.50%) and lowest (0.18%) value of this trait in 2015 year were related to Yoosh and Razmian populations, respectively (Table 3). Previously, Cenkowski et al. (2006) and Gutierrez et al. (2008) reported that myristic acid wasn't present in seed oil of sea buckthorn, whereas Francis (2012) and Li and Beveridge (2003) found that myristic acid was an inseparable part of fatty acid composition of seed oil.

The average of palmitic acid was 15.76% and 17.01% in 2014 and 2015 year, respectively. Based on results of mean comparisons, the highest palmitic acid content were obtained from Astara (18.82%) and Zanjan (21.32%) populations in 2014 and 2015 years and lowest content of this fatty acid were related to Khodkavand (11.68%) and Baladeh (11.11%) in 2014 and 2015 years (Table 3).

Palmitoleic acid had a higher average content in 2015 year (7.72%) in compare with 2014 year (6.46%). Also, maximum Palmitoleic acid content in 2014 and 2015 were reported from Moallemkelaye (12.11%) and Gachsar (11.79%) populations, respectively. Whereas minimum content of this fatty acid were in Kelavenga (1.73%) and Yoosh (3.11%) populations in 2014 and 2015 years, respectively (Table 3). Palmitoleic acid is characteristic of the oils of the fruit coat and pulp, but is low in seed oils (Gao et al. 2000). Vereschagin et al. (1998) reporterd that Palmitoleic acid in the triacylglycerol of pulp/seed oil in wild sea buckthorn fruit of Central Asia and the Baltics (55 and 42%, respectively) higher than in the Caucasian mountain regions (16%).

Mean comparison results showed the stearic acid content (3.86%) in second year was higher than the first year (2.79%). The highest (5.64%) and lowest (1.69%) value of stearic acid in 2014 year were related to Shahrestanak and Gachsar populations, respectively. Also, the highest (6.99%) and lowest (1.99%) value of this trait in 2015 year were related to Moallemkelaye and Zanjan populations, respectively (Table 3).

Same to our work, several studies on sea buckthorn oil showed that both the seeds and soft parts are rich in oleic acid. In first study year, the content of oleic acid cis (15.42%) was more than the second year (9.68%). The maximum and minimum content of this fatty acid in 2014 were existed in Kelavenga (21.19%) and Bozaj (10.03%) populations, respectively. Also, the maximum and minimum content of it in 2015 were in Parachan (13.24%) and Shahmirzad (4.86%), respectively (Table 3). It was found that oleic acid trans content in second year (4.13%) was more than the first year (3.98%). The highest (5.21%) and lowest (2.67%) value of oleic acid trans in 2014 year were related to Razmian and Zarabad populations, respectively. But, the highest (6.34%) and lowest (2.54%) value of this trait in 2015 year were observed in Shahrestanak and Dehdar populations, respectively (Table 3).

The higher content of linoleic acid was observed in second year (33.49%) in compare to first year (31.55%). The maximum and minimum content of this fatty acid in 2014 were occurred in Jajrood (39.72%) and Zanjan (22.21%) populations, respectively. Whereas, the maximum and minimum content of it in 2015 were in Baladeh (42.03%) and Zarabad (28.57%), respectively (Table 3).

The average of linolenic acid content was 23.76% and 23.82% in 2014 and 2015 year, respectively. The highest and lowest linolenic acid content in two years were related to Dehdar (28.28%) and Kejel (18.58%) populations, respectively.

Previously, several studies indicated high content of unsaturated fatty acids (32–40% linoleic, 20–39% linolenic, and 13–17% oleic acids) and lower concentration of saturated fatty acids (8–13% palmitic and 3–8% steric acids) in oil seed of *H. rhamnoides* subspecies *sinensis* and *H. rhamnoides* subspecies *rhamnoides* (Yang and Kallio 2002a; Li and Beveridge 2003; Gutierrez et al. 2008). Linoleic acid (omega 6) and linolenic acid (omega 3) are the essential fatty acids of human body and they carry all fat soluble vitamins i. e., vitamin A, D, E and K and also their important function is to promote cognitive function and bone health (Kumar er al. 2011).

Principal Components Analysis (PCA)

Factor analysis was used based on principal components to provide a reduced dimension model indicating differences measured among groups. PCA allows to evaluate multicollinear data and to determine the traits most suitable for classification. PCA indicated five components with explaining 88.31% of the total variance. The first three components (PC1–PC3) explained 63.86% of the total variation (Table 4). In the first PC (PC1), some characters such as seed oil percentage, and Palmitoleic acid, oleic acid trance and linoleic acid content showed the highest variance. Also, in

Table 4 Eigenvectors of the first three principal component axes from PCA analysis of fatty acid quantity and quality variables in studied *H. rhamnoides* populations

Character	Component		
	1	2	3
Seed Oil	0.81**	0.22	0.25
Palmitic acid	–0.15	–0.01	0.39
Myristic acid	–0.19	0.89**	0.12
Palmitoleic acid	–0.83**	0.10	0.10
Stearic acid	0.61	0.48	0.22
Oleic acid cis	–0.16	0.03	–0.76**
Oleic acid Trance	0.69**	0.22	–0.28
Linoleic acid	0.59**	–0.64**	–0.14
Linolenic acid	–0.03	–0.41	0.67**
Eigenvalue	2.63	1.70	1.42
% of variance	29.20	18.91	15.75
Cumulative%	29.20	48.11	63.86

** Eigenvalues are significant ≥ 0.50

PC2, myristic acid and Linoleic acid contents showed the highest variance. While, the highest variance were observed for oleic acid cis and Linolenic acid contents in PC3.

Cluster Analysis

Cluster analysis based on Wards method at similarity coefficient of 10, divided populations into four main groups with high diversity (Fig. 1). The first main group was divided into six populations. The first group (A) consisted of populations from Dizin, Gachsar, Zanjan, Zarabad, Dehdar and Kelavenga with similar characteristics such as seed oil content and linoleic acid. The second group (B) was comprised of Kejel population. Some of the prominent characteristics of this population are seed oil content, oleic acid cis and linolenic acid which made it distinct among the other populations. The third group (C) was comprised of Parachan, Yoosh, Moallemkelaye, Astara, Sarein, Razmian and Shahrestanak populations. At last fourth group consisted of Khodkavand, Jajrood, Bozaj, Baladeh, Shahrak and Shahmirzad with similar characteristics such as myristic acid and palmitic acid.

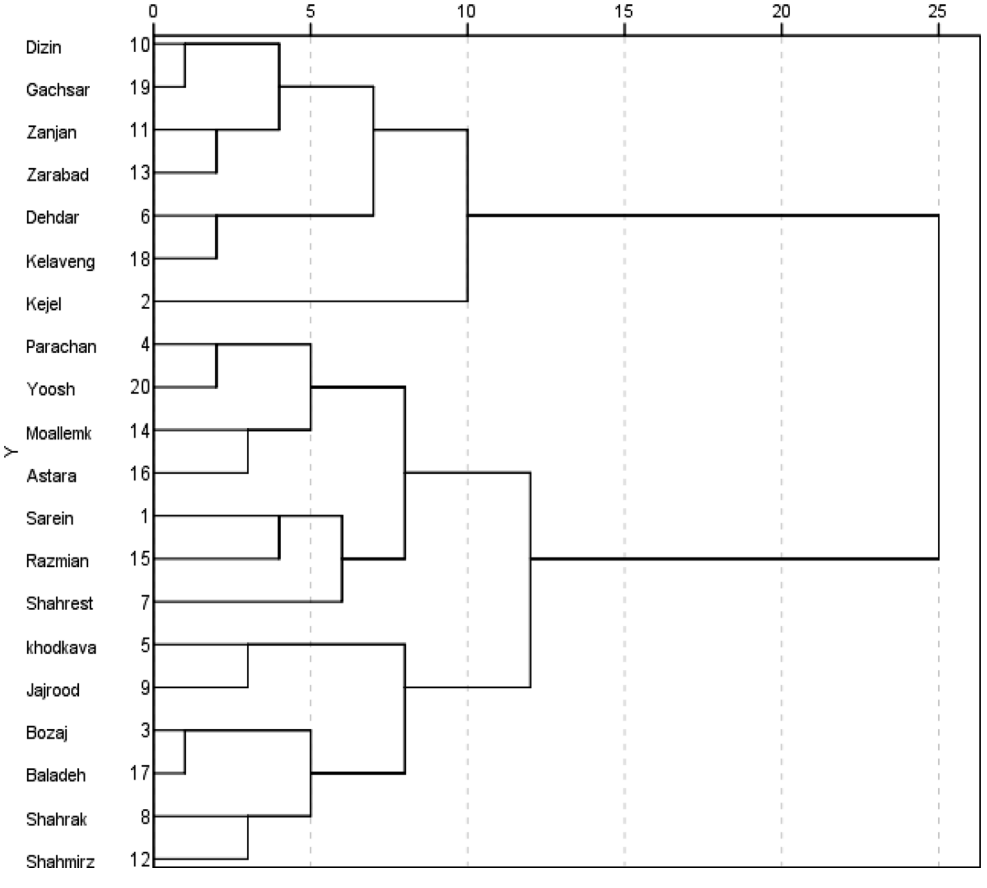

Fig. 1 Wards cluster analysis of *H. rhamnoides* population based on seed oil traits

Table 5 Correlations between seed oil characteristics in *Hippophae rhamnoides* populations

Variables	Altitude	Seed Oil	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid cis	Oleic acid Trance	Linoleic acid	Linolenic acid
Altitude	1	–	–	–	–	–	–	–	–	–
Seed Oil	–0.453*	1	–	–	–	–	–	–	–	–
Myristic acid	0.331	–0.163	1	–	–	–	–	–	–	–
Palmitic acid	–0.431	0.148	–0.007	1	–	–	–	–	–	–
Palmitoleic acid	–0.071	–0.603**	0.105	0.195	1	–	–	–	–	–
Stearic acid	0.153	0.530*	–0.030	0.113	–0.383	1	–	–	–	–
Oleic acid cis	0.211	–0.328	–0.110	–0.097	–0.200	–0.144	1	–	–	–
Oleic acid Trance	0.035	0.328	0.092	0.006	–0.557*	0.415	0.046	1	–	–
Linoleic acid	–0.112	0.368	–0.152	–0.625**	–0.395	–0.091	–0.259	0.282	1	–
Linolenic acid	0.360	–0.012	0.180	–0.283	–0.227	–0.021	–0.229	–0.291	–0.119	1

*$p<0.05$, **$p<0.01$

Correlations Between the Characters

Simple correlation coefficient analysis showed the existence of significant positive and negative correlations among seed oil characteristics (Table 5). We mentioned some of more important correlations between them. Seed oil content exhibited negative correlation with altitude of populations position (r = –0.453 $p < 0.05$) and Palmitoleic acid content (r = –0.603 $p < 0.01$) and positive correlation with stearic acid content (r = 0.530 $p < 0.05$). Palmitic acid had negative correlation with linoleic acid (r = –0.625 $p < 0.01$). Also Palmitoleic acid showed negative correlation with oleic acid trance (r = –0.557 $p < 0.05$).

Same to our results, Yang (1989) explained that region altitude causes decrease in seed oil content above about 2500 m asl. Li and Beveridge (2003) mentioned negatively correlation between proportion of linolenic acid with those of oleic and linoleic acids in seed oil. In present study, similar results were obtained. In accordance to our study, fatty acid composition of fruit pulp and seeds oils was perviously studied in four different cultivars of sea buckthorn in different regions of Himalaya with different altitude ranges. In the seed oils, the unsaturated fatty acids such as linoleic acid, oleic acid and α-linolenic acid constitute higher proportion (86.5–51.7%) with exception in *H. salicifolia*. This investigation results showed that seed oils of *H. rhamnoides* particularly from higher altitude area could be considered excellent sources of linoleic and α-linolenic acids compare with lower altitude. (Singh and Gupta 2015). Also, Shah et al. (2007) reported the variation of seed oil content between different locations of Pakistan (7.03–12.86%). They explained the huge difference in the range of oil content is due to altitude variations and genetics make of sea buckthorn ecotypes as well.

Conclusion

In this study, the nutritional qualities of seed oil from sea buckthorn populations in several region of Iran were evaluated. There was a wide variability in seed oil quality and quantity of different *H. ramnoides* populations in studied regions of Iran. In other word, the results of this research revealed valuable information about the fatty acid quantity and quality in the oils extracted from berry seeds of sea buckthorn wild populations in Iran. So that, the highest value of seed oil content (%) were observed in Sarein population in both experimental years (8.63% and 9.66% for 2010 and 2011 years). Since the seed oil important is because of highly amount of unsaturated fatty acids (oleic acid, linoleic acid and linolenic acid), populations with high content of these fatty acid are more valuable. Maximum content of oleic acid cis in 2014 and 2015 were existed in Kelavenga (21.19%) and Parachan (13.24%) populations, respectively. the highest value of oleic acid trans in 2014 and 2015 years were related to Razmian (5.21%) and Shahrestanak (6.34%) populations, respectively. Maximum content of linoleic acid in 2014 were existed in Jajrood (39.72%) population, whereas maximum content of it in 2015 were in Baladeh (42.03%). the highest linolenic acid content were obtained from Dehdar (30.73%) and Khodkavand (20.02%) populations in 2014 and 2015 years. Factor analysis indicated five components with explaining 88.31% of the total variance. In the first PC some traits such as seed oil percentage, Palmitoleic acid, oleic acid trance and linoleic acid contents showed the highest variance. Hierarchical cluster analysis divided populations into four main groups with high diversity. Some positive or negative correlation between seed oil traits can be used in the future breeding programs. Wide range of variation across the sea buckthorn populations can be exploited for selection of suitable genotypes to improvement and commercial exploitation of this plant.

Acknowledgements This research was supported by Department of Cultivation and Development, Medicinal Plants Research Centre, Institute of Medicinal Plants affiliated with the Academic Center for Education Culture and Research (ACECR) in Karaj, Iran.

Conflict of interest A. Kuhkheil, A. Mehrafarin, V. Abdoosi and H. Naghdi Badi declare that they have no competing interests.

References

AOCS (1989) Official methods and recommended practices of the American oil chemists' society. AOCS, Champaign

Cenkowski S, Yakimishen R, Przybylski R, Muir WE (2006) Quality of extracted sea buckthorn seed and pulp oil. Can Biosyst Eng 48:3.9–3.16

Dong JE, Ma XH, Wei Q, Peng SB, Zhang SC (2011) Effects of growing location on the contents of secondary metabolites in the leaves of four selected superior clones of Eucommia ulmoides. Ind Crops Prod 34:1607–1614. https://doi.org/10.1016/j.indcrop.2011.06.007

El-Adawy TA, Rahma EH, El-Bedawy AA, Gafar AF (1999) Properties of some citrus seeds. Part 3. Evaluation as a new source of protein and oil. Mol Nutr Food Res 43:385–391

Fatima T, Snyder CL, Schroeder WR, Cram D, Datla R, Wishart D, Weselake RJ, Krishna P (2012) Fatty acid composition of developing seabuckthorn (Hippophae rhamnoides L.) berry and the transcriptome of the mature seed. PLOS ONE 7:4

Francis V (2012) Fatty acids in berry lipids of six sea buckthorn (Hippophae rhamnoides L., subspecies carpatica) cultivars grown in Romania. Chem Cent J 6(9):106–118

Gao X, Ohlander M, Jeppsson N, Bjork L, Trajkovski V (2000) Changes in antioxidant effects and their relationships to phytonutrients in fruits of sea buckthorn (Hippophae rhamnoides L.) during maturation. J Agric Food Chem 48:1485–1490

Gutierrez LF, Ratti C, Belkacemi K (2008) Effects of drying method on the extraction yields and quality of oils from Quebec sea buckthorn (Hippophae rhamnoides L.) seeds and pulp. Food Chem 106:896–904. https://doi.org/10.1016/j.foodchem.2007.06.058

Kumar R, Kumar GP, Chaurasia OP, Singh SB (2011) Phytochemical and Pharmalogical profile of sea buckthorn oil: a review. Res J Med Plant 5(5):491–499

Li TSC, Beveridge THJ (2003) Sea buckthorn (Hippophae rhamnoides L.): production and utilization. NRC Research Press, Ottawa, p 133

Rousi A (1971) The genus Hippophae ¨ L. A taxonomic study. Ann Bot Fennici 8:177–227

Sabir SM, Maqsood H, Hayat I, Khan MQ, Khaiq A (2005) Elemental and nutritional analysis of sea buckthorn (Hippophae rhamnoides ssp. turkestanica) berries of Pakistani origin. J Med Food 8:518–522

Shah AH, Ahmad SD, Sabir M, Shazia A, Ishtiaque K, Batool F (2007) Biochemical and nutritional Evaluations of Sea buckthorn (Hippophae rhamnoides ssp. turkestanica) from different locations of Pakistan. Pak J Bot 39:2059–2065

Singh V, Gupta R (2015) Fatty acid composition of fruit pulp and seed oils of Himalayan sea buckthorn. Int J Nutr Food Sci 4:Iss1

Verehschagin AG, Ozerinina OV, Tsydendambaev VD (1998) Occurrence of two different systems of triacyglycerol formation in sea buckthorn fruit mesocarp. J Plant Physiol 153:208–213

Yang B, Kallio H (2002a) Composition and physiological effects of sea buckthorn (Hippophae) lipids. Trends Food Sci Technol 13(5):160–167. https://doi.org/10.1016/S0924-2244(02)00136-X

Yang B, Kallio H (2002b) Effects of harvesting time on triacylglycerols and glycerophospholipids of sea buckthorn (Hippophae rhamnoides L.) berries of different origins. J Food Compost Anal 15(2):143–157. https://doi.org/10.1006/jfca.2001.1041

Yang B, Kallio HP (2001) Fatty acid composition of lipids in sea buckthorn (Hippophae rhamnoides L.) berries of different origins. J Agric Food Chem 49:1939–1947

Yang H (1989) The dynamic distribution of some active components in sea buckthorn fruits growing on Qinghai-Tibet plateau. First International Symposium on Sea Buckthorn, Xi'an, China, 19.10.-23.10.1989, pp 158–162