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Chemical Compositions of Oils from Several Wild Almond Species

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Abstract Chemical compositions of oils extracted from three wild almond species [*Amygdalus scoparia* from Beyza, Iran (AZ); *A. scoparia* from Borazjan, Iran (AJ), and *A. hausknechtii* from the Firuzabad region, (AH)] and a domestic species, *A. dulcis* from Estahban, Iran (AD), as a reference, were investigated. Total oil content ranged from 44.4% in AJ to 51.4% in AD. Saponification numbers were in the range of 173.5 (for AJ) to 192.9 for AD. Oxidative stability, total phenolic contents and total wax contents were found to be within the ranges 11.7–14.0 h, 33.9–43.2 and 2.05–2.53%, respectively. The main monounsaturated fatty acid (MUFA) was oleic acid ranging from 66.7% for AH to 69.7% for AZ. The only polyunsaturated fatty acid (PUFA) was linoleic acid ranging from 18.2% for AZ to 23.0% for AD. The major saturated fatty acid was palmitic acid. MUFA contents and MUFA to PUFA ratio in the oils from wild almond species as well as those in the domestic one were found at higher levels than those in the common vegetable oils such as soybean, various nut oils, and also those from the seeds of pomegranate, grape, date and sesame. Oils from wild almond species investigated here are rich in oleic acid and can be considered as potential vegetable oils in the human diet.

Keywords Fatty acid composition · Oil source · Oil indices · Oxidative stability · Wild almond species

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Introduction

Almonds, *Prunus amygdalus* L., are native to the Western and Central Asia including Western China, Kurdistan, Turkestan, Afghanistan and Iran [1, 2]. The almond is considered a pleasant nut throughout the world with applications in food, pharmaceutical and cosmetic industries. It is used as an ingredient in many snacks and other processed foods [3]. As is the case with other nuts, almonds also reduce the risk of cardiovascular diseases [4]. This is attributed to the hypocholesterolemic effect of high levels of fiber, sterols, ratio of unsaturated fatty acids (USFA) to saturated fatty acids (SFA) and also to the antioxidant capacity of vitamin E and sphingolipids present in almonds [4, 5].

Other than the regular almond, *Prunus dulcis*, there are more than thirty wild or partially cultivated almond species in the world [2]. Around twenty wild almond species have been reported in Iran [6]. Although numerous studies have been reported on the characteristics of the oil and other components of commercial almond species, a complete investigation on the nutritional and chemical compositions of different wild almond species is not found in the literature. Farhoosh and Tavakoli [7] studied some physicochemical properties of kernel oil of *Amygdalus scoparia*, which is a wild species of almond growing in Iran. Despite the wide distribution of these wild species of almonds with high nutritional values, they have not been fully utilized for industrial applications. Therefore, a thorough investigation on the chemical compositions and health benefits of the kernels from wild almond species and their oils will contribute significantly to the current limited data available on the wild almond nuts as potential raw materials for the food industry. In the current study, the chemical, nutritional and some unique properties of oils from three wild almond species (*A. scoparia*, collected from two distinct regions of

Iran and *A. hausknechtii*) were characterized. A commercial species (*A. dulcis*) was also used as the reference.

Materials and Methods

Materials

Fruits of *A. scoparia* were collected from two different regions of Iran (one from the Beyza region (AZ), in the Fars province and the other from the Borazjan region (AJ), in the Bushehr province). Fruits of *A. hausknechtii* (AH) were collected from the Firuzabad region in the Fars province, Iran. Regular almond nuts, *A. dulcis* (AD), were obtained from Estahban, also from the Fars province. All samples were stored under refrigerated conditions (4 °C) until used in the experiments. The solvents and chemicals used in this study were of analytical grade and purchased from Merck Chemical Company (Darmstadt, Germany).

Oil Extraction

Lipid extraction for total fat determination was performed with a Soxhlet apparatus using *n*-hexane as the solvent applying the method reported by Zhang et al. [3]. Five grams of powdered sample from each species was extracted for 6 h and then the solvent was evaporated (at room temperature) and the residue was dried in an oven at 80 °C for 4 h. The oil used for the determination of the fatty acid (FA) compositions and other oil indices were extracted according to the method reported by Kornsteiner et al. [8] (with minor modifications). Briefly, 5.00 g powdered sample was placed in a conical glass containing around 50 ml of *n*-hexane and agitated on a magnetic stirrer for 3 h. Then, the solution was filtered and the remaining solvent was evaporated by using a rotary evaporator at 40 °C. The pure oil was transferred into a small glass vial, flushed with nitrogen and maintained at −18 °C until analyzed for FA compositions, iodine value (IV), saponification number (SN), acid value (AV), peroxide value (PV), oxidative stability (OS), total phenol contents (TP), total wax (TW), and refractive index (RI).

Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters (FAME) of the extracted oils were prepared according to the method previously reported by Metcalfe et al. [9]. Approximately, 0.04 g of each oil was weighed into a glass tube. Then, 5.0 ml of 2.0% (w/v) methanolic sodium hydroxide was added and placed in the boiling water for 10 min. Then, the tubes were cooled and 2.20 ml boron trifluoride (BF_3) was added and boiled again for 3 min. After this stage, the tubes were cooled and 1.5 ml hexane and 1.0 ml saturated NaCl solution were added and the tubes were shaken vigorously and left to stand for 3 min. The upper layer was transferred to a small vial and stored at 0 °C until analyzed. FA compositions were determined using a gas chromatograph (Unicam 4600, UK) equipped with a flame ionization detector (FID) and a BPX70 capillary column (0.25 mm ID × 30 m long × 0.22 μm film thickness). The oven temperature was set at 160 °C and was held there for 6 min. Then, it was increased to 180 °C at 20 °C/min, held for 10 min and increased again to 210 °C at 20 °C/min. Injector and detector temperatures were both set at 250 °C. Helium was used as the carrier gas and the split ratio was set at 1:40. The FAME were identified by comparing their retention times with those of standard samples. The quantification of fatty acids was made according to their relative area percentages.

Oil Indices

IV, SN, AV, PV, OS, TP, TW and RI were the indices measured for the extracted oils from this study. IV and SN were determined according to the methods 920.158 and 920.160, respectively, both from the Official Methods of Analysis of AOAC International [10]. The AV was determined by method Cd 3d-63 of AOCS Official Methods of Analysis [11]. PV was determined spectrophotometrically based on a method from the International Dairy Federation [12]. Approximately, 0.250 g of extracted oil was weighed into a glass tube and dissolved in 9.7 ml of a mixture of chloroform:methanol (4:1, v/v). Then, a drop of ammonium thiocyanate solution (30%, w/v) and a drop of ferrous chloride solution (0.35%, w/v) were added to the tube. After standing for 5 min, the absorbance of the mixture was read at 500 nm using a spectrophotometer. A calibration curve was also obtained using ferric chloride solutions containing 5–20 μg of Fe^{3+}. A Rancimat apparatus (model 679-Metrohm, Herisau, Switzerland) was used to evaluate the OS of the extracted oils. In each cell, about 2.5 g oil was weighed and heated to 110 °C. Air passed through the samples at 20 l/h flow rate and the OS of the oils were reported as their equivalent induction times (h).

TP contents of the extracted oils were determined using a method similar to the one described by Capannesi et al. [13] using Folin–Ciocalteau reagent. The TP was reported as equivalent tannic acid in mg per kg of oil. TW contents of the oils were determined by carrying out a solvent dewaxing process similar to that reported by Mezouari et al. [14]. RI was determined at room temperature by using a refractometer (model Abbe, Bellingham-Stanley Ltd., London, UK).

Statistical Analysis

All experiments were performed in duplicate. Comparison among the means were carried out by using least significant

differences (LSD) at the 95% confidence level from Statistical Analysis System (SAS) release 9.1 (SAS Institute, Inc., Cary, NC).

Results and Discussion

Oil Content and Oil Indices

The results for the oil contents from the different almond species studied here are shown in Table 1. Oil contents varied from 44.4% in AJ to 51.4% in AD. The oil level in AD (i.e., the sweet/domestic almond) was somewhat higher than those in the wild (bitter) genotypes (AZ, AJ and AH). Such findings agree well with the results of Femenia et al. [15], who reported that oil contents of sweet apricot kernels were higher than those of bitter ones. Oil content primarily depends on the genotype of the sample [16, 17]. IV, SN, AV, PV, TP, OS, TW and RI of the extracted oils from this study are also shown in Table 1. IV (g of I_2 per 100 g of oil) demonstrates the levels of unsaturation and potential oxidative sensitivities of the oils [18]. IVs were found at 91.6 level for AD, 88.8 for AH, 90.2 for AZ and 96.1 for AJ. These values indicate that compared to numerous edible oils such as corn and soybean oils, the oils from the almond species studied here are highly unsaturated and therefore susceptible to oxidative degradation. The results of current study on IVs agree well with the previously reported results for almond oil [7, 18]. SN (mg KOH per g of oil), which somehow reflects the average molecular weight of the fatty acids in the oil [15], ranged from 173.5 (for AJ) to 192.9 (for AD). SNs obtained for the oils extracted from wild and domestic almond species in this study were in good agreement with that reported for regular almond (182.5) by Moodley et al. [18]. These values are higher than that reported by Farhoosh and Tavakoli [7] for *A. scoparia* (98.6).

TP contents of the extracted oils are shown in Table 1 (as their equivalent tannic acid in mg per kg oil). They were 37.7, 43.2, 36.3 and 33.9 for the oils extracted from AD, AH, AZ and AJ, respectively, which were not significantly different. Phenolic components display antioxidant activities and protect oils against the oxidation [13, 19]. AV, which indicates the level of free FA as a result of lipase activity in oil [13], ranged from 0.26 to 0.30 mg KOH per g oil. PV, another parameter for evaluating oil quality, in the samples studied here ranged from 0.34 to 0.43 mequiv O_2 per kg oil which confirmed good oxidative stability of oils. TW contents (g per 100 g oil) ranged from 2.05 for AH to 2.53 for AZ.

The results for the OS of the extracted oils are also shown in Table 1 as their equivalent induction times, which were 14.0, 12.9, 12.0 and 11.7 h for AZ, AH, AD and AJ, respectively. The values obtained for OS of the oils from the species studied here are somewhat lower than those reported for almond, hazelnut and pistachio by other researchers [19, 20]. The temperature applied in the above studies (100 °C) was lower than that used in the current study (110 °C). Many factors such as FA profile, tocopherols and other antioxidants of oils influence OS [20, 21]. However, no relationships among the FA profile, TP contents and OS were found in this study. In spite of significant differences among the levels of OS (14.0 for AZ and 11.7 for AJ), such trends were not found in the levels of TP and FA classes. RI is an important physical property for oils. As shown in Table 1, RI level was significantly higher in AJ (1.467) than those in the other species (1.462 for AD, 1.462 for AZ and 1.463 for AH).

Fatty Acid Composition

FA compositions of the oils extracted from the studied almond species are presented in Table 2. Major monounsaturated fatty acid (MUFA) among all studied species was oleic acid ranging from 66.7% (for AH) to 69.7% (for AZ). Also, oleic acid contents in AD and AJ were 68.5 and 67.9%, respectively. Palmitoleic acid was another MUFA,

Table 1 Oil contents (on a dry basis) and some physicochemical characteristics of oils from almond species investigated in this study

Sample*	Oil content (%)	IV**	SN**	RI**	AV**	PV**	OS**	TP**	TW**
AD	51.4 ± 2.1^{a}	$91.6^{a} \pm 2.0$	$192.9^{a} \pm 9.1$	$1.462^{b} \pm 0.000$	$0.28^{ab} \pm 0.02$	$0.34^{b} \pm 0.03$	12.0 ± 0.3	$37.7^{a} \pm 1.5$	$2.15^{a} \pm 0.21$
AH	47.8 ± 1.4^{ab}	$88.8^{a} \pm 0.4$	$177.9^{a} \pm 12.7$	$1.463^{b} \pm 0.000$	$0.27^{ab} \pm 0.01$	$0.43^{a} \pm 0.02$	12.9 ± 0.1	$43.2^{a} \pm 3.4$	$2.05^{a} \pm 0.21$
AZ	47.1 ± 0.3^{b}	$90.2^{a} \pm 2.6$	$179.8^{a} \pm 7.4$	$1.462^{b} \pm 0.000$	$0.30^{a} \pm 0.02$	$0.37^{ab} \pm 0.03$	14.0 ± 0.3	$36.3^{a} \pm 4.3$	$2.53^{a} \pm 0.31$
AJ	44.4 ± 0.7^{b}	$96.1^{a} \pm 11.2$	$173.5^{a} \pm 6.2$	$1.467^{a} \pm 0.000$	$0.26^{b} \pm 0.02$	$0.38^{ab} \pm 0.02$	11.7 ± 0.4	$33.9^{a} \pm 1.0$	$2.20^{a} \pm 0.14$

*AZ (*A. scoparia* from Beyza, Fars, Iran), AJ (*A. scoparia* from Borazjan, Bushehr, Iran), AH (*A. hausknechtii* from Firuzabad, Fars) and AD (*A. dulcis* from Estahban, Fars)

***IV* iodine value (g I_2 per 100 g of oil), *SN* saponification number (mg KOH per g of oil), *AV* acid value (mg KOH per g of oil), *PV* peroxide value (mequiv O_2 per kg of oil), *OS* oxidative stability (reported as induction time, h), *TP* total phenolics content (equivalent tannic acid, mg per kg of oil), *TW* total wax content (g per 100 g oil). *RI* refractive index (at room temperature)

[a,b] In each column, means with the same letter are not significantly different ($P > 0.05$)

Table 2 Fatty acid compositions (%, w/w) of the oils extracted from different almond species investigated in this study

Sample*	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	SFA**	USFA**	MUFA/PUFA**
AD	$7.1^{b} \pm 0.1$	$0.4^{a} \pm 0.0$	$1.0^{c} \pm 0.0$	$68.5^{ab} \pm 0.4$	$23.0^{a} \pm 0.3$	8.0 ± 0.1	92.8 ± 0.1	3.04 ± 0.06
AH	$9.5^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$2.6^{a} \pm 0.1$	$66.7^{b} \pm 0.4$	$20.7^{b} \pm 0.4$	12.1 ± 0.8	87.8 ± 0.7	3.24 ± 0.10
AZ	$9.5^{a} \pm 1.0$	$0.6^{a} \pm 0.3$	$1.9^{b} \pm 0.2$	$69.7^{a} \pm 1.4$	$18.2^{c} \pm 0.3$	11.4 ± 0.2	88.5 ± 0.2	3.86 ± 0.08
AJ	$9.4^{a} \pm 0.4$	$0.3^{a} \pm 0.1$	$2.1^{b} \pm 0.1$	$67.9^{ab} \pm 0.4$	$20.3^{b} \pm 0.0$	11.5 ± 0.5	88.4 ± 0.5	3.34 ± 0.02

*AZ (*A. scoparia* from Beyza, Fars, Iran), AJ (*A. scoparia* from Borazjan, Bushehr, Iran), AH (*A. hausknechtii* from Firuzabad, Fars) and AD (*A. dulcis* from Estahban, Fars)

***SFA* saturated fatty acid, *USFA* unsaturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

[a,b,c] In each column, means with the same letter are not significantly different ($P > 0.05$)

which was found at very low levels (~0.8%). Linoleic acid as the only polyunsaturated fatty acid (PUFA) was found at 18.2% for AZ, 20.3% for AJ, 20.7% for AH and 23.0% for AD. The main SFA among the three species studied here was palmitic acid ranging from 7.1% (in AD) to 9.5% (in AZ and AH). Stearic acid, another SFA that was found in the oils, was within 1.0–2.6%. AH indicated significantly higher level of stearic acid (2.6%) than other species. Linoleic acid was found at lower levels among the wild species (18.2–20.7% for AH) than that in the regular almond (23.0% for AD), while total SFA was found at higher levels in the wild species (AZ, AJ and AH) than those in the domestic one studied here (AD).

The ratio of MUFA to PUFA is an important parameter for oil stability in highly unsaturated oils [16]. In the current study, AZ had the highest ratio of MUFA to PUFA (3.86) mainly due to the lower concentration of linoleic acid. The corresponding values for this ratio were 3.24 and 3.34 for AH and AJ, respectively. Because of its higher level of linoleic acid, AD had the lowest MUFA to PUFA ratio. AZ with a higher ratio of MUFA to PUFA also had a significantly higher OS value than did the others.

Results of this study on the oleic acid levels in the wild almond species, as well as that of the domestic one, are in good agreement with those reported by Maguire et al. [5] (69.2%) and Miraliakbari and Shahidi [22] (69.9%) for commercial almond. Venkathacalam and Sathe [23], Zhang et al. [3] and Zacheo et al. [24] reported oleic acid levels of 60.9, 62.9 and 61.0%, respectively, for domestic almonds, which are lower than those from the wild and domestic almond species used in the present study. Nanos et al. [17] and Kodad and Socias [16] reported oleic acid levels in the ranges of 72–80% and 69–78%, respectively, for domestic almonds, both of which are higher than those from the oleic acid levels in the wild and domestic almonds studied here. Zacheo et al. [24] reported oleic acid levels of up to 77.5% for some almond varieties.

FA compositions in the wild almond species from this study indicated slight differences from that reported by Farhoosh and Tavakoli [7], who studied the oil from *A. scoparia* kernel from a region different from those of this study. They reported oleic acid concentration at 62.8% (compared to 66.7–69.7% in this study). In case of the domestic species, AD, our results agree well with those of the published studies [22, 25]. Chemical compositions such as FA profile of kernels depend on the genotype, climatic conditions, maturity level and soil composition [8, 15, 16]. Nanos et al. [17] reported that oleic acid levels in the almonds collected from irrigated plots were higher than those collected from non-irrigated plots. So, a greater amount of oleic acid in AZ may be due to the differences in the climate conditions, amounts of annual rainfall and soil compositions of the harvesting regions in the wild almond species studied here (Beyza and Firuzabad regions with 1,654 and 1,368 m altitudes, respectively, in the Fars province, and Borazjan region with 73 m altitude in the Bushehr province of Iran).

Comparing the results of this study with those reported by Maguire et al. [5] and Venkathacalam and Sathe [23], it can be interpreted that MUFA to PUFA ratio in the oils extracted from AZ, AJ and AH species are at higher levels than those in some other popular nuts such as peanut, walnut, pistachio, pine nut and Brazil nut (Table 3). Compared with some health-promoting infrequent oils

Table 3 Fractions of mono- and polyunsaturated fatty acids reported for some popular nuts in the literature

Nut	Fatty acid[a]					
	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:5}$	$C_{22:6}$
Brazil nut[b]	0.29	28.75	45.43	0.18	ND	ND
Peanut[c]	0.15	38.41	44.60	0.58	ND	O.75
Pine nut[b]	0.11	27.44	46.84	1.51	ND	ND
Pistachio[b]	1.18	50.29	33.43	0.86	ND	ND
Walnut (Cork-Ireland)[c]	0.23	21.00	57.46	11.58	0.06	ND

ND not detected

[a] $C_{16:1}$ (palmitoleic acid), $C_{18:1}$ (oleic acid), $C_{18:2}$ (linoleic acid), $C_{18:3}$ (linolenic acid), $C_{20:5}$ (eicosapentaenoic acid)

$C_{22:6}$ (docosahexaenoic acid)

[b] Venkathacalam and Sathe [23]

[c] Maguir et al. [5]

(such as pomegranate seed oil and grape seed oil), oils from the wild almond species investigated here showed certain unique properties. Predominant FAs in the wild almond species were completely different from those of pomegranate seed and grape seed oils [26, 27]. However, USFA content and SFA to USFA ratio were almost similar. Pomegranate seed oil contains high levels of punicic acid (a conjugated linolenic acid) at approximately 80% [26] and grape seed oil is rich in linoleic acid at approximately 66.3% [27]. MUFA contents in the oils of wild almond species from this study were higher than those reported for both pomegranate seed oil (8.1%) [26] and grape seed oil (approximately, 20%) [27]. Wild almond species from this study had much lower SFA content (8.1–12.8%) as compared to 44.3% and 27.0% of SFA in the oils of the seeds from two date cultivars [28]. In addition, as expected, the ratio of MUFA to PUFA as well as the MUFA contents in the oils of wild almonds in the current study were higher than those reported for raw sesame oil (1.3 and 45.4%, respectively) by Elleuch et al. [29]. SFA contents in the oils from the wild almond species (11.0–12.1%) were also lower than that reported for raw sesame oil (18.5%) [29].

MUFAs and USFA to SFA ratios of oils from wild almonds of this study were also higher than those reported for virgin olive oil (62.2% and 4.61%, respectively) [7]. Compared to canola oil, the wild almond species from the present study indicated similar FA compositions [30]. However, MUFA levels in the oils of wild almond species were much higher than those in the oils of soybean (21.3%), sunflower (20.5%), corn (29.9%), palm (37.1%) and safflower (12.0%) [30]. Due to the higher ratios of MUFA to PUFA, the oils of AZ, AH, and AJ are expected to be more stable than prevalent culinary oils with similar saturation levels.

Despite the fact that SFA render the oils containing them more stable, they, in particular palmitic acid, are harmful FA for the cardiovascular system [16]. Compared to the oils of peanut, coconut, palm and soybean [30], the oils of wild almond species from the current study had much lower SFA levels (mean levels of SFA reported for the oils from peanut, coconut, palm and soybean are 20.0, 86.5, 47.8 and 15.6%, respectively). Ratios of USFA to SFA in the oils of wild almond species from the current study were comparable with those reported for domestic almonds and other nuts [5, 22, 23].

In accordance with the published reports, a MUFA-rich diet [17, 24] and daily consumption of 100 g almond [4] resulted in the reduction of low density cholesterol (LDL) and total cholesterol in the blood. However, such diets did not alter the level of high density cholesterol (HDL). Therefore, consuming wild almond species and their oils could have a positive impact on the performance of the cardiovascular system. Moreover, lack of linolenic acid and high ratio of MUFA to PUFA results in a lower enzymatic and non-enzymatic oil degradation and subsequent off flavor.

Regardless of the taste, wild almond species can be considered as potential sources of vegetable oil. More studies are needed to evaluate possible hazard of anti-nutritional components from these sources, which was outside the scope of this study. Gandhi et al. [31] in a 13-week feeding trial on rats observed that the oil of wild apricot (with a bitter kernel) showed no toxic potential, when it was compared with groundnut oil as a control. They showed that food consumption, growth rate and food efficiency ratio of rats fed a diet containing 10% of wild apricot seed oil were similar to those fed a diet containing groundnut oil.

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