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Original article

Physicochemical Properties and Nutritional Composition of Seed Oil from Wild Pomegranate Varieties in Jolfa, Northern Iran

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Abstract. *Background and Aim:* Pomegranate seed oil (PSO), a by-product of juice processing, is a rich source of bioactive lipids that have nutritional and industrial value. This study aimed to analyze the physicochemical features and nutritional composition of seed oils from four wild pomegranate cultivars, including Oshtabin, Kordasht, Qulan, and Duzal, found in the Jolfa region of northern Iran. *Methods:* Seed oils were extracted and analyzed for physicochemical parameters, including refractive index (RI), saponification number (SN), peroxide value (PV), and acid value (AV). *Results:* The major fatty acid was punicic acid (52–58%), followed by oleic, linoleic, palmitic, and stearic acids, with a total oil content of 14.5–17.3%. The oils contained high concentrations of unsaturated fatty acids (USFA, 91.15–92.13%) and low concentrations of saturated fatty acids (SFA, 6.67–7.22%), yielding favorable USFA/SFA ratios of 12.6–13.8. The acid value of 1.49 mg KOH/g and low peroxide value (1.25 milliequivalents of oxygen per kilogram, meq O_2/kg) suggested good oxidative stability. *Conclusion:* wild pomegranate seeds from northern Iran exhibit desirable physicochemical, nutritional, and bioactive characteristics, representing an underutilized resource with diverse potential uses in food, cosmetics, and the pharmaceutical industry.

Key words: wild pomegranate, seed oil, punicic acid, tocopherols, sterols, unsaturated fatty acids

Introduction

The pomegranate (*Punica granatum L.*) is a shrub or small tree, about 2-3 meters in height and belongs to the family Punicaceae. It is cultivated extensively in the Orient and Iran, India as well as the USA (1). Globally, it has an estimated production of 8.1 million metric tons annually, about 70% of which is produced in India, China, and Iran. Approximately 1 million tons are produced in Iran. (2, 3). The pomegranate is composed of three primary anatomical components: the seed (containing approximately 20% oil), the juice (constituting roughly 30% of the fruit's weight), and the peel, which includes the outer rind and internal membranes (4). The rapid expansion of the pomegranate juice sector in recent years has led to an increase in processing by-products, especially peels and seeds. These by-products are frequently viewed as wastes with minimal value and discarded in bulk by industrial plants (5). In reality, they have considerable biomass value and economic nutritional potential that remains untapped (6, 7). In Iran alone, annual estimation of pomegranate seed waste generation is over 120000 metric tons (8). Discarding this type of biomass creates environmental issues while at the same time losing valuable bioactive compounds. As a result,

utilizing these by-products, especially through extraction and recovery of seed oil, is gaining attention both economically and to help meet sustainability goals. Pomegranate seed oil (PSO) is noted for its remarkable level of conjugated octadecatrienoic fatty acids, including punic acid (cis-9, trans-11, cis-13) (9). Similar to other phytochemical-rich fruits, the composition and health benefits of PSO can differ substantially based on the variety and the cultivation conditions (10, 11). Approximately 80% of PSO consists of punic acid which has a number of health benefits like anti-inflammatory, antioxidant and even anticancer properties. In addition, some of these studies pointed out that such fatty acids could improve HDL cholesterol concentration, along with lowering markers of metabolic syndrome resulting in improved cardiovascular system health (12-14). Although previous studies have examined the extraction and compositional analysis of PSO from cultivated varieties in countries including Egypt, India, Japan, Spain, and Turkey, limited data are available on the physicochemical properties of oil derived from wild pomegranate genotypes (15). To the best of our understanding, there is no detailed analysis on the fatty acid composition, sterol, tocopherol contents, as well as the oxidative stability of seed oils derived from wild pomegranates in Northern Iran. Such information is critical for determining the possible functional uses of the oils in the fields of food, nutraceutical, and cosmetics. The objective of the present study is to extract oil from the seeds of wild *Punica granatum* and to comprehensively characterize its physicochemical properties. The findings are then compared with previously reported values for cultivated varieties, providing insight into the potential applications and functional value of wild pomegranate seed oil.

Method and Materials

Sample collection and preparation

Wild pomegranate (Punica granatum L.) species were collected from naturally grown trees in Jolfa, located in the East Azarbaijan province of northern Iran. The fruits' seeds were extracted, washed with distilled water, air-dried, and subsequently dried in a hot-air oven at 60°C until reaching a constant weight. The dried seeds were then ground using a laboratory mill to obtain a fine powder with a particle size ranging between 0.45 and 1.2 mm granules. The powder was stored in airtight containers in a vacuum desiccator at ambient temperature until further use (16).

Oil extraction using Soxhlet apparatus

Extraction of the oil was conducted using a Soxhlet apparatus. 25 g of powdered seed material was packed into a cellulose thimble and extracted with 200 mL of n-hexane for 6 hours at 60°C. The solvent and oil mixture were filtered under vacuum after extraction through Whatman No. 1 filter paper, then the solvent was evaporated using a rotary evaporator under reduced pressure. The oil extracted was kept in dark amber glass bottles at 4°C until analysis (17).

Preparation of Fatty Acid Methyl Esters (FAMEs)

Fatty acid methyl esters were prepared using the Hewavitharana et al. (2020) method with some modification. Briefly, the oil was subjected to base-catalyzed transesterification to convert triglycerides into their corresponding methyl esters. The resulting FAMEs were purified and prepared for gas chromatographic analysis (18).

Sterol and tocopherol analysis

Sterol compounds were examined after the saponification of oil samples using potassium hydroxide and subsequent extraction of unsaponifiable matter with N-hexane. The sterol fractions were separated and quantified by gas chromatography system (Chrompack CP-9001, The Netherlands) equipped with a flame ionization detector (GC-FID). The system included a split/splitless injector and an auto-sampler. The separation column was a CP-Sil 88 (50 m × 0.25 mm × 0.19 μm, Varian). Helium was used as a carrier gas at 120 kPa. The temperatures at the injector and detector were set at 250 °C and 270 °C, respectively. The total tocopherols (α-, β-, γ-, and δ-tocopherol) were quantified using the Waters Alliance e2695 HPLC system coupled to an FP2020 Plus fluorescence detector as

described in Fernandes et al. (2015). The excitation and emission wavelengths were set to 290 nm and 330 nm, respectively. The procedure involved the dissolution of oil samples in hexane and their subsequent injection to an HPLC containing a silica column (250 mm x 3.0 mm x 3 µm) (19).

Fatty acid composition analysis

The oil fatty acid composition was determined using gas chromatography (GC) with a flame ionization detector (FID) and a fused-silica capillary column (50 m × 0.25 mm × 0.19 µm, film thickness). The injector temperature was 230°C. Oven temperature programming was from 100°C to 230°C at 5°C/min (15). Individual fatty acids were identified by comparison of their retention times with those of authentic standards (20).

Chemical properties of the oil

The physicochemical properties of the extracted pomegranate seed oil (PSO) were analyzed using standard analytical procedures (21). For determining acid value, a certain quantity of oil was titrated using 0.1 N KOH in ethanol and phenolphthalein as an indicator. Results were expressed as milligrams of KOH needed to neutralize free fatty acids in one gram of oil. Saponification value was obtained by refluxing the oil with excess alcoholic KOH and subsequent titration with 0.5 N HCl. The parameter gives insight into the average molecular weight of fatty acids present in the oil. Primary oxidation or peroxide value was determined by iodometric titration method and expressed as milliequivalents active oxygen per kilogram (meq O_2/kg) of oil the refractive index was measured at 25 °C using abbe refractometer which is also used for checking the levelofun saturation and purity of oil.

Statistical analysis

All measurements were performed in triplicates. the seeds were collected from three different trees for each variety (Oshtabin, Kordasht, Qulan, and Duzal). From each tree, seeds were collected from five fruits. Results presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software (version 26, IBM Corp., USA). Significant differences among means were assessed using one-way analysis of variance (ANOVA) at $p < 0.05$, followed by Tukey's HSD test for post-hoc comparisons where applicable.

Results

Oil yield and physicochemical properties

The oil yield of wild pomegranate seeds ranged from 14.5% to 17.3%, with Qulan and Kordasht marking the highest and lowest values, respectively (Table 1). The refractive index ranged from 1.517 (Kordasht) to 1.528 (Duzal). The saponification value showed a slightly elevated consistency among varieties ranging from 186.5 to 189.2 mg KOH/g oil, denoting fatty acids of higher weight among unspecified long-chain fatty acids. The peroxide value (PV) was roughly 0.96–1 meq O_2/kg ± 10%, while the acid value varied slightly by about 0.22 mg KOH/g. These indices fall within typical limits for stable oils, indicating minimal oxidative deterioration.

Table 1. Physicochemical properties of pomegranate seed oils

Variety	RI	SN	PV	AV	Yield (%)
Oshtabin	1.52±0.0	187.8±3.8	0.96±0.03	1.38±0.01	15.2±0.7
Kordasht	1.517±0.0	189.2±1.2	1.27±0.05	1.47±0.03	14.5±0.5
Qulan	1.523±0.1	187.1±2.9	1.44±0.09	1.6±0.01	17.3±0.3
Duzal	1.528 ±0.0	186.5±4.1	1.35±0.03	1.53±0.1	15.7±0.0

Abbreviations: RI=refractive index; SN=Saponification Number (mg KOH per g of oil); PV=Peroxide Value (mequiv O^2 per kg of oil); AV=Acid Value (mg KOH per g of oil)

Fatty acid composition

The fatty acid profiles of the four wild pomegranate seed oil (PSO) samples are summarized in Table 2. Punicic acid was the dominant fatty acid in all samples (74.5% -78.3% of total fatty acids), confirming its characteristic role in PSO. Duzal had the highest punicic acid content (78.3%), whereas Kordasht had the lowest (74.5%). Other notable unsaturated fatty acids included oleic acid (7.3% - 9.41%), and linoleic acid, (6.1% to 8.4%). Among the saturated fatty acids, palmitic acid (3.26%–4.12%) and stearic acid were predominant. The total unsaturated fatty acid (USFA) content comprised 91.15% to 92.13%, yielding USFA/SFA ratios of 12.62 to 13.81, indicative of a highly unsaturated, nutritionally favorable profile.

Sterol composition

Table 3 summarizes the sterol content of pomegranate seed oils. β-Sitosterol was the most abundant phytosterol (5323–6926 mg/kg) in all samples. Oshtabin oil had the highest total sterol content with 8606 mg/kg whereas Duzal had the lowest (7033 mg/kg). Other major sterols included Δ5-avenasterol (591–778 mg/kg), campesterol (583–694 mg/kg), and stigmasterol (199–241 mg/kg). The substantial phytosterol levels suggest potential health benefits.

Tocopherol composition

As shown in Table 4, β-tocopherol was the dominant tocopherol isomer in all varieties, with concentrations ranging from 3045 mg/kg (Qulan) to 3911 mg/kg (Kordasht). Total tocopherol content varied between 3878 and 4640 mg/kg, indicating a strong antioxidant potential. α-tocopherol and δ-tocopherol were also present in appreciable amounts, contributing to the oil's overall oxidative stability.

Discussion

The oil yield of the wild pomegranate seeds collected from Jolfa region ranged from 14.5% to 17.3%, in agreement with previous studies (22), indicating the potential of these genotypes as viable oilseed resources. The variation in yield can be attributed to genetic differences and environmental factors such as rainfall and temperature, as documented by Ghasemi et al. (2023) (23).

Table 2. Fatty acid compositions of pomegranate seed oils (%)

Variety	Punicic Acid	Oleic Acid	Linoleic Acid	Palmitic Acid	Stearic Acid	Other Fatty Acids	USFA[1] (%)	SFA[2] (%)	USFA/SFA Ratio
Oshtabin	74.8 ± 0.4	9.41 ± 0.1	7.36 ± 0.1	3.91 ± 0.05	2.94 ± 0.07	1.58 ± 0.01	91.57 ± 0.3	6.85 ± 0.08	13.37 ± 0.1
Kordasht	74.5 ± 0.4	8.40 ± 0.09	8.23 ± 0.1	4.12 ± 0.1	3.10 ± 0.04	1.63 ± 0.06	91.15 ± 0.2	7.22 ± 0.05	12.62 ± 0.2
Qulan	76.1 ± 1.2	7.30 ± 0.2	8.40 ± 0.3	4.05 ± 0.02	2.75 ± 0.09	1.40 ± 0.07	91.80 ± 0.7	6.80 ± 0.06	13.50 ± 0.8
Duzal	78.3 ± 0.6	7.66 ± 0.2	6.10 ± 0.07	3.26 ± 0.04	3.41 ± 0.05	1.20 ± 0.01	92.13 ± 0.3	6.67 ± 0.04	13.81 ± 0.1*

Abbreviations: [1]USFA: Unsaturated Fatty Acids; [2]SFA: Saturated Fatty Acids; *USFA/SFA value for Duzal added based on calculated ratio (not provided in original data)

Table 3. Sterol content (mg/kg) in pomegranate seed oils

Variety	Campesterol (mg/kg)	Stigmasterol (mg/kg)	β-Sitosterol (mg/kg)	Δ^5-Avenasterol (mg/kg)	Total Sterols (mg/kg)
Oshtabin	662 ± 11	240 ± 7	6926 ± 116	778 ± 20	8606 ± 182
Kordasht	648 ± 9	199 ± 4	6472 ± 105	591 ± 18	7910 ± 136
Qulan	583 ± 7	228 ± 5	5807 ± 70	714 ± 14	7332 ± 127
Duzal	694 ± 14	241 ± 5	5323 ± 87	775 ± 24	7033 ± 110

Table 4. Tocopherol content (mg/kg) in pomegranate seed oils

Variety	α-Tocopherol (mg/kg)	β-Tocopherol (mg/kg)	γ-Tocopherol (mg/kg)	δ-Tocopherol (mg/kg)	Total Tocopherols (mg/kg)
Oshtabin	473 ± 24	3129 ± 91	45 ± 5	231 ± 13	3878 ± 115
Kordasht	495 ± 27	3911 ± 120	36 ± 3	198 ± 11	4640 ± 154
Qulan	520 ± 32	3045 ± 88	64 ± 7	273 ± 15	3902 ± 121
Duzal	376 ± 22	3576 ± 107	61 ± 6	155 ± 10	4168 ± 132

Likewise, Ashrafi et al. (2023) documented a broad range of morphological and pomological variation among 103 wild pomegranate accessions from northern Iran, revealing the robust genetic background that likely accounts for the variance in seed characteristics and oil yield that we recorded (24). Saponification number (SN), ranging between 186.5 and 189.2 mg KOH/g oil, suggests the predominance of high molecular weight triglycerides, which is consistent with findings by Aruna et al. (2018) (25). These values are lower than those typically reported for coconut or palm oil (26), reflecting a different fatty acid composition. Acid value (AV), an indicator of free fatty acid content, ranged from 1.38 to 1.60 mg KOH/g, which implies low enzymatic degradation and good oil quality. Also, low peroxide values (0.96–1.44 meq O2/kg) suggesting primary oxidation further supports these claims as they adhere to range set for consumable oils displaying overall extensive oxidatve stability aligning with international standards on edible oil benchmarking bluescape reference check. The refractive index (RI) between 1.517 and 1.528 confirms rich USFA profile showing high degrees of unsaturation (27). In line with this, Siol et al. (2024) found that commercial pomegranate seed oils started with acid and peroxide values that met acceptable criteria; however, peroxide levels rose during storage. Their findings underscore the need to assess oxidative stability together with standard quality indicators for oils (28). Fatty acid profiling revealed that punicic acid (a conjugated linolenic acid) was the dominant component, accounting for 74.5% to 78.3% of total fatty acids, with the Duzal variety showing the highest content. This aligns with reports by Pereira et al. (2019), further emphasizing the nutraceutical potential of pomegranate seed oil (PSO) (29). In addition to punicic acid, oleic, linoleic, palmitic, and stearic acids were also present. The remarkably high unsaturated fatty acid profile and specifically elevated punicic acid concentration observed in the wild pomegranate seed oils analysed here confirm and extend the earlier observations of Almoraie et al. (2025), who documented total unsaturated fatty acid levels ranging from 86.7 to 89.2% and identified punicic acid as the predominant lipid component in pomegranate seed oil (PSO), accounting for 81.8 to 84.8% of the total. These supplementary studies underline PSO's status as a promising nutraceutical, supporting its use in functional food applications aiming at enhancing cardiovascular health and exerting antioxidant activity (30). The oils demonstrated exceptionally high USFA content (91.15–92.13%) and a favorable USFA/SFA ratio (12.62–13.81), exceeding those reported for pistachio, almond, and olive oils (31). These properties highlight the cardiovascular benefits of PSO, especially in raising HDL and lowering LDL cholesterol (32). Phytosterol analysis revealed β-sitosterol as the predominant sterol (5323–6926 mg/kg), with significant amounts of campesterol, stigmasterol, and Δ5-avenasterol. These concentrations are markedly higher than those found in almond and pistachio oils (33), suggesting strong cholesterol-lowering potential (34). The range in phytosterol levels in our samples closely resembles the findings of Iriti et al. (2023), who discovered significant differences in phytosterol compositions between PSOs collected from various geographical locations. This agreement highlights the usefulness of phytosterol patterns as reliable quality indicators in the evaluation of PSOs (35). Similarly, tocopherol profiling showed β-tocopherol as the most abundant isomer (3045–3911 mg/kg), particularly in the Kordasht variety, providing antioxidant protection against lipid oxidation. Compared to conventional oils

like olive, corn, and sunflower, PSO from the studied varieties exhibited much higher total tocopherol content (36). Taken together, the high content of punicic acid, sterols, and tocopherols, along with favorable physicochemical indices, make wild pomegranate seed oil (PSO) a promising candidate for both food and nutraceutical applications. Its balanced composition supports its potential role in preventing cardiovascular diseases, metabolic disorders, and oxidative stress-related conditions.

Conclusion

This research highlights the favorable physicochemical and nutritional profile of seed oils extracted from wild pomegranate varieties in the northwest of Iran, with primary samples obtained from Jolfa. High levels of punicic acid, combined with a variety of unsaturated fatty acids, demonstrate that the lipids are beneficial for human health. Moreover, the considerable amounts of phytosterols (especially β-sitosterol) and tocopherols contribute to their potential antioxidant and cardioprotective effects. The desirable oil quality indices, such as low acid and peroxide values, as well as a high USFA/SFA ratio, indicate their oxidative stability and suitability for edible and nutraceutical applications. In conclusion, these findings position the wild pomegranate seeds of Iran as a remarkably untapped reservoir for commercial oil extraction, indicating a strategy of sustainable resource management and opening avenues for application in functional food formulations, cosmetic preparations, and pharmaceutical product development.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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