EXTRACTION AND CHARACTERIZATION OF POMEGRANATE (PUNICA GRANATUM L.) SEED OIL

Z.N. Kuziyeva[1], M.J. Oripova[1], K.A. Raimova[1], S.B. Karabayeva[1], F.Z. Orifov[2], N.G. Abdullajanova[1]
Institute of Bioorganic Chemistry, Uzbekistan Academy of Sciences, Mirzo Ulughbek St. 83, 100125 Tashkent, Uzbekistan[1]
Center of pharmacy-innovation[2]
https://doi.org/10.5281/zenodo.20793518

Abstract. *Pomegranate (Punica granatum L.) seeds are an important by-product of the juice industry and represent a valuable source of bioactive compounds, particularly oil rich in conjugated fatty acids (FA). The present study aimed to extract pomegranate seed oil and characterize its FA composition using gas chromatography–mass spectrometry (GC–MS). Oil was extracted from dried and ground pomegranate seeds using Soxhlet extraction with n-hexane as solvent. Fatty acid methyl esters (FAMEs) were prepared by acid-catalyzed transesterification and analyzed by GC–MS on a Supelco SP-2560 capillary column. The chromatographic analysis revealed that pomegranate seed oil was predominantly composed of unsaturated fatty acids. Punicic acid methyl ester was identified as the major fatty acid component, accounting for 77.78% of the total fatty acid content. Other identified fatty acids included linoleic acid (8.53%), oleic acid (6.65%), palmitic acid (4.16%), and stearic acid (2.89%). The high proportion of punicic acid confirms that pomegranate seed oil is an excellent natural source of conjugated linolenic acid with potential nutritional and pharmacological benefits. The obtained results demonstrate that pomegranate seed oil possesses a unique FA profile characterized by a predominance of bioactive polyunsaturated fatty acids. These findings support the potential application of pomegranate seed oil as a functional ingredient in food, nutraceutical, pharmaceutical, and cosmetic products. Furthermore, the utilization of pomegranate seeds contributes to the valorization of agro-industrial by-products and promotes sustainable resource use.*

Keywords: *punica granatum, seed oil, fatty acid composition, GS/MS, punicic acid, conjugated linolenic acid.*

Introduction

Pomegranate (Punica granatum L.) is one of the oldest cultivated fruit crops and is widely recognized for its nutritional, medicinal, and economic importance. In recent decades, global pomegranate production has increased considerably due to growing consumer demand for functional foods and natural health-promoting products. During industrial processing, particularly juice production, substantial quantities of by-products are generated, including peels and seeds, which represent valuable sources of bioactive compounds [1]. Pomegranate seeds constitute approximately 10–20% of the total fruit weight and contain significant amounts of oil, proteins, dietary fiber, minerals, and phenolic compounds. These components make pomegranate seeds a promising raw material for the development of value-added products and nutraceutical ingredients [2].

Among the various seed constituents, pomegranate seed oil (PSO) has attracted considerable scientific interest because of its unique fatty acid composition and biological

properties. PSO is particularly rich in punicic acid (cis-9, trans-11, cis-13 octadecatrienoic acid), a conjugated linolenic acid that typically accounts for 60–80% of the total fatty acids. In addition, the oil contains tocopherols, phytosterols, polyphenols, and other minor bioactive compounds that contribute to its antioxidant, anti-inflammatory, anticancer, cardioprotective, and antidiabetic activities [3, 4].

The extraction technique significantly influences the yield, chemical composition, physicochemical characteristics, and biological activity of pomegranate seed oil. Conventional extraction methods, such as Soxhlet and solvent extraction, generally provide high oil recovery but may require large volumes of organic solvents and extended extraction times. In contrast, environmentally friendly approaches, including cold pressing and supercritical carbon dioxide extraction, have gained increasing attention because they can preserve thermolabile compounds and improve the quality of the extracted oil [5, 6]. Therefore, optimization of extraction conditions remains essential for maximizing oil yield while maintaining its nutritional and functional properties. Recent studies have demonstrated the potential of pomegranate seed oil as a natural source of bioactive lipids for the prevention and management of chronic diseases associated with oxidative stress, inflammation, obesity, diabetes, and cardiovascular disorders [4]. Moreover, the utilization of pomegranate seeds for oil production contributes to sustainable processing practices by converting agro-industrial by-products into value-added products. Such an approach is consistent with the principles of the circular bioeconomy and supports the efficient use of natural resources. Despite the growing body of research on pomegranate seed oil, further investigations are required to improve extraction efficiency, characterize its FA composition and bioactive constituents under different processing conditions, and expand its industrial applications.

Therefore, the aim of the present study was to investigate the extraction and characterization of pomegranate seed oil and to evaluate its potential as a valuable natural source of bioactive compounds.

Materials and methods

Extraction of Pomegranate seed oil (PSO). Pomegranate seeds were separated from their juice sacs, washed several times with distilled water and air-dried at ambient temperature (4–6 days). Afterwards, the dried seeds were ground with a high-speed mill (MMB 1000/05, Bosch). Ground seeds were further extracted for 6 h using a Soxhlet apparatus applying hexane as solvent at four times volume to weight ratio. The defatted residue obtained after oil extraction was air-dried prior to the extraction of proteins and polyphenolic compounds.

Preparation of Fatty Acid Methyl Esters (FAMEs). The fatty acid composition of pomegranate seed oil was determined after methylation of the oil sample. Briefly, the oil was treated with 1% HCl in methanol and incubated at 60°C for 1 h in a sealed 22 mL vial. After completion of the reaction, fatty acid methyl esters were extracted with n-hexane (1 × 5 mL). The resulting organic phase was directly subjected to GC–MS analysis.

GC–MS Analysis. Fatty acid methyl esters were analyzed using a Shimadzu Nexis GC-2030 gas chromatograph coupled with a Shimadzu QP-2020NX mass spectrometer operating in electron ionization (EI) mode at 70 eV. Separation was achieved on a Supelco SP-2560 capillary column (100 m × 0.20 mm i.d., 0.25 μm film thickness), specifically designed for FAME analysis. Helium was used as the carrier gas at a constant flow rate of 2.0 mL min^{-1}. The injector temperature was maintained at 250°C, and samples were injected in split mode (1:20) with an injection volume of 1 μL. The GC oven temperature program was as follows: initial temperature of 100°C held for 4 min, increased to 200°C at 25°C min^{-1} and held for 8 min, followed by heating to 250°C at 5°C

min⁻¹ and held for 7 min. Mass spectra were recorded in scan mode over the m/z range of 40–400. The ion source and interface temperatures were maintained at 200°C and 250°C, respectively. Compound identification was performed by comparing the obtained mass spectra with those available in the NIST Mass Spectral Library. Relative fatty acid composition was calculated using peak area normalization and expressed as percentage of the total chromatographic area.

Results and Discussion

The fatty acid composition of pomegranate seed oil was determined by GC–MS analysis following transesterification of triglycerides into their corresponding FAMEs. The chromatographic analysis revealed that the oil was predominantly composed of unsaturated **FAs**, with punicic acid being the major constituent**.** A typical GC chromatogram of the FA separation of pomegranate seed oil is shown in Figure, and the FA composition of the pomegranate seed oil, obtained by GC/MS analysis, is presented in Table.

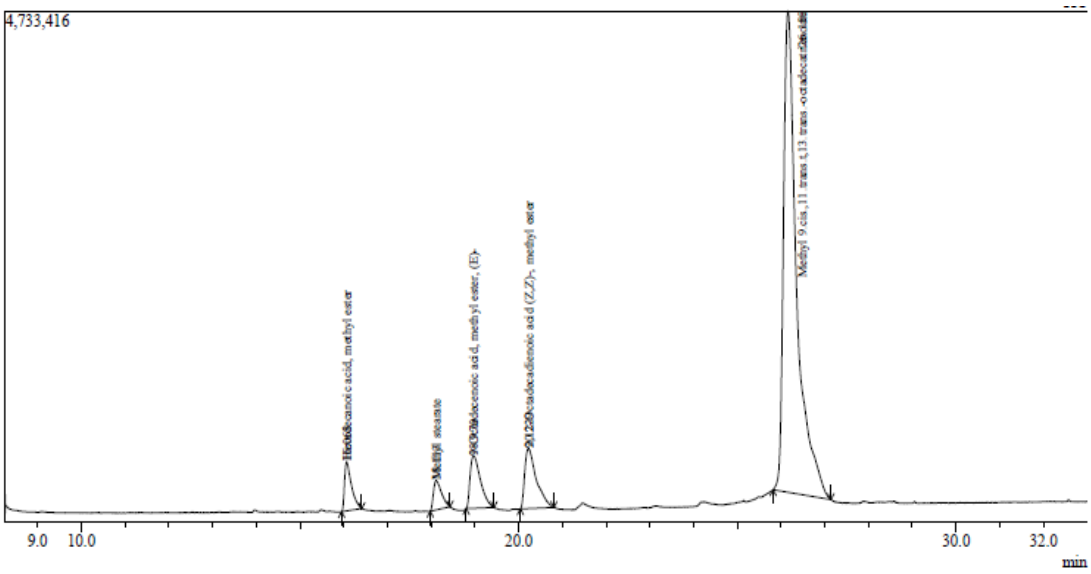


Figure. GC–MS chromatogram of FAMEs obtained from pomegranate (Punica granatum L.) seed oil.

Table. Fatty acid composition of pomegranate seed oil determined by GC–MS

Peak	Retention Time	Common name of fatty acid	Systematic name of fatty acid	Numerical symbol	Peak area percentage in oil (mean wt % ±SD)
1	16.068	Palmitic acid	Hexadecanoic acid	C16:0	4,16
2	18.113	Stearic acid	Octadecanoic acid	C18:0	2,89
3	18.970	Oleic acid	9-Octadecenoic acid	C18:1; ω-9	6,65
4	20.223	Linoleic acid	9Z,12Z)-octadeca-9,12-dienoic acid	C18:2; ω -6	8,53
5	26.159	Punicic acid	(9Z,11E,13Z)-octadecatrienoic acid	C18:3	77,78

Among the identified compounds, methyl punicate (methyl 9-cis,11-trans,13-cis-octadecatrienoate), corresponding to punicic acid, was detected at a retention time of 26.159 min and accounted for 77.78% of the total fatty acids. Punicic acid is a conjugated linolenic acid (CLnA) recognized for its antioxidant, anti-inflammatory, antidiabetic, and anticancer properties. Other major fatty acids identified included linoleic acid (8.53%), oleic acid (6.65%), palmitic acid (4.16%), and stearic acid (2.89%). The predominance of unsaturated fatty acids (>92%) indicates the high nutritional and functional value of the oil. The relatively low levels of saturated fatty acids (palmitic and stearic acids) further enhance its potential application in food, nutraceutical, and pharmaceutical formulations.

The obtained fatty acid profile agrees well with previously published reports on pomegranate seed oil. Johanningsmeier and Harris [1] reported that punicic acid represents approximately 65–80% of total fatty acids in pomegranate seed oil. Similarly, Verardo et al. [5] identified punicic acid as the predominant fatty acid, with concentrations exceeding 70% depending on cultivar and geographical origin. Differences in fatty acid composition among studies may be attributed to cultivar, climatic conditions, seed maturity, extraction method, and analytical procedures.

The exceptionally high content of punicic acid (77.78%) observed in the present study confirms that the investigated pomegranate seed oil is a rich natural source of conjugated linolenic acid. Such a composition supports its potential utilization as a functional ingredient with significant biological activity and health-promoting effects.

The predominance of punicic acid is particularly important because this conjugated FA exhibits diverse biological activities. Numerous studies have demonstrated that punicic acid possesses antioxidant, anti-inflammatory, anti-obesity, antidiabetic, and anticancer properties [7, 8, 9]. Furthermore, it has been reported to modulate lipid metabolism and reduce oxidative stress by regulating cellular signaling pathways. Therefore, the high concentration of punicic acid observed in the present study highlights the potential of pomegranate seed oil as a valuable source of functional lipids for food, nutraceutical, and pharmaceutical applications.

The fatty acid profile obtained in this study confirms that pomegranate seed oil is rich in unsaturated fatty acids, particularly conjugated polyunsaturated fatty acids. Such a composition is desirable because unsaturated fatty acids contribute to various health-promoting effects and enhance the commercial value of the oil. The presence of bioactive lipids supports the utilization of pomegranate seed oil as a natural ingredient in functional foods and health-related products. Overall, the GC–MS analysis demonstrated that punicic acid was the dominant FA in the analyzed pomegranate seed oil sample, accounting for 77.78% of the total FA composition. These findings further support the growing interest in pomegranate seed oil as a promising source of biologically active compounds with potential industrial and therapeutic applications.

Conclusions. In the present study, pomegranate (Punica granatum L.) seed oil was successfully extracted using Soxhlet extraction with n-hexane and characterized by GC–MS analysis. The obtained results demonstrated that the oil possesses a distinctive fatty acid profile dominated by unsaturated fatty acids. Punicic acid was identified as the major fatty acid constituent, representing 78.60% of the total fatty acid content, followed by linoleic acid (7.74%), oleic acid (6.18%), palmitic acid (3.82%), and stearic acid (3.02%). The exceptionally high concentration of punicic acid confirms that pomegranate seed oil is a rich natural source of conjugated linolenic acid with significant nutritional and biological value. The predominance of

bioactive unsaturated fatty acids suggests potential antioxidant, anti-inflammatory, cardioprotective, and metabolic health-promoting properties of the oil.

These findings highlight the potential application of pomegranate seed oil as a valuable ingredient in food, nutraceutical, pharmaceutical, and cosmetic formulations. Moreover, the utilization of pomegranate seeds, an agro-industrial by-product, contributes to waste valorization and supports sustainable processing practices. Further studies should focus on the optimization of extraction technologies, isolation of individual bioactive compounds, and evaluation of their biological activities to expand the industrial and therapeutic applications of pomegranate seed oil.

REFERENCES

1. Johanningsmeier, Harris. 2011. *Pomegranate as a Functional Food and Nutraceutical Source*. Annual Review of Food Science and Technology, 2, 181–201.
2. Elfalleh et al. 2011. *Total Phenolic Contents and Antioxidant Activities of Pomegranate Peel, Seed and Pulp Extracts*. Journal of Medicinal Plants Research, 5(20), 4724–4730.
3. Lansky, Newman. 2007. *Punica granatum and its Potential for Prevention and Treatment of Inflammation and Cancer*. Journal of Ethnopharmacology, 109(2), 177–206.
4. Melo et al. 2018. *Punicic Acid: Chemistry, Biological Activities and Health Benefits*. Food Research International, 106, 611–625.
5. Verardo et al. 2014. *Natural Product Recovery from Pomegranate Seed Oil*. Food Research International, 65, 445–452.
6. Goula et al. 2017. *Ultrasound-Assisted Extraction of Pomegranate Seed Oil*. Journal of Food Engineering, 206, 119–127.
7. Caligiani, A., Bonzanini, F., Palla, G., Cirlini, M., Bruni, R. (2010). Characterization of a potential nutraceutical ingredient: pomegranate seed oil and related minor compounds. *Food Chemistry*, 123(2), 347–353.
8. Fernandes, L., Pereira, J.A., López-Cortés, I., Salazar, D.M., Ramalhosa, E., Casal, S. (2015). Fatty acid, vitamin E and sterol composition of pomegranate (Punica granatum L.) seed oils. *Journal of Food Composition and Analysis*, 39, 1–6.
9. Grossmann, M.E., Mizuno, N.K., Schuster, T., Cleary, M.P. (2010). Punicic acid is an omega-5 fatty acid capable of inhibiting breast cancer proliferation. *International Journal of Oncology*, 36, 421–426.