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Fatty acid profile, tocopherol content, and phenolic compounds of pomegranate (*Punica granatum* L.) seed oils

Manal Almoraie [a,b], Jeremy Spencer [a,*], Carol Wagstaff [a]

[a] Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading RG6 6DZ, UK
[b] Department of Food Science and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah 21589, Saudi Arabia

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ABSTRACT

Pomegranate (*Punica granatum* L.) seeds are typically treated as by-products of fruit processing; however, pomegranate seed oil (PSO) has recently gained attention owing to its potential health benefits. In this study, we investigated the fatty acid profile, micronutrient profile, and polyphenol composition of PSO in fresh samples, extracted via cold pressing, and also in commercial oil samples. Results showed high levels of polyunsaturated fatty acids across samples (86.71 %-89.21 %), punicic acid was the main lipid (81.81 %-84.86 %), along with smaller amounts of linoleic and oleic acids. Total tocopherol content ranged between 362.69 and 397.48 mg/100 g of oil, γ-tocopherol being the major form. Catechin, gallic, vanillic, ellagic, *p*-coumaric, and ferulic acids were the major phenolic compounds present in PSO, particularly gallic acid, which was the most abundant (15.82–24.81 mg/100 g of oil). Our findings indicate the untapped potential of PSO as a nutraceutical or food ingredient with valuable potential health benefits.

1. Introduction

Pomegranate (*Punica granatum*) is a commonly grown and consumed crop belonging to the family Punicaceae and originating in western Asia. It is cultivated in Asian, European, Middle Eastern, and South American countries and is usually grown in tropical and subtropical regions with diverse climatic conditions (Viuda-Martos et al., 2010; Akbar et al., 2015). The fruit comprises three main parts: outer peel, inner peel (mesocarp), and edible arils, which contain sacs of pulp and pomegranate seeds (Holland et al., 2009). Pomegranate pulp is sweet and contains high concentrations of bioactive substances, particularly phenolic acids (Lansky and Newman, 2007). In addition to being consumed fresh, the primary commercial application of the arils lies in the juice processing, with the seeds, the sources of pomegranate seed oil (PSO), often discarded in the process (Teixeira da Silva et al., 2013; Kaseke et al., 2020; Paul and Radhakrishnan, 2020). This wastage is not only an inefficient use of resources but also causes environmental pollution (Banerjee et al., 2017).

Seeds generally are a rich source of lipids, fibres, and proteins, constituting approximately 12 %–20 % of the fruit weight (Elfalleh et al., 2011; Fernandes et al., 2015; Talekar et al., 2018). In particular, PSO represents 10 %–25 % of the total seed weight and has a high concentration of conjugated linolenic acids (cLnA), which are polyunsaturated fatty acids (PUFAs). CLnA isomers can be either cis- or trans-, with a single bond separating the double bonds (Cao et al., 2006; Boroushaki et al., 2016). PSO exhibits a unique fatty-acid profile, with punicic acid (PA), an omega-5 cLnA (*cis*-9, *trans*-11, *cis*-13 18:3; Fig. Supplementary 1), being the most abundant fatty acid in PSO (Grossmann et al., 2010), at approximately 64 %–83 % of the total fatty acid content (Vroegrijk et al., 2011; Boroushaki et al., 2016), followed closely by linoleic and oleic acids (Paul and Radhakrishnan, 2020; Iriti et al., 2023). Furthermore, PA has been previously shown to have an impact on diabetes, obesity, inflammation, and cancer (Hora et al., 2003; Nekooeian et al., 2014; Aruna et al., 2016; Khajebishak et al., 2019). Other bioactive compounds in PSO include tocopherols and phenolic compounds (Hernández-Corroto et al. 2022; Costa et al., 2019). Polyphenols, such as gallic acid and ellagic acid, exhibit significant anti-cancer, anti-inflammatory, antioxidant and hypolipidaemic effects (Pandey and Rizvi, 2009). Tocopherols, particularly α-, γ- and δ-tocopherols, function as lipid-soluble antioxidants, protecting cell membranes from oxidative damage and contributing to skin health, immune function and a reduced risk of atherosclerosis (Szewczyk et al., 2021). Beta-carotene also

Abbreviations: PSO, pomegranate seed oil; CLnA, conjugated linolenic acids; PA, punicic acid; FAME, fatty acids methyl ester; PS, pomegranate seeds; GC-MS, gas chromatography-mass spectrometry; PVDF, polyvinylidene difluoride; PUFAs, polyunsaturated fatty acids.

* Corresponding author.
E-mail addresses: m.almoraie@pgr.reading.ac.uk (M. Almoraie), j.p.e.spencer@reading.ac.uk (J. Spencer), c.wagstaff@reading.ac.uk (C. Wagstaff).

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contributes to these health benefits by acting as a powerful antioxidant, supporting immune function, promoting skin health and reducing the risk of chronic diseases (Bufka et al., 2024). The synergistic action of these compounds in natural food sources enhances their potential to promote overall health and prevent disease. The quantity of bioactive molecules present in PSO is influenced by various factors, including species and variety (Elfalleh et al., 2011; Okere et al., 2022), harvest period (Peng, 2019; Zaouay et al., 2020), and extraction method (Costa et al., 2019; Silva et al., 2019; Kaseke et al., 2020; Liu et al., 2022). Although PSO is increasingly being recognised as a functional food, previous studies have primarily focused on the effects of different extraction techniques on its chemical properties (Liu et al., 2009; Aruna et al., 2018; Liu et al., 2022), whereas, to date, information available on the comprehensive chemical composition of commercial cold-pressed PSO remains limited, hindering the assessment of its bioactivity in comparison to other widely consumed edible oils. Therefore, we performed a chemical analysis of four PSO samples from different origins, with the aim of comparing the fatty acid profiles and bioactive compound content (phenolics, tocopherols, and carotenoids) across these samples. We have chosen a fresh sample, commercial seeds, and two commercial oils to reflect the variability of available market products, given the limited edible options. This comparison was made to understand how the variations in PSO composition, influenced by their different origins, might impact their potential health benefits.

2. Materials and methods

2.1. Samples and chemicals

Fresh pomegranates, serife variety, grown in Turkey were obtained from Asda Supermarket (Reading, UK), sun-dried commercial pomegranate seeds grown organically in Turkey were purchased from Hatton Hill Organic (UK), and cold-pressed commercial PSO was obtained from Fushi, UK (South African variety) and Pödör, USA (Iranian variety).

Fatty acid standards (37 Component FAME mixture from C4 to C24) and standard phenolic components (vanillic acid, trans-ferulic acid, gallic acid, (+) —catechin, caffeic acid, *p*-coumaric acid, (—) —epicatechin, ellagic acid, quercetin, and punicalagin) and α-, δ-, γ-tocopherol and β- carotene were all purchased from Sigma-Aldrich (Dorset, UK). 11(E), 13 (*Z*)-Octadecatrienoic Acid methyl ester was purchased from Cambridge Bioscience. All other chemicals used were of analytical grade and sourced from Sigma-Aldrich (Dorset, UK).

2.2. PSO extraction

Pomegranates were processed in a pilot plant at the University of Reading. Following fruit selection, washing, and sanitisation, pomegranates were cut and manually processed to separate the peels and arils. Subsequently, arils were manually squeezed using a cheesecloth to separate juice from the seeds, which were then washed with distilled water to remove any adhered pomegranate flesh. Pomegranate seeds (PS) were frozen at −80 °C for 36—48 h, and freeze-dried (CoolSafe Pro 110–4, Korea) at −105 °C for 72 h. Dried samples were stored at —20 °C until further use. PS (500 g) were pressed using a single-screw press (KK20, Oil Press GmbH & Co. KG, Germany). The maximum capacity of the expeller press was approximately 20 kg/h. The press head was preheated to 65 ± 5 °C prior to oil extraction using a detachable heating element. PSO was centrifuged for 15 min at 4000 rpm (Thermo Scientific Heraeus® Multifuge® 3SR Plus Centrifuge, Germany) to remove any sediments or fines. The oil samples were placed in brown bottles and kept at −20 °C before further analysis to minimize oxidation.

Oil yield (%) = (Mass of extracted oil (g))/(Total mass of starting material (g)) × 100(1)

2.3. Analytical methods

2.3.1. Preparation of fatty acid methyl esters (FAME)

Pomegranate seed oil was converted to FAMEs according to the method outlined by O'Fallon et al. (2007); Briefly, PSO (40 μL) was mixed in a tube with 0.7 mL of 10 N potassium hydroxide (KOH) in water and 5.3 mL of MeOH. The tube was placed in a water bath at 55 °C for 1.5 h with vigorous shaking every 20 min to ensure proper sample permeation, dissolution, and hydrolysation. Then, samples were cooled in a tap-water bath and 0.58 mL of 24 N sulphuric acid (H_2SO_4) in water was added, vortexed, and incubated in a 55 °C water bath for 1.5 h, with periodic shaking every 20 min for 5 s. After FAME synthesis, the tube was cooled. Subsequently, 3 mL of hexane was added and the mixture was vortexed for 5 min. The tube was centrifuged for 5 min and the upper layer (hexane) was transferred to a clean tube. Then, the clean tube was placed at −20 °C before GC-MS analysis.

2.3.2. GC-MS analysis

Fatty acid-methyl esters (FAME) were analysed via gas chromatography (GC, Agilent 7693) and separated in a FAME column HP-88 (100 m x 0.25 mm×0.20 μm film thickness, Chrompack, London). Helium was utilised as the carrier gas at a 1.3 mL/min flow rate. The inlet temperature of the GC was 260 °C, while the oven temperature was 120 °C (hold for 0.50 min). The volume of the injected sample was 1 μL with a split ratio of 50:1. Fatty acids were identified using standards: 37 fatty acids from C4 to C24 and 9 (Z), 11(E) and 13 (*Z*)-octadecatrienoic acid methyl ester. The composition of individual fatty acids was represented as a percentage of the total identified fatty acids.

2.4. Bioactive compounds in PSO

2.4.1. Phenolic compound extraction

Phenolic compounds were extracted and detected according to the method outlined by Tsimidou (1998) and da Silveira et al. (2017). Briefly, 100 mg of each PSO sample was mixed with 1 mL of hexane for 5 min using a vortex mixer. After adding 1 mL of methanol: water (60/40), the mixture was centrifuged for 10 min at 3500 rpm (Centrifuge 5427 R; Eppendorf, Hamburg, Germany), and the polar extract was transferred to a new tube. The extraction was repeated three times to ensure completion. All extractions were combined and then evaporated in a SpeedVac vacuum (Thermo Vacuum Evaporator, Thermo Scientific, UK) at 35 °C and then dissolved with methanol/water (50:50 v/v) to a volume of 1 mL. After centrifugation (3000 rpm, 5 min), the supernatant was filtered using a 0.25 μm filter (PVDF) membrane and transferred to a clean tube. The extracts were stored at −80 °C until LC analysis.

LC analysis was conducted in negative ion mode using an Agilent 1200 Series LC system, which included a binary pump, degasser, autosampler, thermostat, column heater, and diode array detector (DAD). Compound separation was achieved on a ZORBAX Eclipse Plus C18 column (2.1 mm × 150 mm, 3.5 μm; Agilent, Santa Clara, CA, USA). The mobile phase consisted of 0.1 % (v/v) formic acid in water (A) and 0.1 % (v/v) formic acid in methanol (B), at a flow rate of 0.3 mL/min. An injection volume of 5 μL was used. The gradient was optimized as follows: 1–4 min, 1 %–5 % B; 4–20 min, 5 %–27 % B; 20–50 min, 27 %–60 % B; and 50–57 min, 95 % B.

Phenolic compounds were identified by comparing their retention times with those of authentic commercial standards. Quantification was performed using a DAD detector based on UV absorbance at 280 nm (for catechin, epicatechin, ellagic acid, gallic acid, *p*-coumaric acid, and vanillic acid) and 330 nm (for caffeic acid and ferulic acid). Calibration curves were prepared for each standard, with correlation coefficients (R^2) ranging from 0.98 to 1. Results were expressed as mg/100 g of oil.

2.4.2. Total carotenoids

The total carotenoid content was determined using the method outlined by Ranjith et al. (2006). Briefly, 0.2 g of PSO was dissolved in

5 mL hexane before adding 0.5 mL of sodium chloride solution (NaCl, 0.5 %, w/v). This mixture was centrifuged for 5 min at 4000 rpm (Centrifuge 5427 R; Eppendorf, Hamburg, Germany). The absorbance values were measured at λ = 460 nm using a UV/VIS spectrophotometer (Lambda XLS, UK), and the results were presented as μg β-carotene/100 g of PSO.

2.4.3. Determination of tocopherols

Following the method used by Gimeno et al. (2000), 100 mg of samples were dissolved in 900 μL hexane and thoroughly vortexed before centrifuging (Centrifuge 5427 R, Eppendorf, Hamburg, Germany) at 3000 rpm, for 5 min; then, the supernatant was filtered using a 0.25 μm filter (PVDF) membrane and transferred to a clean tube.

HPLC-DAD (Agilent 1200, Manchester, UK) was performed using a ZORBAX SB-C-18 column (4.6 cm × 150 mm, 5 μm). The injection volume was 5 μL. The mobile phase was methanol: water (96:4, v/v), with elution carried out at a flow rate of 1 mL/min. The analytical column was maintained at 23°C. For compound identification, working standard solutions were analysed together with the samples. The standard curve (α-, γ-, and δ-tocopherols), had a linearity range of 0.1–50 μg/mL and $R^2 = 0.99$ for all isomers. Tocopherol content was expressed as mg/100 g of oil. Detection was performed at 295 nm and each run lasted 18 min (Gimeno et al., 2000).

2.5. Statistical analysis

Statistical analysis of the data was performed using the SPSS software, version 27.0. Parameter measurements were performed in triplicate. Data on continuous variables in the text and tables are means ± standard deviation (SD); categorical variables were frequency and percentage. One-way ANOVA followed by Tukey's Honest Significance Difference test was used to determine the significance of the relationships among the different groups. All probability tests with $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Total oil content

Cold pressing of fresh pomegranate seeds resulted in an oil yield of 11.49 %, compared to 11 % for commercial seeds, on a dry weight basis. Interestingly, our yield from cold-press extraction was higher than that reported by Khoddami et al. (2014) and Ghorbanzadeh and Rezaei (2017). The variations in the oil yield between this study and the others might have been due to the specific variety of pomegranate, the environmental conditions in which the plants were grown, and/or harvest time (Liu et al., 2022). Moreover, different extraction techniques affect oil yield (Tian et al., 2013; Goula et al., 2018). The main disadvantage of the cold pressing method is the incomplete extraction of oil from raw materials (Dąbrowski et al., 2020).

3.2. Chemical properties and composition of PSO

3.2.1. Fatty acid composition

Fatty acid composition in PSO enhances glucose intake (Khajebishak et al., 2019), controls inflammatory diseases linked to obesity (Harzallah et al., 2016; Hontecillas et al., 2009), and regulates insulin resistance (Nekooeian et al., 2014). Using FAME analysis with a GC-MS system, 13 fatty acids were found in the oil samples processed in this study (Table 1). The fatty acids identified were palmitic (C16:0), stearic (C18:0), margaric (C17:0), arachidic (C20:0), behenic (C22:0), oleic (C18:1), linoleic (C18:2), eicosenoic (C20:1), punicic (C18:3-c9, t11, c13), cis 11- octadecanoic (C18:1), and cis-trans 12-octadecanoic (C18:2) (Table 1). In this study, the main component of PSO was found to be PA (84.86 %—81.81 %). This finding agreed with previous reports, which found percent PA contents in PSO of 71 %—78 %, 72 %–78 %, 64.47 %-82.81 %, 70.41 %-74.57 %, and 82 %–86 % in Turkish, Chinese, Iranian, South African, and Tunisian pomegranate varieties, respectively (Juhaimi et al., 2017; Peng, 2019; Khemakhem et al., 2021; Iriti et al., 2023). In this study, the proportions of other fatty acids were as follows: 2.45 %—2.72 % palmitic, 1.64 %—1.82 % stearic, 3.92 %—4.73 % oleic, and 4.02 %—4.84 % linoleic acid. These results are in accordance with previous studies, which used pomegranate seeds produced in Turkey, China, Spain, Tunisian, and Iran (Kýralan et al., 2009; Jing et al., 2012; Verardo et al., 2014; Zaouay et al., 2020; Liu et al., 2022). In addition, some studies have detected other fatty acid compounds, such as nervonic, arachidic, erucic, lignoceric, and myristic acids (Jing et al., 2012; Amri et al., 2017; Khemakhem et al., 2021), although these were generally found in trace concentrations (< 1 % of the total fatty acids). Çelenk et al. (2017) found that PSO had a higher PUFAs percentage, mainly in the form of triacylglycerol, compared to other edible oils. Overall, these results show that PSO represents a dietary fat source with excellent nutritional qualities owing to its high PUFA levels and lower levels of saturated fatty acids.

3.2.2. Polyphenols

Phenolic compounds in PSO largely determine its quality and are responsible for its taste (Badr et al., 2020; Tavakoli et al., 2024). In this study, PSO showed a distinct phenolic profile comprised of two primary categories: phenolic acids and flavonols (Table 2 and Fig. Supplementary 2). Gallic acid was the main component in all PSO samples (24.81–15.82 mg/100 g), followed by ellagic acid

Table 1
Fatty acids (%) identified in PSO.

Fatty acid %	Fresh Seed-oil (Turkey)	Commercial Seed-oil (Turkey)	Commercial oil (South Africa)	Commercial oil (Iran)
Palmitic acid $C_{16:0}$	2.68 ± 0.01^{b}	2.65 ± 0.03^{b}	2.72 ± 0.03^{bc}	2.45 ± 0.03^{a}
Margaric acid $C_{17:0}$	0.09 ± 0.00^{b}	0.08 ± 0.01^{b}	0.11 ± 0.01^{bc}	0.47 ± 0.05^{a}
Stearic acid $C_{18:0}$	1.70 ± 0.02^{a}	1.79 ± 0.09^{a}	1.82 ± 0.03^{a}	1.64 ± 0.12^{a}
Arachidic acid $C_{20:0}$	0.38 ± 0.02^{a}	0.37 ± 0.03^{a}	0.45 ± 0.02^{b}	0.45 ± 0.02^{b}
Behenic acid $C_{22:0}$	0.11 ± 0.00^{a}	0.09 ± 0.00^{a}	0.35 ± 0.01^{a}	0.38 ± 0.04^{a}
Oleic acid $C_{18:1\ (cis)}$ (n−9)	3.92 ± 0.04^{a}	4.03 ± 0.01^{b}	4.73 ± 0.04^{c}	4.45 ± 0.02^{d}
Octadecanoic acid $C_{18:1\ (cis)}$	0.41 ± 0.01^{a}	0.43 ± 0.02^{a}	0.64 ± 0.02^{b}	1.12 ± 0.06^{c}
Eicosenoic acid $C_{20:1\ (cis)}$ (n−9)	0.59 ± 0.01^{a}	0.65 ± 0.03^{ab}	0.68 ± 0.03^{b}	1.76 ± 0.02^{c}
Octadecanoic acid $C_{18:2\ (cis9\text{-}trans12)}$	0.26 ± 0.02^{a}	0.27 ± 0.01^{a}	0.51 ± 0.07^{c}	0.40 ± 0.01^{b}
Linoleic acid $C_{18:2\ (cis9\text{-}cis\text{-}12)}$ (n−6)	4.16 ± 0.02^{a}	4.02 ± 0.09^{a}	4.39 ± 0.11^{c}	4.84 ± 0.04^{b}
Punicic acid $C_{18:3\ (cis9\text{-}trans11,cis13)}$	84.79 ± 0.03^{c}	84.86 ± 0.24^{c}	81.81 ± 0.14^{a}	82.05 ± 0.03^{b}
Total SFA	4.96	4.98	5.45	5.39
Total MUFA	4.92	5.11	6.05	7.33
Total PUFA	89.21	89.15	86.71	87.29

Different letters within the same row indicate significant differences (p < 0:05. All data are expressed as mean ± standard deviation (SD). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Fresh Seed-oil: oil extracted from fresh pomegranate seeds, Commercial Seed-oil: oil extracted from commercial dry seeds.

Table 2
Phenolic compounds composition (mg/100 g of extract) of PSO.

Phenolic Compounds	[M-H]⁻ *m/z*	Fresh Seed-oil (Turkey)	Commercial Seed-oil (Turkey)	Commercial oil (South Africa)	Commercial oil (Iran)
Gallic acid	169	24.81 ± 0.12^{a}	18.59 ± 0.15^{b}	15.82 ± 0.04^{d}	18.02 ± 0.06^{c}
(+)- Catechin	288.9	3.66 ± 0.14^{a}	3.21 ± 0.07^{b}	3.38 ± 0.22^{a,b}	3.49 ± 0.17^{a,b}
Vanillic acid	166.8	7.61 ± 0.08^{a}	6.67 ± 0.11^{b}	6.73 ± 0.21^{b}	6.02 ± 0.10^{c}
Caffeic acid	178.9	ND	ND	1.69 ± 0.06	1.56 ± 0.05
Epicatechin acid	289	4.86 ± 0.31^{a}	4.48 ± 0.12^{a}	3.75 ± 0.22^{b}	3.85 ± 0.22^{b}
P-coumaric acid	162.9	4.17 ± 0.04^{a}	4.15 ± 0.03^{a}	3.27 ± 0.07^{c}	3.71 ± 0.07^{b}
Ferulic acid	192.9	2.39 ± 0.01^{a}	1.86 ± 0.01^{b}	1.77 ± 0.03^{b}	1.71 ± 0.02^{b}
Ellagic acid	301	9.26 ± 0.28^{a}	8.42 ± 0.10^{b}	8.48 ± 0.03^{b}	8.25 ± 0.11^{b}
Quercetin−3-O-rhamnoside	447.3	4.08 ± 0.11^{a,b}	4.19 ± 0.02^{a,b}	4.42 ± 0.12^{a}	3.78 ± 0.44^{b}

Different letters within the same row indicate significant differences ($p < 0:05$. All data are expressed as mean ± standard deviation (SD) of at least three replicates of each sample. ND: not detected. Fresh Seed-oil: oil extracted from fresh pomegranate seeds, Commercial Seed-oil: oil extracted from commercial dry seeds.

(9.26–8.25 mg/100 g). Different ranges of phenolic fractions, including (+)- catechin, epicatechin, and vanillic, *p*- coumaric, and ferulic acids, were detected in all samples, whereas caffeic acid was detected only in PSO from the South African and Iranian varieties (1.69 mg/100 g and 1.56 mg/100 g respectively). Phenolic compound profiles are known to vary among pomegranate fruit varieties owing mainly to the specific environmental conditions in which fruits grow (Iriti et al., 2023).

Fresh PSO from Turkey showed higher gallic acid, (+)-catechin, vanillic acid, ferulic acid, and ellagic acid contents than those of commercial PSO, presumably because fresh PS were processed by freeze-drying, which helps maintain their bioactive ellagitannins and other components, compared to other processing technique (sun-drying) (John et al., 2017). In contrast to other pomegranate fruit parts, such as peels, mesocarps, seeds, arils, and juice, and even leaves (Fischer et al., 2011; Ambigaipalan et al., 2016; Shahkoomahally et al., 2023), few studies have examined the polyphenolic profile of PSO. Moreover, most previous studies have relied on a low-selectivity spectrophotometric assay to determine total phenolic content (Liu et al., 2022; Iriti et al., 2023), and diverse methods were used to extract the oil. Therefore, direct comparisons with other studies are difficult, although the phenolic component profiles presented here are in accordance with the results obtained by Badr et al. (2020). Similarly to the results presented by Costa et al. (2019), vanillic, ferulic, *p*-coumaric acids, and quercetin-3-O-rhamnoside were the main phenolic compounds detected in PSO. However, gallic and ellagic compounds were not found. This might be due to variations in cultivars, location of the pomegranate plants, and/or oil extraction techniques used. These differences likely caused variations in specific phenolic components (Zaouay et al., 2020; Liu et al., 2022).

In this study, PSOs showed significantly higher phenolic concentration compared to commonly consumed vegetable oils, including corn, sunflower, and olive oils (Wildermuth et al., 2016; Çelenk et al., 2017; Medjkouh et al., 2018), which typically contain low phenolic concentrations, further underscoring the phenolic richness of PSOs and their potential health-promoting properties.

3.2.3. Tocopherols

The presence of tocopherol (vitamin E) is important because of its antioxidant activity, which shields PUFAs from oxidative degradation, and its biological activity, which shields cells from oxidative stress (Tucker and Townsend, 2005; Clarke et al., 2008). Additionally, tocopherol has anti-inflammatory properties which contribute to heart health and cancer prevention (Jiang et al., 2022). Total tocopherol content in PSO ranged from 362.69 to 397.48 mg/100 g. Moreover, across all PSOs, γ-tocopherol was the main component of total tocopherols, reaching 334.32–363.51 mg/100 g (on average 98.9 % of total tocopherol), followed by δ-tocopherol (18.48–25.16 mg/100 g), and α-tocopherol (5.51–8.81 mg/100 g) (Table 3). β-tocopherol was not detected in this study, consistent with the findings of Costa, et al., (2019) and Verardo et al.,(2014), while other authors have reported low levels (Melo et al., 2016). In previous studies (Caligiani et al., 2010; Fernandes et al., 2015; Melo et al., 2016; Liu et al., 2022; Iriti et al., 2023) total PSO tocopherol contents varied, ranging from 135 to 564 mg/100 g oil. Although the values found here were within the anticipated range, notable variations were observed in the distribution of isoforms; thus, γ-tocopherol showed the highest contents in PSO (Fernandes et al., 2015; Costa et al., 2019; Badr et al., 2020; Hajib et al., 2021; Liu et al., 2022; Iriti et al., 2023). In contrast, Pande and Akoh (2009) and Jing et al. (2012) reported that δ- and α-tocopherols were predominant. Overall, tocopherol contents of PSOs in this study were found to be much greater than those of other widely used vegetable oils, including corn (91.34 mg/100 g), sunflower (74.31 mg/100 g), and olive (20.2 mg/100 g) oils (Martakos et al., 2019; Wen et al., 2020).

3.2.4. Beta-carotenoids

Carotenoids exhibit antioxidant and cytoprotective properties, helping to combat diseases such as cancer, diabetes, cardiovascular conditions and obesity (Bufka et al., 2024). Beta-carotenoids can be found in PSOs at concentrations ranging from 0.03 to 0.04 μg/100 g (Table 3). These results were similar to those reported by Badr et al. (2020). In contrast, Yoshime et al. (2018) did not identify any carotenoids in PSO. The difference between these outcomes might be caused by variations in the oil extraction method, geographical location, fruit maturity, and/or cultivar used.

Future PSO studies should include comprehensive in vitro and animal studies. Such studies would provide valuable insights into assessing PSO safety and biological activity and could progress to human trial studies to determine PSO's therapeutic efficacy. Sensory studies are also essential to evaluate PSO's taste, aroma and overall acceptability. Understanding sensory attributes is paramount for consumer acceptance

Table 3
Biochemical composition of PSO.

	Fresh Seed-oil (Turkey)	Commercial Seed-oil (Turkey)	Commercial oil (South Africa)	Commercial oil (Iran)
ß Carotenoids (μg/100 g)	0.04 ± 0.01^{b}	0.04 ± 0.02^{b}	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}
α tocopherol (mg/100 g)	8.81 ± 0.92^{b}	7.71 ± 0.12^{b}	5.51 ± 0.44^{a}	6.15 ± 0.12$^{a,\ b}$
γ tocopherol (mg/100 g)	363.51 ± 3.98^{c}	334.32 ± 2.17^{a}	346.69 ± 3.21^{b}	345.94 ± 4.38^{b}
δ tocopherol (mg/100 g)	25.16 ± 1.04^{c}	20.66 ± 1.12$^{a,\ b}$	22.29 ± 0.34^{b}	18.48 ± 0.99^{a}
Total tocopherol (mg/100 g)	397.48 ± 5.94^{c}	362.69 ± 3.41^{a}	374.49 ± 3.99^{b}	370.57 ± 5.49$^{a,\ b}$

Different letters within the same row indicate significant differences ($p < 0:05$. All data are expressed as mean ± standard deviation (SD) of at least three replicates of each sample. Fresh Seed-oil: oil extracted from fresh pomegranate seeds, Commercial Seed-oil: oil extracted from commercial dry seeds.

and can considerably influence PSO's marketability and dietary integration. These findings will aid the development of PSO products tailored according to both health benefits and consumer preferences.

4. Conclusions

Pomegranate seed oil is an emerging nutraceutical and valuable oil source that is economically and nutritionally important. In this study, the chemical compositions of fresh PSO and various commercial PSOs were determined. All PSO samples had substantial levels of PUFAs, primarily PA, which have various health benefits and contain vitamin E and phenolic components, with differences in composition depending on parameters such as the environment of origin. However, extraction conditions may differ between commercial samples and fresh produce, while also the cultivars of commercial oil are unidentified. Therefore, this assessment must be validated in future studies using a variety of cultivars and oils obtained under similar extraction conditions, although that was not the aim here. Few studies have used HPLC to analyse the phenolic profile of PSO. Employing this method in our study allowed the identification of individual phenolic compounds in PSO rather than total phenolic content, thus providing a more detailed phenolic profile.

CRediT authorship contribution statement

Wagstaff Carol: Writing – review & editing, Supervision, Project administration, Conceptualization. **Manal Almoraie:** Writing – original draft, Funding acquisition, Formal analysis, Data curation, Methodology. **Spencer Jeremy:** Writing – review & editing, Supervision, Conceptualization.

Ethical approval

Not Applicable

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Manal Almoraie reports financial support was provided by King Abdulaziz University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.107788.

Data Availability

Data will be made available on request.

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