

The Fatty Acid Composition of Pomegranates Grown in Turkey

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ABSTRACT

The pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and is one of the oldest known edible fruits. It is accepted as a fruit that is a source of health and recovery in many countries in the world. Therefore, recently scientific studies have concentrated on pomegranate and pomegranate seed oil. The objective of this study the dispersion of fatty acids in the seed oil of a type of pomegranate called Hicaznar which is widely grown in Turkey. It has been determined that pomegranate seed oil contains palmitic acid (5.30±0.38%), stearic acid (2.78±0.51%), oleic acid (12.34±2.88%), linoleic acid (13.45±2.01%) and punicic acid (64.65±4.51%) using GC. Pomegranate seed oil contains a high incidence of punicic acid or omega-5. This type of fatty acid is effective in preventing the formation of acidic cancer cells. Punicic acid also has antioxidant, antitumor, anti-atherosclerotic and serum lipid-lowering properties. Thus, this study indicated that fatty acid in the composition of pomegranate has positive health effects.

Keywords: Fatty acid, Linolenic acid, Pomegranate seed, *Punicagranatum*, Punicic acid

INTRODUCTION

Pomegranate is a perennial from the Punicaceae family (*Punica granatum* L.). It grows to a height of 2-5 in the form of a bush. It can commonly be grown in tropical and subtropical regions up to an altitude of 1000 m. Pomegranate is classified as sweet and sourish in terms of taste, and it has fruits with many granular seeds. Pomegranate with fleshy seeds can be found in different colors, from dark red to white [1]. Pomegranate is a symbolic fruit with a rich history regarding art, medicine, and religion. It originates from Iran, and India, China, and Turkey are its primary producers today. In addition to these countries, it is grown in Spain, Morocco, Egypt, the United States, Malaysia, Israel, Tunisia, Saudi Arabia, Azerbaijan, Pakistan, Afghanistan, Africa, Japan, and Russia [2,3].

Though this fruit can be eaten fresh, it is hard to consume it so in general. It is necessary to process the pomegranate for different products in the food industry, such as pomegranate juice, pomegranate juice concentrate, syrup, canned pomegranate, grain from dried pomegranate, pomegranate syrup, jelly, jam, liqueur, and wine. The composition of pomegranate is important to know in order to determine the product into which it will be processed. The diversity of its

usage area has increased the amount of production and consumption of pomegranate around the world [4-6].

Much research has been conducted on the composition of pomegranate fruit in the various countries from which it is produced [7,5]. The fruit composition of pomegranate can vary depending on the type, growing regions, climate, maturity, cultural application and conservation conditions [4].

Pomegranate seeds are rich in lipid sources and pomegranate seed weight is composed of 12-20% lipid.

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Pomegranate seed oil is characterized by polyunsaturated (n-3) fatty acids such as linolenic, linoleic, and other fatty acids such as punicic acid, oleic acid, stearic acid and palmitic acid [8,9].

It is known that pomegranate consumption has an effect on preventing disease by decreasing blood pressure, especially in patients with high blood pressure. It prevents LDL oxidation and increases paraoxonase activity, as well as high blood pressure, inflammation, arteriosclerosis, prostate cancer, cardiac disease and HIV [10-12]. As a home remedy, pomegranate preparations, dried pericarp, root, bark and fruit juice are used for colic, colitis, and dysentery [2]. The pomegranate seed, the by-product of pomegranate juice processing, contains a range of nutraceutical components such as sterols, γ -tocopherol, punicic acid and hydroxyl benzoic acids [13]. The extract of pomegranate seeds was reported to have antidiarrhoeal and antioxidant bioactivities [14].

Pomegranate seed oil consists of approximately 80% conjugated octadecatrienoic fatty acids, with a high content of punicic acid (c9-t11-c13,18:3), a fatty acid specific to this oil. Punicic acid is also known as trichosanic acid (an omega-5 long chain polyunsaturated fatty acid) and an isomer of conjugated α -linolenic acid (CLnA) with structural similarities to conjugated linoleic acid (CLA) and α -linolenic acid (LnA) in ways such as carbon composition, atomic arrangement and the number of carbon double bonds [15,16]. Chemical structure of punicic acid is given **Figure 1** [17].

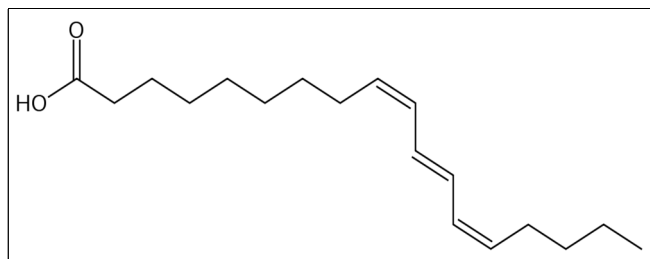


Figure 1. Chemical structure of punicic acid (cis-9,trans-11, cis-13 octadecatrienoic acid)

Punicic acid has increasingly attracted scientific interest because of its several potential health benefits including antioxidant, antitumor, anti-atherosclerotic and serum lipid-lowering properties [18,19]. Overall, the effectiveness using of pomegranate seed oil promotes good health and possibly helps in preventing inflammation, diabetes, brain disorders, oxidative stress, hypoxia, hyperlipidemia (possibly decreased low-density lipoprotein and increased high-density lipoprotein), cardiac disease, AIDS, ischemia and cancer, especially skin, colon, breast, prostate, and lung [20]. The objective of this work is to determine the composition of pomegranate fatty acid which has positive effects on health.

OBJECTIVES

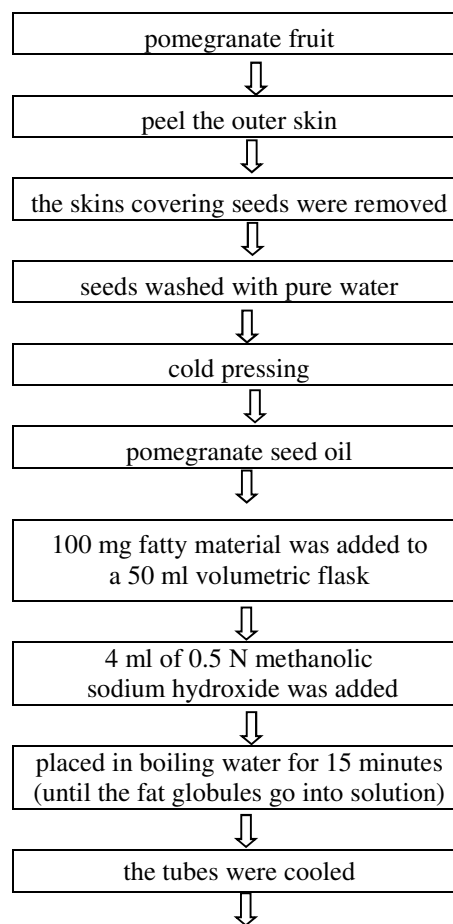
The main aim of this study was to evaluate the dispersion of fatty acids in the seed oil of pomegranate which is widely grown in Turkey. Pomegranate seed oil contains a high incidence of punicic acid which has antioxidant, antitumor, anti-atherosclerotic and serum lipid-lowering properties. The specific objective was fatty acid in the composition of pomegranate has positive health effects.

MATERIALS AND METHODS

Collection and preparation of samples: Approximately 5 kg (n=5) of medium-sized pomegranate fruits (350-400 g) from Hicaznar cultivars in the 2016 autumn season were selected from mature fruits grown in the district of the Fethiye (Mugla) in Turkey.

Analytical procedure:

Preparation of the sample for determination of fatty acids: Fatty acid methyl esters of the extracted oils were prepared according to the method previously reported by Metcalfe et al. [21]. The sample preparation method is given as a schematic diagram in **Figure 2**.



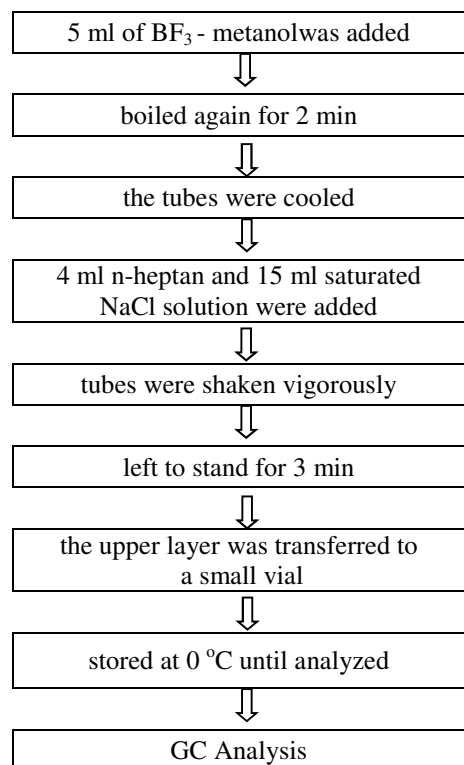


Figure 2. Schematic diagram of the sample preparation method

Fatty acid methyl esters were determined by comparison of their retention times with the individual reference standards of palmitic acid (C_{16:0}), stearic acid (C_{18:0}), oleic acid (C_{18:1}), linoleic acid (C_{18:2}) and punicic acid (C_{18:3}) from Sigma Chemical Co. (St. Louis, MO, USA). The quantification of fatty acids was made according to their relative area percentages compared with their calibration curves.

GC-MS instrument: Fatty acid composition was determined using Gas Chromatography (Agilent 6890 N) equipped with a flame ionization detector (FID) and a ZB-1 column (10 m, 0.53 mm, 2.65 μm). The oven temperature was set at 100°C, which was held for 10 minutes. Then, it was increased at 15°C/min intervals, held for 25 minutes and increased again to 250°C. Detector temperatures were set at 350°C. The injection type was an auto injector and the temperature of the injection was 350°C. The volume of the injection was 1.0 μl, and helium was used as the carrier gas with a linear velocity of 50 cm/second.

Statistical Analysis: Analysis of variance (ANOVA) tests were performed using SAS (SAS Version 6.12). Data were expressed as means ± Standard Error (SE). Samples were studied in two replication.

RESULTS AND DISCUSSION

The fatty acid composition of pomegranate seed oils was determined, and five different fatty acids were identified in

all samples. The content of saturated and unsaturated fatty acids are presented in **Tables 1** and **Table 2**.

Table 1. Fatty acid composition of Hicaznar cultivars pomegranate seed oil

Fatty acid composition (%)	Hicaz nar cultivars				
	N1	N2	N3	N4	N5
Palmitic (C _{16:0})	5.05	5.32	5.93	5.30	4.92
Stearic (C _{18:0})	3.04	2.86	3.47	2.35	2.20
Oleic (C _{18:1})	10.01	17.28	10.60	11.87	11.95
Linoleic (C _{18:2})	12.68	17.00	11.91	12.84	12.85
Punicic (C _{18:3})	67.14	56.61	67.01	66.23	66.30

Punicic acid, a geometric isomer of linolenic acid, was the predominant fatty acid in all pomegranate cultivars. Its amount ranged between 56.61 to 67.14% for N2 and N1 cultivars, respectively. Linoleic acid and oleic acid were the next most abundant fatty acids in these samples. Their amounts ranged between 11.91 to 17.00% (N3 and N2 cultivars, respectively) and 10.01 to 17.28% (N1 and N2 cultivars, respectively). Palmitic and stearic acids were determined in all pomegranate varieties. Their amounts ranged between 4.92-5.93% and 2.20-3.47% (N5 and N3 cultivars respectively) (**Table 1**).

Table 2. Fatty acid composition of pomegranate seed oil

Fatty acid composition (%)	Variation limits			
	Min	Max	Mean	S. D.
Palmitic (C _{16:0})	4.92	5.93	5.30	0.38
Stearic (C _{18:0})	2.20	3.47	2.78	0.51
Oleic (C _{18:1})	10.01	17.28	12.34	2.88
Linoleic (C _{18:2})	11.91	17.00	13.45	2.01
Punicic (C _{18:3})	56.61	67.14	64.65	4.51

The mean palmitic, stearic, oleic, linoleic and punicic acid content of pomegranate seed oil was 5.30 ± 0.38%, 2.78 ± 0.51%, 12.34 ± 2.88%, 13.45 ± 2.01%, and 64.65 ± 4.51%, respectively (**Table 2**). It has been determined that the unsaturated fatty acid content is 90.44% and saturated fatty acid is 8.08% in total. In addition, the rate of saturated fatty acids to unsaturated fatty acids, which is used as an important quality criterion in vegetable oils, has been calculated as 0.09 in pomegranate seed oil. Gölükcü et al. [22] found this rate to be 0.08 in pomegranate seed oil.

The fatty acid composition and related ratios are summarized in **Table 2**. **Figure 3** shows the chromatogram of the punicic acid standard and Figure 4 demonstrates the chromatogram of fatty acids in pomegranate seed oil. As shown, the main constituent of the oil was polyunsaturated fatty acids. Up to 80 percent of the total polyunsaturated fatty acids' profile in pomegranate seed oil was punicic acid (**Table 2**, **Figure 3** and **Figure 4**).

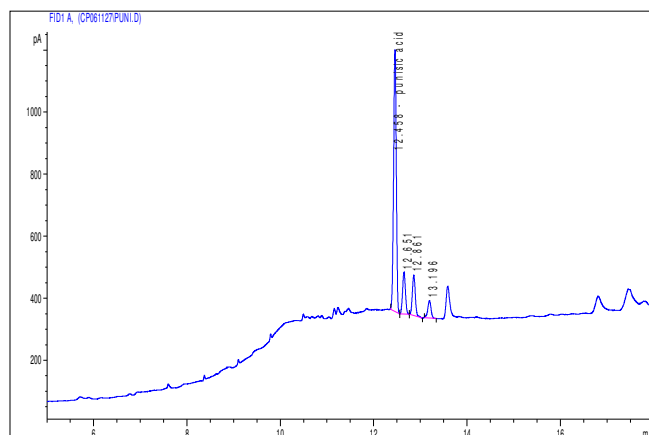


Figure 3. GC Chromatogram of punicic acid standard

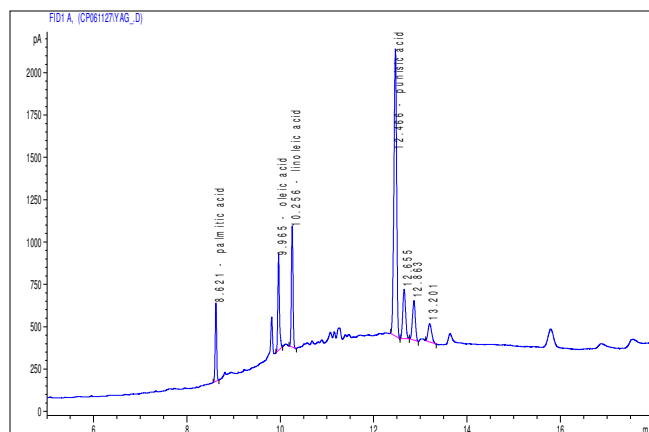


Figure 4. GC Chromatogram of fatty acids in pomegranate seed oil

Prior studies have shown that unsaturated fatty acids were predominant in pomegranate seed oils. El-Shaarawy et al. [23] reported that 8% of fatty acids were saturated, 10% were monounsaturated, 10% diunsaturated and approximately 70% would probably be punicic acid. Melgarejo et al. [24] studied seven clones of a pomegranate from Spain and found that 4.16-26.65% of the fatty acids in seed oils were saturated, and 73.4-95.8% of fatty acid were unsaturated. It was reported that the predominant fatty acid was the polyunsaturated (n-3) linolenic acid (43.4-88.2%), followed by linoleic (5.3-16.5%), oleic (3.7-20.3%) and palmitoleic (traces to 2.9%) acids. To a lesser extent the saturates palmitic (2.6-14.9%) and stearic (1.2-9.0%) acids

were also found. Melgarejo et al. [25] determined the fatty acid composition of the seed oils of six pomegranate cultivars. In all varieties, the predominant fatty acids were linoleic (25.2-38.6%) and oleic acid (24.8-35.5%), followed by palmitic (18.2-22.6%), stearic (6.9-10.4%) and linolenic acid (0.6-9.9%). Elfalleh et al. [26] reported that the predominant fatty acid was linolenic acid (44.51-86.14%), followed by linoleic acid (3.57-13.92%), oleic acid (3.03-12.88%), palmitic acid (3.13-11.82%) and stearic acid (1.68-15.64%) in 21 pomegranate cultivars.

Hernandez et al. [27] have reported palmitic, stearic, oleic, linoleic and punicic acid content of the fatty acid composition of seed oil from three commercial pomegranate cultivars as 2.99-3.83%, 1.6-2.38%, 4.09-4.82%, 4.98-7.74%, and 66.76-79.29%, respectively. Kıralan et al. [28] identified the fatty acid composition of 15 commercially important pomegranate cultivars grown in Turkey. They stated that the palmitic and stearic acid contents of the oils ranged between 2.10-2.77% and 1.35-2.01%, respectively. They also reported that the predominant unsaturated fatty acid was punicic acid (70.42-76.17%). Özgül-Yücel [8] has also declared that punicic acid was found to be dominant (57.3%) in pomegranate oil seeds grown in Turkey. Palmitic acid (2%), stearic acid (1.6%), oleic acid (3.7%), and linoleic acid (3.3%) were also found. Turtygin et al. [29] studied the triglyceride composition of pomegranate seed oil. They obtained the palmitic, stearic, oleic, linoleic and punicic acid contents of the oils ranging between 1.781-2.218%, 1.009-1.296%, 2.825-4.056%, 2.030-2.742% and 70.24-89.68%, respectively. In another study, punicic acid was found to be dominant (76.57%), and the pomegranate seed oil also contained linoleic acid (4.73%), oleic acid (3.91%), stearic acid (1.82%) and palmitic acid (2.49%) [30].

In an earlier attempt to determine the fatty acid composition of pomegranates grown in Turkey, palmitic (4.62%), stearic (2.77%), oleic (6.83%), linoleic (5.81%), and punicic acid (78.83%) have been reported by Gölükçü et al. [22]. Pande et al. [31] found punicic acid (78.3-83.4%) to be the predominant fatty acid and palmitic acid (2.8 - 4.8%), stearic acid (2.1-3.6%), oleic acid (3.6-7.7%) and linoleic acid (3.2-5.8%) were composed of lipid profiles of six pomegranate cultivars.

Parashar et al. [32] studied that the predominant fatty acid was linolenic acid ($C_{18:3}$) and its content was about 31.8-86.6%, followed by linoleic acid (0.7-24.4%), oleic acid (0.4-17.4%), stearic acid (2.8-16.7%) and palmitic acid (0.3-9.9%) in 25 pomegranate varieties obtained from India. Fernandes et al. [33] announced that pomegranate seed lipid fractions consisted mainly of punicic acid (c9, t11, c13 $C_{18:3}$), a geometric isomer of linolenic acid, ranging between 77.3% and 83.6% of the total fatty acids, followed by small amounts of linoleic acid (3.9-5.4%), oleic acid (3.1-5.7%)

and palmitic acid (3.1-4.0%). Parashar [34] determined the fatty acid composition of the seed oils of six pomegranate cultivars by gas chromatography. Eleven fatty acids were identified. In all cultivars, the predominant fatty acids were linoleic (25.2-38.6%) and oleic acid (24.8-35.5%), followed by palmitic (18.2-22.6%), stearic (6.9-10.4%), and linolenic acid (0.6-9.9%). Melo [35] reported that pomegranate seed lipid fractions consisted mainly of punicic acid (55%). Soetjijto et al. [36] determined the composition of fatty acids in the seed oil of red and purple pomegranates. Both showed the same major fatty acids as palmitic, stearic, oleic, linoleic and punicic acid. Oleic acid (19-21%) and linoleic acid (20-21%) were found to be the most dominant fatty acids in red pomegranate, whereas purple pomegranate seed oil was dominated by oleic acid (41-43%) and punicic acid (0-25%). The punicic acid content of the total lipids of purple pomegranate seed oil (0-5%) was higher than red pomegranate (9-16%). El-Nemr et al. [37] stated that stearic acid (22.5%), linoleic acid (10.3%), and oleic acid (5.1%) were found in pomegranate cultivars grown in Egypt.

The results of the research indicate that the compounds identified were the saturated fatty acids palmitic acid (2.38-4.37%) and stearic acid (1.59-3.64%), monounsaturated fatty acid oleic acid (7.10-19.86%), and polyunsaturated fatty acids alpha-linolenic acid (50.90-78.24%) and linoleic acid (5.30-8.76%) [20]. Rowayshed et al. [38] announced that palmitic acid (4.2%), stearic acid (3.2%), oleic acid (6.5%), linoleic acid (9.4%), and punicic acid (59.4%) were found in pomegranate seed oil. Dadashi et al. [39] declared that punicic acid was the highest level among all fatty acids and found it ranging from 72.07-73.31%. Palmitic acid (4.04-4.46%), stearic acid (2.81-3.00%) and oleic acid (8.31-9.77%) were also found in pomegranate seed oil.

Verardo et al. [40] informed the pomegranate seed oil contained significant amounts of conjugated linolenic acids (CLnA), such as punicic acid, in the range of 74-85% of the total fatty acid content. Other major fatty acids were oleic, linoleic and palmitic acid, respectively. Tianet al. [41] reported that the fatty acid compositions were significantly different among the PSO extracted by soxhlet extraction, supercritical fluid extraction, and ultrasonic-assisted extraction, with punicic acid (>65%) being the most dominant using ultrasonic-assisted extraction. They discovered palmitic acid ranging from 5.34-5.97%, stearic acid ranging from 3.85-4.65%, oleic acid ranging from 9.45-10.34%, linoleic acid ranging from 12.87-14.89%, and punicic acid ranging from 65.04-67.85%.

Our results were in agreement with the literature. Qualitatively, in this study, the fatty acid composition was similar to previous findings [20,38]. Punicic acid ranged between 56.61 to 67.14% of total fatty acids in the present study. These results are in accordance with previous reports, once Özgül-Yücel [8] obtained values for this fatty acid at

57.3% in pomegranate seed oil grown in Turkey; Melo [35] determined 55% punicic acid in pomegranate seed oil, Tianet al. [41] found punicic acid ranging from 65.04 to 67.85%. However, in prior studies, the punicic acid content was found to be higher than our results. Hernandez et al. [27] found punicic acid at 66.76-79.29% in seed oil from three commercial pomegranate cultivars; Gölükçüet al. [22] determined punicic acid at 78.83% in pomegranates grown in Turkey; Kiralan et al. [28] identified punicic acid ranged from 70.42-76.17% in 15 commercial pomegranate cultivars in Turkey. Turtyginet al. [29] obtained value for punicic acid ranged between 70.24 to 89.68%; Dadashi et al. [39] found punicic acid ranging from 72.07-73.31%; Verardo et al. [40] found punicic acid at 74-85%; Topkafaet al. [30] reported that punicic acid was found to be 76.57%.

CONCLUSION

Pomegranate is a symbolic fruit with a rich history in terms of art, medicine, and religion. It is grown in many countries like Turkey, Iran, India, and China. In this study, five pomegranate cultivars grown in Turkey were analyzed for fatty acid composition. Palmitic acid, stearic acid, oleic acid, linoleic acid and punicic acid were determined to be in pomegranate seed oil. All cultivar samples included a high rate of punicic acid. Punicic acid is also known as omega-5 and is a conjugated isomer of a-linolenic acid. It has increasingly attracted scientific interest because of its several potential health benefits. Consequently, pomegranate seed oil is found to be very valuable in terms of health.

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