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ORIGINAL PAPER

Oil and Conjugated Linolenic Acid Contents of Seeds from Important Pomegranate Cultivars (*Punica granatum* L.) Grown in Turkey

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Abstract In the present study, seed oil content and fatty acid composition of 15 commercially important pomegranate cultivars were determined. The oil content of pomegranate seeds ranged between 13.95 and 24.13% (d.b). Palmitic, stearic, arachidic, and behenic acid contents of the oils ranged between 2.10–2.77, 1.35–2.01, 0.33–0.48, and 0.16–0.22%, respectively. The predominant unsaturated fatty acid was punicic acid (70.42–76.17%) and a minor unsaturated fatty acid was gadoleic acid (0.42–0.75%). The analysis on unsaturated fatty acids particularly showed significant amounts of punicic acid, which is considered to enhance the oil quality and is of importance to health.

Keywords Pomegranate oil · Fatty acid · Conjugated linolenic acid

Introduction

The pomegranate (*Punica granatum* L.) belongs to the Punicacea family and is generally grown in tropical and subtropical regions. It is native to Iran and has been cultivated extensively in that country, India and the USA, and to a lesser extent in most near and far eastern countries [1]. Iran produced approximately 670,000 tonnes of pomegranates in 2005 [2]. Turkey is the third largest producer with 60,000 tonnes being produced in 2004 [3]. Pomegranate is mostly consumed fresh, however, in the food industry it may be use to produce fruit juice, wine, liqueur, pekmez (condensed juices) and canned food [4, 5]. Pomegranate seeds remain as a waste product after processing. The fruit contains considerable amounts of seeds, ranging between 40 and 100 g kg^{-1} of fruit weight depending on cultivar [6, 7]. Considering the amount of seed that is produced, oil content and composition might be of interest.

Pomegranate seed oil contains considerable amounts of polyunsaturated fatty acid (PUFA) including conjugated linolenic acid (CLA) isomers as well as a low saturated fatty acid content [8]. CLA isomers may prevent diseases such as cancer [9] and [10]. Moreover, consumption of oils, rich in highly unsaturated fatty acids (HUFA), protects against diseases such as colorectal, breast, colon and prostate cancers [11]. Essential fatty acids such as omega 3-, and 6-fatty acids, decrease plasma triacylglycerols and LDL cholesterol, which are risk factors for cardiovascular disease [12].

CLA is a mixture of octadecatrienoic fatty acid isomers. These isomers are positional (8,10,12–18:3; 9,11,13–18:3; 10,12,14–18:3; 11,13,15–18:3, etc.) and geometric (*cis* and *trans*) forms of linolenic acid. CLA in tung oil is mainly α-eleostearic acid (9c,11*t*, 13*t*-18:3) and β-eleostearic acid (9*t*,-11*t*,13*t*-18:3) [8, 13, 14].

The aim of this study was to determine the total lipid (oil) content as well as fatty acid composition, particularly CLA of the oils extracted from commercially important pomegranate cultivars grown in Turkey.

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Experimental Procedures

Material

Approximately 15 kg ($n = 40$) of fully mature, medium size (350–400 g) fruit from pomegranate cultivars [Fellahyemez (01N04), Katırbaşılı (31N07), Ekşilik (01N07), Hicaznar (07N08), İzmir-1264, İzmir-1499, İzmir-1513, Erdemli Aşınar (33N11), İzmir-23, İzmir-26, Ernar (07N03), Lefan (31N06), Silifke Aşısı (33N16), Ekşi Göknar (33N12) and Mayhoş-IV (07N14)] grown in Turkey were harvested by hand in 2006 (Table 1). Four fruits located externally and four located internally on each tree were harvested from five randomly selected trees for each pomegranate cultivar. Fellahyemez, Katırbaşılı, Ekşilik, Hicaznar, Erdemli, İzmir-23, İzmir-26 and Ernar cultivars from the West Mediterranean Agricultural Research Institute (Antalya); İzmir-1264, İzmir-1499 and İzmir-1513 cultivars from the Agean Agricultural Research Institute (İzmir); Erdemli, İzmir-23, İzmir-26, Ernar, Lefan, Silifke, Ekşi Göknar and Mayhoş IV cultivars from the Alata Horticultural Research Institute (Mersin) were harvested according to their skin color following parameters from our previous study [15].

Methods

This study was conducted at the West Mediterranean Agricultural Research Institute (Antalya), Agean Agricultural Research Institute (İzmir) and the Alata Horticultural Research Institute (Mersin) in 2006. During the crop cycle, the average minimum, maximum temperature, and relative humidity were recorded as 15.25, 25.59 and 62.74 °C in Antalya; 3.71, 29,78 and 59.33 °C in İzmir; 4.63, 24.66 and 61.24 °C in Mersin, respectively (Table 2).

Table 1 Harvest time and place of pomegranate fruits

Cultivar	Harvest date	Place
Fellahyemez	10 October 2006	Antalya-Serik (BAARI)
Katırbaşılı	10 October 2006	Antalya-Serik (BAARI)
Ekşilik	10 October 2006	Antalya-Serik (BAARI)
Hicaznar	10 October 2006	Antalya-Serik(BAARI)
İzmir-1264	28 September 2006	İzmir-Menemen (EARI)
İzmir-1499	28 September 2006	İzmir-Menemen (EARI)
İzmir-1513	28 September 2006	İzmir-Menemen (EARI)
Erdemli	2 October 2006	Antalya-Serik (BAARI)
İzmir-23	2 October 2006	Antalya-Serik (BAARI)
İzmir-26	2 October 2006	Antalya-Serik (BAARI)
Ernar	2 October 2006	Antalya-Serik (BAARI)
Lefan	17 October 2006	Mersin-Erdemli (BAARI)
Silifke	17 October 2006	Mersin-Erdemli (AHRI)
Ekşi Göknar	17 October 2006	Mersin-Erdemli (AHRI)
Mayhoş IV	17 October 2006	Mersin-Erdemli (AHRI)

BAARI West Mediterranean Agricultural Research Institute; *EARI* Agean Agricultural Research Institute; *AHRI* Alata Horticultural Research Institute

Harvested fruits were transported to the laboratory and the pomegranate fruits were sorted by appearance (i.e., free from sunburn, crack). Seeds were separated from their juice sacs, washed in distilled water, and dried in a vacuum oven at 55 °C to a constant weight. Afterwards, the dried seeds were ground in a coffee grinder (Sinbo, SCM-2909, Turkey). The oil from the dried seeds was successively extracted by hexane using a Soxhlet extractor for 5 h. The oil content was calculated as a percentage of the dry matter content.

Fatty acids were converted to their methyl esters [16] and injected into a Shimadzu (Kyoto, Japan) gas chromatograph, fitted with an FID detector. Separations were performed using an Omegawax 250 fused-silica capillary column (30 m, 0.25-mm i.d., 0.25 μm film thickness) (Bellefonte, PA, USA). Helium was used as the carrier gas at a flow rate of 1.25 mL/min. Injector and detector temperatures were 250 and 260 °C, respectively. The column temperature was maintained at 205 °C for 37 min. Samples of 0.5 μL were injected by autoinjector with a split mode (1:100).

FAMEs (Fatty acid methyl esters) were identified by comparison of their retention times with individual reference standards (palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), gadoleic acid (C20:1), behenic acid (C22:0) from Sigma Chemical Co. (St. Louis, MO, USA). The other fatty acids (catalpic, punicic, α-eleostearic and β-eleostearic acids) were identified using the following procedures: catalpic acid is the major fatty acid in catalpa tree seeds which were ground and extracted with hexane and esterified to the methyl esters. Tung oil containing large amounts of α-eleostearic and β-eleostearic acids was obtained from Sigma Chemical Co. and converted to the methyl esters. These methyl esters were injected into a GC and the retention times of catalpic, α-eleostearic and β-eleostearic acids were identified. The content (percentage by weight) of fatty acids was calculated from their corresponding integration data.

Statistical Analysis

Analysis of variance (ANOVA) and Duncan's multiple range test were performed using SAS (SAS Version 6.12) to evaluate the significance of differences between the oil contents and individual fatty acids of samples at the level of $p < 0.05$. Samples were studied in two replication (the fruit was selected from five different trees and then pooled

Table 2 Climatic conditions of growing places during 2006 growing season

Month	Antalya-Serik (BAARI)			İzmir-Menemen (EARI)			Mersin-Erdemli (AHRI)		
	Min	Max	RH	Min	Max	RH	Min	Max	RH
January	5.4	14.2	55.1	−6.5	14.7	72	4.9	15.0	48.9
February	6.9	15.9	63.3	−5.5	19.4	70	5.6	15.6	59.3
March	9.1	18.2	71.4	−3.4	21.1	70	7.9	18.5	68.5
April	12.6	22.4	63.7	0.9	26.2	64	1.9	22.5	65.6
May	15.4	26.9	64.2	2.2	36.9	58	1.1	25.5	60.4
June	20.4	31.5	57.9	8.2	38.8	49	2.1	28.8	65.6
July	23.3	35.2	55.6	12.6	37.1	44	2.2	31.3	60.2
August	24.1	33.9	66.9	14.2	38.2	51	2.6	32.3	66.1
September	19.8	31.8	60.8	10.7	35.6	56	2.2	30.7	54.4
October	15.5	25.9	68.5	6.6	29.7	69	15.8	26.4	63.4
November	8.7	20.2	60.7	−3.5	21.2	72	8.4	20.5	51.6
December	8.3	17.6	56.2	−6.4	17.7	69	4.4	17.5	43.1

Min minimum temperature (°C); *Max* maximum temperature (°C); *RH* relative humidity (%)

for replication 1 and then fruit from another five trees were selected and then pooled for replication 2). Data were expressed as means ± Standard Error (SE).

Results and Discussion

The oil contents of pomegranate seeds are shown in Table 3. The amount of total lipids of the dry matter ranged between 13.95 (Ekşilik) and 24.13% (Fellahyemez). The seed oil content was determined as 18.1% of the dry weight [8]. Other studies indicated that total lipid contents varied between cultivars grown in Spain and Iran, i.e. 6.90–10.49% and 6.63–19.3%, respectively [6, 7]. Our results are in agreement with findings of a study carried out in Turkey, but higher than the Spanish and some of the Iranian varieties [6, 7]. Particularly, Fellahyemez, İzmir-1264, Hicaznar, Mayhoş IV, Ernar, İzmir-1513 and İzmir-23 cultivars had a higher oil content than other cultivars previously reported.

Fatty acid composition of pomegranate seed oils was determined and 11 different fatty acids were identified in all samples (Fig. 1). The content of saturated and unsaturated fatty acids are presented in Tables 4 and 5, respectively.

The major saturated fatty acid in the cultivars was palmitic acid which ranged between 2.10 (Mayhoş IV) and 2.77% (Fellahyemez). Palmitic acid was previously reported to be between 2.58–14.91, 2.0, and 0.30–9.90% [6, 8, 17]. It was followed by stearic acid that was between 1.35 (Mayhoş IV) and 2.01% (İzmir-1499). The amounts of arachidic and behenic acids were less and were between 0.33 (Ekşilik)–0.48% (Ernar) and 0.16 (İzmir-1264 and Mayhoş IV)–0.22% (İzmir-1499), respectively. Stearic acid was reported as 2.8–16.7, 1.6, 1.16–8.98 and 1.6–2.38% [6–8, 17]. Our findings for palmitic and stearic acids are similar to these references. Yücel [8] and Melgarejo and Artes [17] determined arachidic acid contents as being 3.0 and 0–2.76%, respectively. However, these researchers did not detect behenic acid in pomegranate seed oil. However, behenic acid content of pomegranate seed oils was determined to be in the range of 0–3.9% by Fadavi et al. [6]. There are no agreements for arachidic and behenic acid between our findings and the results of these researchers.

Punicic acid was the major unsaturated fatty acid in all samples and ranged between 70.42 and 76.17%. The

Table 3 Oil content of pomegranate seeds (%, means ± SE)

Cultivars	Oil content
Fellahyemez	$24.13^{a} \pm 0.740$
Katırbaşılı	$15.93^{de} \pm 0.560$
Ekşilik	$13.95^{f} \pm 1.030$
Hicaznar	$21.03^{bc} \pm 0.525$
İzmir-1264	$22.64^{ab} \pm 0.220$
İzmir-1499	$14.69^{ef} \pm 0.505$
İzmir-1513	$20.19^{c} \pm 0.260$
Erdemli	$16.83^{d} \pm 0.355$
İzmir-23	$19.67^{c} \pm 0.005$
İzmir-26	$16.38^{de} \pm 0.145$
Ernar	$20.22^{c} \pm 0.320$
Lefan	$17.56^{d} \pm 0.940$
Silifke	$14.65^{ef} \pm 0.690$
Ekşi Göknar	$17.81^{d} \pm 0.735$
Mayhoş IV	$20.39^{c} \pm 0.330$

Different superscript letters in the same column indicate significant difference between values at the $p < 0.05$ level

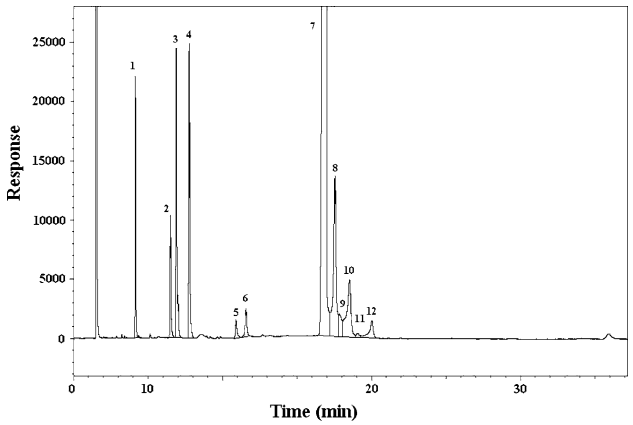

Fig. 1 GC chromatogram of fatty acids of a pomegranate oil. Peaks correspond to (*1*) palmitic acid; (*2*) stearic acid; (*3*) oleic acid; (*4*) linoleic acid; (*5*) arachidic acid; (*6*) gadoleic acid; (*7*) punicic acid; (*8*) α-eleostearic acid; (*9*) unidentified; (*10*) catalpic acid; (*11*) behenic acid; (*12*) β-eleostearic acid

punicic acid content of the samples varied significantly depend on cultivar. The highest punicic acid content was determined in Mayhoş IV cultivar. α-Eleostearic acid was determined as the second most abundant fatty acid in all samples and ranged between 5.94 (Katırbaşılı) and 6.85% (İzmir-1499). Linoleic, catalpic, and oleic acids were found in all cultivars and their contents were determined to be 3.44 (Mayhoş IV)–5.27% (Fellahyemez), 3.06 (Ekşilik)–4.40% (Fellahyemez), and 3.20 (Mayhoş IV)–5.28% (İzmir-1499), respectively. β-Eleostearic (0.73–1.35%) and gadoleic acids (0.42–0.75%) were lowest among the unsaturated fatty acids. Melgarejo and Artes [17], Fadavi et al. [6] and Hernandez et al. [7] found punicic acid and the other CLA isomers and declared all of them as linolenic acid. Yücel [8] identified punicic, catalpic and β-eleostearic acids in pomegranate seed oil. But α-eleostearic acid was not detected. Our results do not correspond with the other studies [6–8, 17]. In our study, in addition to punicic, catalpic and β-eleostearic acids, the α-eleostearic isomer of CLA was identified in pomegranate seed oil.

Linoleic and oleic acid contents were similar to previous studies [6, 7, 17]. However, in one report, the linoleic acid content was higher (5.19–16.50%) than our results [17]. Gadoleic acid was not detected by other researchers. The fatty acid content of our samples ranged between 0.42 and 0.75%.

Some results on oil content and fatty acids of samples were different from the referncrs cited above. Some researchers [18–23] indicated that oil content and fatty acid composition of vegetable oils vary depending on genotype, location, harvest time and climatic conditions etc. Lajara et al. [24] determined variation in the oil content and fatty acid composition of different sunflower varieties from different locations. Oil content and fatty acid composition varied according to temperature, location and varieties. Thompson et al. [25] determined fatty acid composition of four corn genotypes grown in five environments. Environment conditions influenced fatty acid composition of corn genotypes. Robertson et al. [26] studied the effect of maturity on oil content and fatty acid composition of sunflower. They are influenced by the stage of maturity of the sunflower seeds. Our samples were different cultivars and collected from three locations which have different climatic conditions and as well as geographical properties. These

Table 4 Saturated fatty acid contents of pomegranate seed oils (%, means ± SE)

Cultivars	Palmitic (C16:0)	Stearic (C18:0)	Arachidic (C20:0)	Behenic (C22:0)
Fellahyemez	$2.77^{a} \pm 0.030$	$1.52^{g} \pm 0.000$	$0.35^{bc} \pm 0.005$	$0.21^{abc} \pm 0.005$
Katırbaşılı	$2.36^{gh} \pm 0.015$	$1.72^{c} \pm 0.005$	$0.44^{ab} \pm 0.065$	$0.18^{abcd} \pm 0.005$
Ekşilik	$2.72^{b} \pm 0.005$	$1.76^{b} \pm 0.005$	$0.33^{c} \pm 0.010$	$0.17^{bcd} \pm 0.010$
Hicaznar	$2.25^{j} \pm 0.010$	$1.59^{e} \pm 0.005$	$0.40^{abc} \pm 0.020$	$0.18^{abcd} \pm 0.015$
İzmir-1264	$2.34^{hı} \pm 0.010$	$1.63^{d} \pm 0.000$	$0.42^{abc} \pm 0.000$	$0.16^{d} \pm 0.005$
İzmir-1499	$2.54^{e} \pm 0.010$	$2.01^{a} \pm 0.010$	$0.43^{abc} \pm 0.005$	$0.22^{a} \pm 0.040$
İzmir-1513	$2.39^{g} \pm 0.030$	$1.63^{d} \pm 0.005$	$0.39^{abc} \pm 0.000$	$0.18^{abcd} \pm 0.000$
Erdemli	$2.60^{d} \pm 0.000$	$1.40^{ı} \pm 0.005$	$0.39^{abc} \pm 0.005$	$0.21^{ab} \pm 0.000$
İzmir-23	$2.54^{e} \pm 0.000$	$1.56^{f} \pm 0.015$	$0.42^{abc} \pm 0.000$	$0.19^{abcd} \pm 0.000$
İzmir-26	$2.49^{f} \pm 0.005$	$1.51^{g} \pm 0.005$	$0.38^{bc} \pm 0.005$	$0.18^{abcd} \pm 0.000$
Ernar	$2.65^{c} \pm 0.020$	$1.52^{g} \pm 0.010$	$0.48^{a} \pm 0.085$	$0.19^{abcd} \pm 0.025$
Lefan	$2.33^{hı} \pm 0.005$	$1.52^{g} \pm 0.005$	$0.36^{bc} \pm 0.000$	$0.18^{abcd} \pm 0.000$
Silifke	$2.35^{gh} \pm 0.005$	$1.49^{h} \pm 0.005$	$0.36^{bc} \pm 0.005$	$0.17^{bcd} \pm 0.010$
Ekşi Göknar	$2.30^{ı} \pm 0.005$	$1.53^{g} \pm 0.005$	$0.37^{bc} \pm 0.005$	$0.17^{bcd} \pm 0.000$
Mayhoş IV	$2.10^{k} \pm 0.020$	$1.35^{j} \pm 0.000$	$0.39^{abc} \pm 0.005$	$0.16^{cd} \pm 0.000$

Different superscript letters in the same column indicate significant difference between values at the $p < 0.05$ level

Table 5 Unsaturated fatty acid contents of pomegranate seed oils (%, means ± SE)

Varieties	Oleic (C18:1)	Linoleic (C18:2)	α-eleostearic (9c, 11*t*,13*t*-18:3)	β-eleostearic (9*t*,11*t*, 13*t*-18:3)	Catalpic (9*t*,11*t*,13c-18:3)	Punicic (9 c,11*t*,13c-18:3)	Gadoleic (C20:1)
Fellahyemez	$4.87^{abc} \pm 0.015$	$5.27^{a} \pm 0.040$	$6.72^{ab} \pm 0.500$	$1.35^{a} \pm 0.135$	$4.40^{a} \pm 0.565$	$70.83^{c} \pm 1.17$	$0.42^{g} \pm 0.005$
Katırbaşılı	$4.92^{ab} \pm 0.005$	$5.21^{a} \pm 0.040$	$5.94^{b} \pm 0.030$	$1.00^{bcde} \pm 0.015$	$3.26^{bc} \pm 0.020$	$73.38^{b} \pm 0.06$	$0.56^{ef} \pm 0.030$
Ekşilik	$4.36^{de} \pm 0.360$	$4.61^{bc} \pm 0.005$	$6.04^{b} \pm 0.080$	$0.73^{f} \pm 0.000$	$3.06^{c} \pm 0.080$	$74.69^{ab} \pm 0.34$	$0.57^{ef} \pm 0.035$
Hicaznar	$4.09^{ef} \pm 0.045$	$4.22^{h} \pm 0.005$	$6.10^{ab} \pm 0.000$	$1.07^{bcde} \pm 0.040$	$3.39^{bc} \pm 0.000$	$74.97^{ab} \pm 0.05$	$0.71^{ab} \pm 0.035$
İzmir-1264	$4.46^{cde} \pm 0.160$	$4.41^{ef} \pm 0.055$	$6.21^{ab} \pm 0.075$	$0.96^{cde} \pm 0.015$	$3.34^{bc} \pm 0.035$	$74.38^{b} \pm 0.10$	$0.69^{abc} \pm 0.015$
İzmir-1499	$5.28^{a} \pm 0.020$	$5.26^{a} \pm 0.040$	$6.85^{a} \pm 0.640$	$1.23^{ab} \pm 0.195$	$3.84^{ab} \pm 0.500$	$70.42^{c} \pm 1.38$	$0.69^{abc} \pm 0.010$
İzmir-1513	$4.54^{bcd} \pm 0.310$	$4.40^{efg} \pm 0.050$	$6.20^{ab} \pm 0.155$	$1.16^{abc} \pm 0.040$	$3.63^{bc} \pm 0.125$	$73.76^{b} \pm 0.12$	$0.68^{b}c \pm 0.000$
Erdemli	$3.79^{fg} \pm 0.055$	$3.96^{i} \pm 0.010$	$6.64^{ab} \pm 0.260$	$1.13^{abcd} \pm 0.025$	$3.58^{bc} \pm 0.145$	$74.79^{ab} \pm 0.49$	$0.53^{f} \pm 0.005$
İzmir-23	$3.78^{fg} \pm 0.070$	$4.70^{b} \pm 0.045$	$6.28^{ab} \pm 0.000$	$0.91^{def} \pm 0.020$	$3.12^{c} \pm 0.015$	$74.84^{ab} \pm 0.04$	$0.62^{de} \pm 0.010$
İzmir-26	$3.51^{gh} \pm 0.050$	$4.53^{cd} \pm 0.040$	$6.48^{ab} \pm 0.000$	$1.00^{bcde} \pm 0.040$	$3.25^{bc} \pm 0.000$	$74.88^{ab} \pm 0.08$	$0.62^{de} \pm 0.005$
Ernar	$3.46^{h} \pm 0.020$	$4.28^{gh} \pm 0.060$	$6.36^{ab} \pm 0.110$	$1.16^{abc} \pm 0.005$	$3.46^{bc} \pm 0.075$	$74.91^{ab} \pm 0.14$	$0.51^{f} \pm 0.015$
Lefan	$4.46^{cde} \pm 0.030$	$4.45^{de} \pm 0.035$	$6.44^{ab} \pm 0.095$	$0.86^{ef} \pm 0.020$	$3.18^{bc} \pm 0.070$	$74.56^{ab} \pm 0.21$	$0.64^{cd} \pm 0.015$
Silifke	$3.64^{g} \pm 0.005$	$4.29f^{gh} \pm 0.020$	$6.66^{ab} \pm 0.040$	$0.92^{def} \pm 0.025$	$3.41^{bc} \pm 0.095$	$75.09^{ab} \pm 0.14$	$0.60^{de} \pm 0.000$
Ekşi Göknar	$4.52^{bcde} \pm 0.020$	$4.33^{efgh} \pm 0.000$	$6.56^{ab} \pm 0.030$	$0.98^{cde} \pm 0.040$	$3.64^{bc} \pm 0.055$	$73.98^{b} \pm 0.04$	$0.61^{de} \pm 0.020$
Mayhoş IV	$3.20^{h} \pm 0.025$	$3.44^{j} \pm 0.045$	$6.60^{ab} \pm 0.035$	$1.00^{bcde} \pm 0.055$	$3.61^{bc} \pm 0.095$	$76.17^{a} \pm 0.04$	$0.75^{a} \pm 0.025$

Different superscript letters in the same column indicate significant difference between values at $p < 0.05$ level

differences between samples and literatures may be sourced from different factors mentioned above.

Conclusion

Pomegranate seeds have considerable amounts of oil depending on the cultivar. The seeds of all cultivars had FA compositions that were statistically different ($p < 0.05$). The saturated/unsaturated fatty acid ratio ranged between 0.042 and 0.055. The oil is very rich in CLA, particularly punicic acid, which are protective against some cancer types. Therefore, pomegranate seed oil is very valuable in terms of health. The seeds, remaining after processing the fruits into juice, can be subjected to oil extraction. This is important for making additional profit from the plants.

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